MONONUCLEAR PHAGOCYTES (KUPFFER CELLS) AND ENDOTHELIAL CELLS

Identification of Two Functional Cell Types in Rat Liver Sinusoids by Endogenous Peroxidase Activity

JEAN-JACQUES WIDMANN, RAMZI S. COTRAN, and H. DARIUSH FAHIMI

From the Harvard Pathology Unit, Mallory Institute of Pathology at Boston City Hospital, Boston, Massachusetts 02118

ABSTRACT

The fine structural characteristics and phagocytic properties of peroxidase-positive and peroxidase-negative cells in rat hepatic sinusoids were investigated. Cells with a positive peroxidase reaction in the endoplasmic reticulum and the nuclear envelope make up approximately 40% of cells in rat hepatic sinusoids and have abundant cytoplasm containing numerous granules and vacuoles, and occasional tubular, vermiform invaginations. After intravenous injection of colloidal carbon, the luminal plasma membrane of these cells shows continuous sticking of carbon, and there is evidence of avid phagocytosis of colloidal carbon particles. Peroxidase-positive cells are the only cells in hepatic sinusoids which phagocytize large (0.8 μ in diameter) latex particles. In contrast, the peroxidase-negative endothelial cells, which make up 48% of cells, have scanty perinuclear cytoplasm and organelles, and their long cytoplasmic extensions that form the lining of the hepatic sinusoids have fenestrations; these cells ingest small amounts of colloidal carbon, principally by micropinocytosis, exhibit no sticking of carbon particles to their plasma membranes, and do not ingest the larger (latex) particles. The so-called fat-storing cells are peroxidase negative and totally nonphagocytic. The peroxidase reaction thus distinguishes the typical mononuclear phagocytes or Kupffer cells of rat liver from the endothelial-lining cells.

Since the initial observation of phagocytic activity in cells lining the hepatic sinusoids (1), and the description of phagocytic Kupffer cells by Von Kupffer and others (2–4), the question: "Whether hepatic sinusoids are lined by a single cell type or by two or more different distinct types of cells" has been the subject of controversy. Several comprehensive reviews of the literature have noted that since all hepatic sinusoidal cells are capable of a certain degree of phagocytosis, they must be functional variants of the same single cell type (5–7). Although several recent fine structural studies (8–12) indicate the presence of at least two types of lining cells, i.e. a wall-forming endothelial cell and a phagocytic Kupffer cell, other authors emphasize that there are numerous intermediate forms and, on ultrastructural grounds alone, raise again the question of two variants of the same cell type (6).

In a recent cytochemical study from this labora-

tory, Fahimi reported that approximately 40% of sinusoidal-lining cells in rat liver showed a characteristic pattern of endogenous peroxidase activity in the endoplasmic reticulum and the perinuclear cisternae (13). A similar pattern of localization of peroxidase has also been described in peritoneal macrophages of guinea pigs and rats by Cotran and coworkers (14, 15). The present study was undertaken in order to determine whether such peroxidase activity in hepatic sinusoidal-lining cells could be correlated with distinct morphological and functional (phagocytic) properties and thus could be used as a marker to differentiate Kupffer cells from endothelial cells. The results indicate that peroxidase-positive cells, as compared to the peroxidase-negative cells, (*a*) ingest significantly larger amounts of relatively small particles (carbon 0.02–0.05 μ), (*b*) exclusively phagocytize larger particles (latex 0.8 μ), and (*c*) possess morphological characteristics that have been ascribed by others (8–12) to typical Kupffer cells. The peroxidase reaction can thus clearly differentiate two types of sinusoidal-lining cells: Kupffer cells and endothelial cells.

MATERIALS AND METHODS

Cytochemical Studies

Normal female adult albino rats (Charles River strain) weighing 200–250 g each and fasted for 24 hr were used. The liver was fixed by perfusion through the portal vein (16) with 2 or 2.25% purified glutaraldehyde (17), in 0.1 M sodium cacodylate buffer, pH 7.3, for approximately 10 min. After several rinses in the same buffer, 40-μ-thick sections were cut on a Sorvall TC2 tissue sectioner (Ivan Sorvall Inc., Norwalk, Conn.) (18), collected in 0.05 M Tris-HCl buffer, pH 7.6, and incubated in a modified Graham and Karnovsky (19) medium containing 0.1% 3,3′-diaminobenzidine-tetrahydrochloride (DAB) (Sigma Chemical Co., St. Louis, Mo.), and 0.01% H_2O_2 in 0.1 M Tris-HCl buffer, pH 7.6, for 60 min at room temperature. After 90 min of postfixation with 2% osmium tetroxide in distilled water (20), sections were dehydrated in graded ethanol solutions and embedded in Epon 812 (21). 1-μ-thick sections were examined by light microscopy both unstained and stained with 1% toluidine blue. Thin sections for electron microscopy were cut on an LKB ultrotome III (LKB Instruments, Rockville, Md.) and examined either unstained or lightly counterstained with lead citrate in a Philips EM 200 electron microscope. Cytochemical controls for the peroxidase activity were performed by incubation of sections from all experimental animals in media in the absence of hydrogen peroxide.

Phagocytosis Experiments

COLLOIDAL CARBON: Under light ether anesthesia, animals were injected intravenously with 0.05 ml/100 g weight of filtered, shellac-free, nontoxic colloidal carbon (Gunther Wagner, Hannover, Germany, Lot c-11-1431/a, containing 100 mg carbon/ml), and the livers were fixed by perfusion at 2, 5, and 30 min. Such carbon particles measure approximately 0.03 μ in diameter (22).

LATEX: In another group of animals, 0.05 ml/100 g weight of latex particles with mean diameter of 0.8 μ (Bacto-Latex 0.81, code 3102 from Difco Laboratories, Detroit Mich.) were injected directly into the portal vein of anesthetized and laparotomized rats; the livers were fixed by perfusion 2 min and 5 hr after the injection of particles.

RESULTS

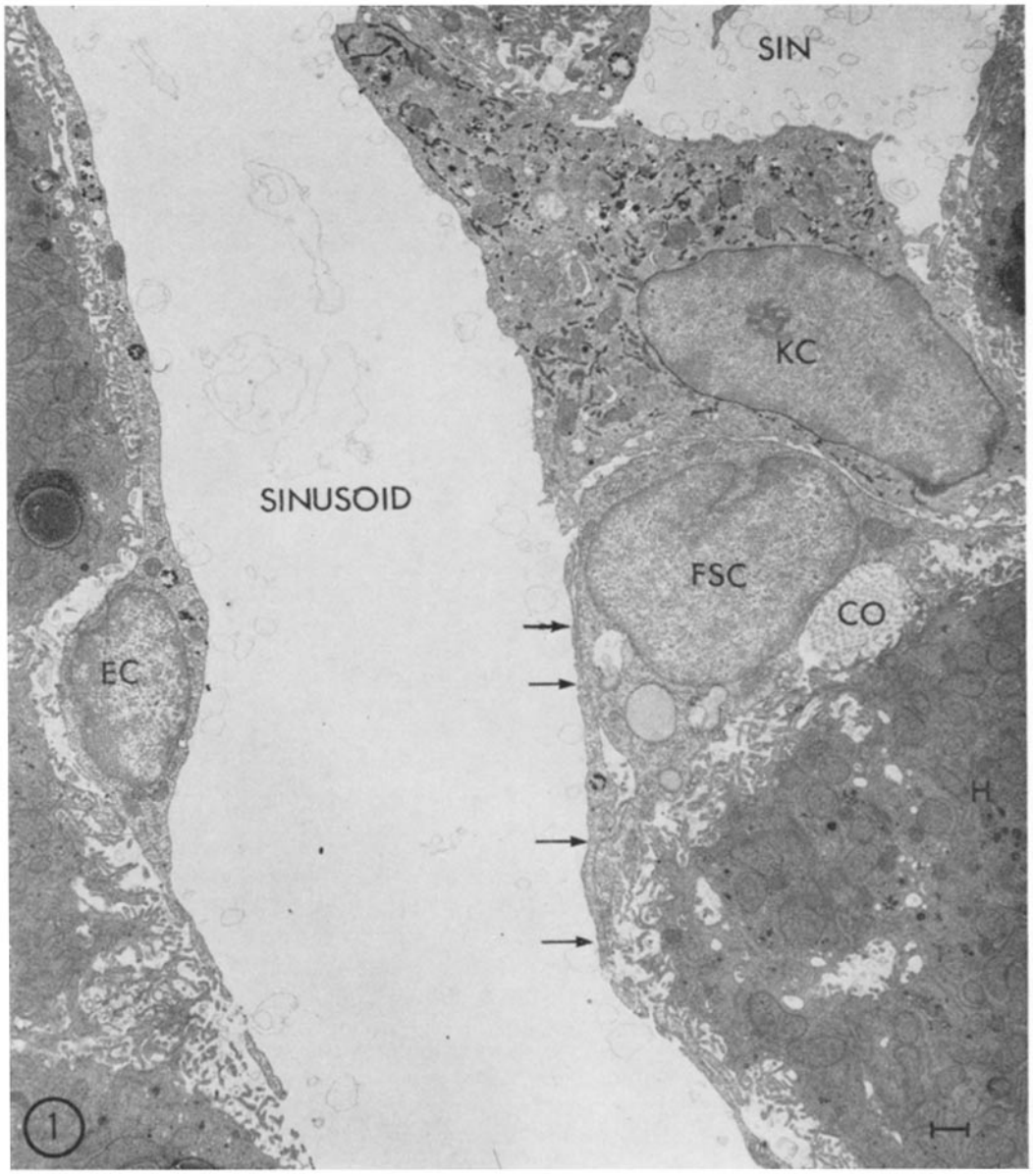
Fine Structural Cytochemical Characteristics of Various Sinusoidal-Lining Cells

On the basis of ultrastructural characteristics and peroxidase activity, three different types of cells could be differentiated (Fig. 1):

(*a*) The typical peroxidase-positive cells (*KC* in Fig. 1) exhibited electron-opaque reaction product in the nuclear envelope and segments of the endoplasmic reticulum. These cells had relatively abundant cytoplasm, and were rich in cytoplasmic organelles, and had numerous granules of varying density and shape. Peroxidase-positive cells were seen overlying the extensions of typical peroxidase-negative endothelial cells and were found occasionally in direct contact with microvilli of hepatocytes.

(*b*) The typical wall-forming endothelial cells (*EC* in Fig. 1) were peroxidase negative, possessed a smaller nucleus, and had less cytoplasm and fewer organelles. The cytoplasmic extensions of these cells showed many micropinocytotic vesicles and, in proper sections, exhibited fenestrations or sieve plates (10) (Figs. 1, 5, and 7). In some areas, there were larger discontinuities in the endothelium as described by several investigators (23, 47), but we could not determine whether such discontinuities were preexisting gaps which increased in size owing to high perfusion pressure. This matter was not pursued further in the present study.

(*c*) The third cell type was the so-called fat-



FIGURES 1–7 are from livers of rats injected with colloidal carbon; the livers were fixed at different time intervals by perfusion and processed for peroxidase activity. All sections were counterstained with lead citrate. Length of scale line for all figures is 1 μ.

FIGURE 1 This electron micrograph illustrates the three types of cells encountered in rat hepatic sinusoids. A Kupffer cell (*KC*), which is seen at the bifurcation of the sinusoid (*SIN*), shows peroxidase reaction product in the perinuclear cisternae and ER. A typical wall-forming endothelial cell (*EC*), elongated in shape, shows no evidence of staining for peroxidase. A "fat-storing cell" (*FSC*) contains a few fat droplets and lacks peroxidase reaction product. Note that fine extensions of an endothelial cell cover the luminal surface of the fat-storing cell (arrows), indicating the true perisinusoidal location of such cells. Kupffer cells and endothelial cells are thus the only two cell types which line the sinusoids. Hepatocytes, *H*; collagen, *CO*. × 5700.

storing cell (*FCS* in Fig. 1) (24–26). These cells were characterized by the presence of a few lipid droplets, a few mitochondria, and a prominent granular endoplasmic reticulum. They were always peroxidase negative (Fig. 1). Such cells were separated from the sinusoidal lumen by thin extensions of endothelial cell cytoplasm (Fig. 1), and thus can be considered as truly perisinusoidal cells.

Of over 1000 sinusoidal-lining cells counted, 39% of cells were peroxidase-positive Kupffer cells, 48% endothelial cells, and 13% fat-storing cells.

Uptake of Colloidal Carbon by Sinusoidal-Lining Cells

Light microscope examination of thick Epon sections from livers of animals injected with 0.05 ml/100 g weight of colloidal carbon revealed that larger amounts of carbon particles were picked up by sinusoidal-lining cells around the portal tract as compared to those in the center of hepatic lobules. Studies with electron microscopy revealed a striking difference in distribution of injected carbon particles between peroxidase-positive and peroxidase-negative cells (Fig. 2).

PEROXIDASE-POSITIVE CELLS: 2 and 5 min after the injection of carbon particles, the peroxidase-positive cells in the periportal region of hepatic lobules exhibited a continuous line of carbon particles attached to the cell membrane facing the luminal aspect of the hepatic sinusoid (Fig. 2). A narrow space of approximately 150–200 A separated the carbon particles from the cell membrane of the peroxidase-positive cells (Figs. 2 and 3). Occasional pseudopods projecting from the luminal surface membrane of such cells also showed evidence of sticking of carbon. At these intervals, fairly large numbers of carbon particles were phagocytized by peroxidase-positive cells and were contained in membrane-bounded phagosomes and phagolysosomes close to the luminal surface of the cell. 30 min after injection, there was no more evidence of carbon sticking on the surface of peroxidase-positive cells, but the particles appeared in large vacuoles located deep in the cytoplasm (Fig. 4) close to the nucleus. Occasional tubular structures beneath the luminal surface of peroxidase-positive cells, the so-called "micropinocytosis vermiformis" (27, 28), also contained carbon particles at 30 min (Fig. 4).

PEROXIDASE-NEGATIVE CELLS: Of the two other cell types which border the hepatic sinusoids and which are peroxidase negative, the fat-storing cells never showed any evidence of uptake of carbon particles. The endothelial cells, however, did ingest carbon particles but considerably less so than the peroxidase-positive cells (see quantitative data below). 2 and 5 min after the injection, there was no linear sticking of carbon to the luminal surface membrane. Fig. 2 demonstrates the contrast between a peroxidase-positive cell with carbon sticking to the luminal surface and a peroxidase-negative cell without such linear sticking of carbon. Occasional micropinocytotic vesicles containing carbon particles were seen below the surface membrane of peroxidase-negative cells (Figs. 2, 5, and 6). Rarely, larger intracytoplasmic vacuoles with an approximate diameter of 0.3–0.5 μ were also seen to contain carbon (Figs. 2 and 6). In comparison to vacuoles of about the same size in peroxidase-positive cells, the vacuoles of peroxidase-negative cells contained consistently fewer particles of carbon, which were distributed at the periphery of such vacuoles such that the center of the vacuoles was electron lucent (Fig. 6). No marked difference was noted in the amount of carbon in vacuoles of peroxidase-negative cells at 5 and 30 min after the injection.

QUANTITATIVE DATA ON CARBON PHAGOCYTOSIS: In an attempt to obtain a quantitative estimate of the difference in phagocytic activity between peroxidase-positive and peroxidase-negative sinusoidal-lining cells, we examined approximately 1000 cells in sections prepared from different blocks of animals injected with carbon and sacrificed at 2 and 5 min. Peroxidase-positive and -negative cells were divided arbitrarily into four categories (0 to 3+) according to the number and size of carbon-containing vacuoles in each cell type. The results of such counts are presented in Table I. It can be seen that greater than 1+ carbon was seen in 40% of peroxidase-positive cells in contrast to only 5% of peroxidase-negative cells. The relatively large proportion of peroxidase-positive cells exhibiting no phagocytosis is believed to be due to the small dose of carbon used in these experiments; such cells were seen principally in the central part of the lobules. These quantitative results indicate that although peroxidase-negative cells are able to ingest small amounts of carbon particles, most of the cells

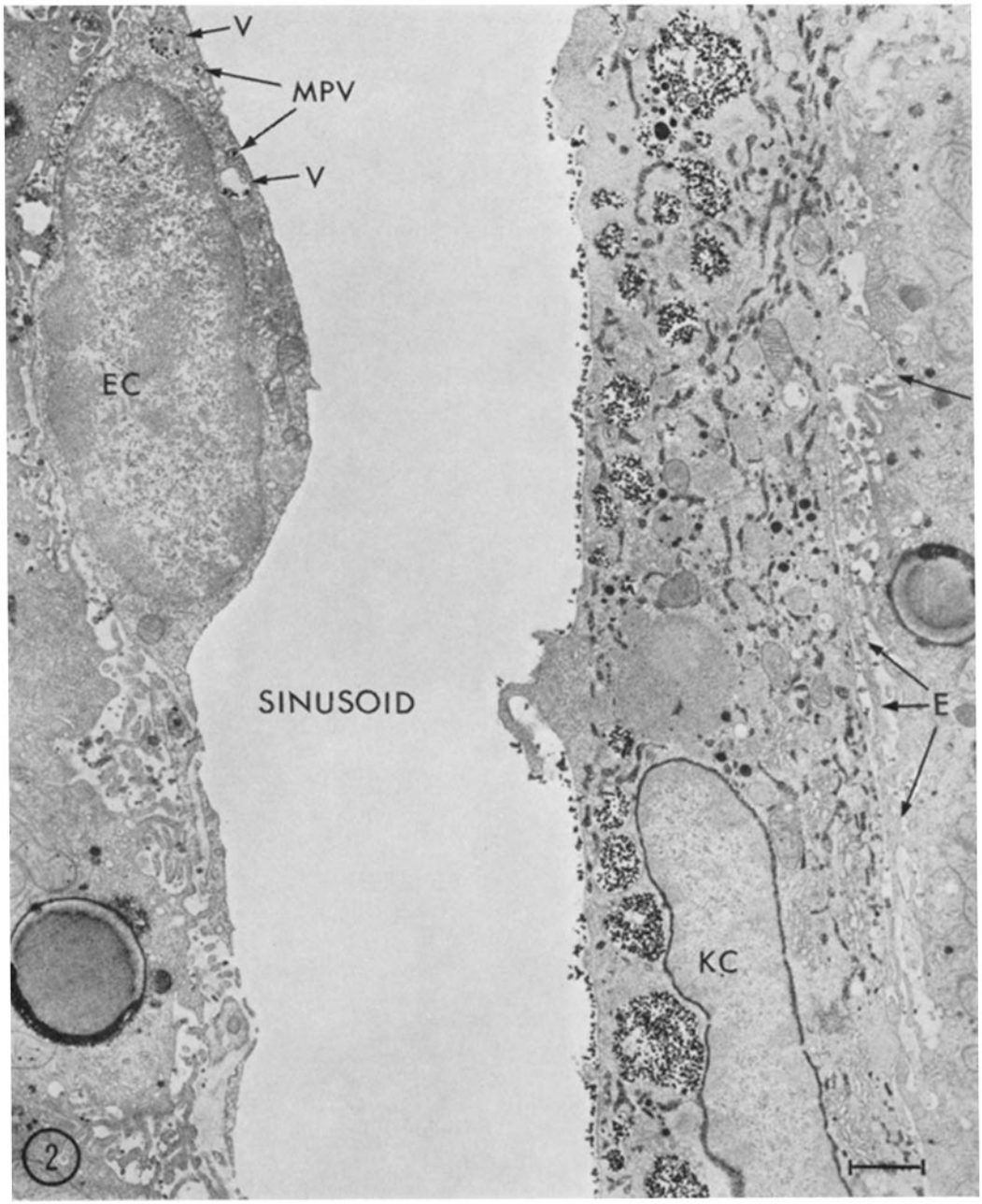


FIGURE 2 Electron micrograph of hepatic sinusoid 5 min after injection of carbon, showing the linear sticking of particles on the luminal surface of a peroxidase-positive Kupffer cell (*KC*) and the contrasting absence of such sticking on the endothelial cell (*EC*). In the Kupffer cell, carbon particles are also contained in phagosomes situated close to the luminal cell surface, whereas the endothelial cell shows only scant particles in micropinocytotic vesicles (*MPV*) or in larger intracytoplasmic vacuoles (*V*). The Kupffer cell is separated from the hepatocellular microvilli by discontinuous endothelial cytoplasmic processes (*E*), but also shows occasional direct contact with the hepatocyte (arrow). Sinusoidal lumen, *sinusoid*. × 9800.

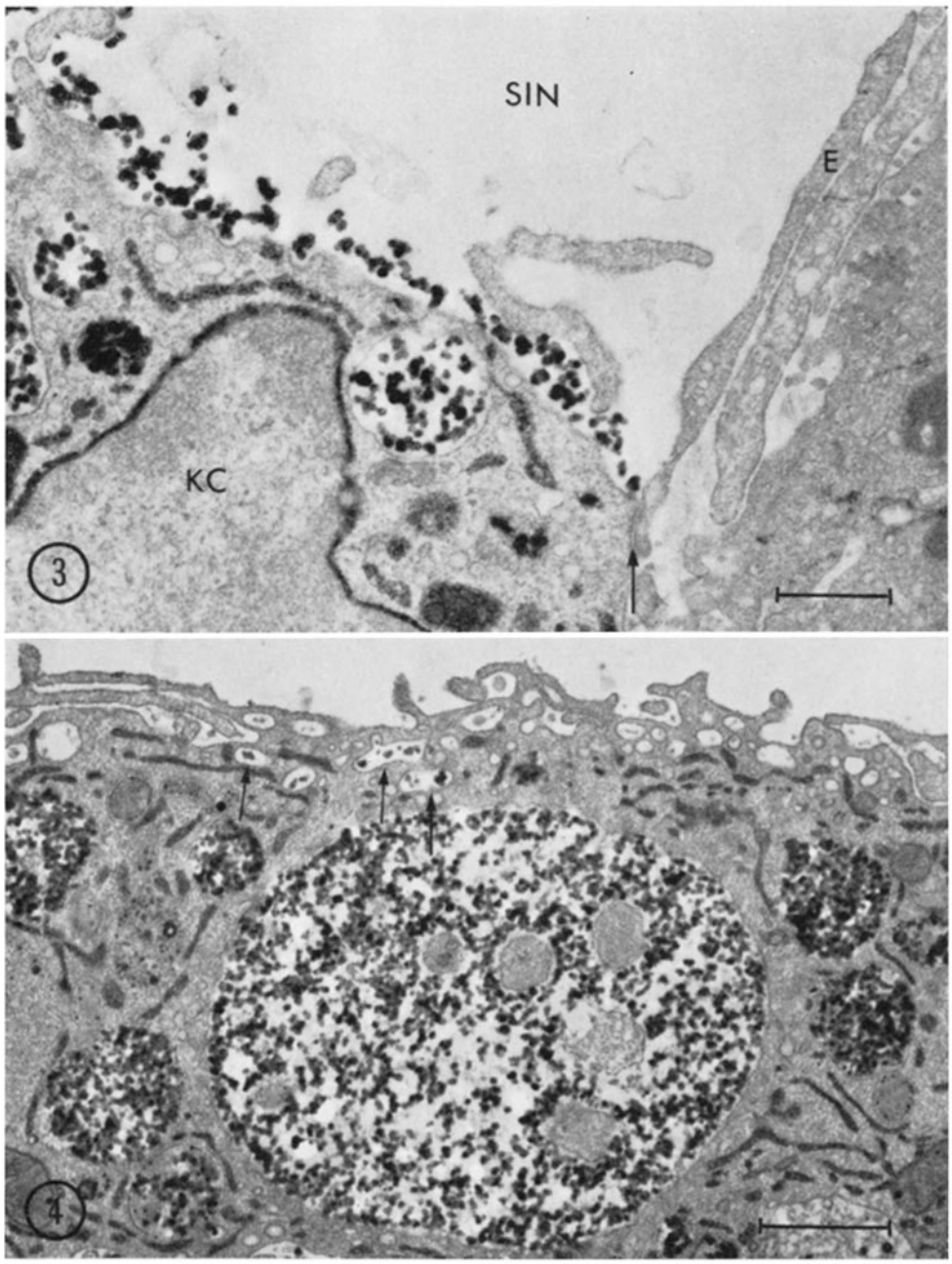


FIGURE 3 Electron micrograph of the junction (arrow) between a peroxidase-positive Kupffer cell (*KC*) and a cytoplasmic process of an endothelial cell (*E*), 5 min after injection of carbon. Sticking particles are exclusively present on the luminal surface of the Kupffer cell. Carbon-containing phagosomes are found close to the cell surface. Sinusoidal lumen, *SIN*. × 15,600.

FIGURE 4 High-power view of a peroxidase-positive Kupffer cell 30 min after injection of carbon particles. Carbon is found in large phagosomes and phagolysosomes deep in the cytoplasm, and in the "micropinocytosis vermiformis" (arrows) close to the luminal cell surface. × 17,900.

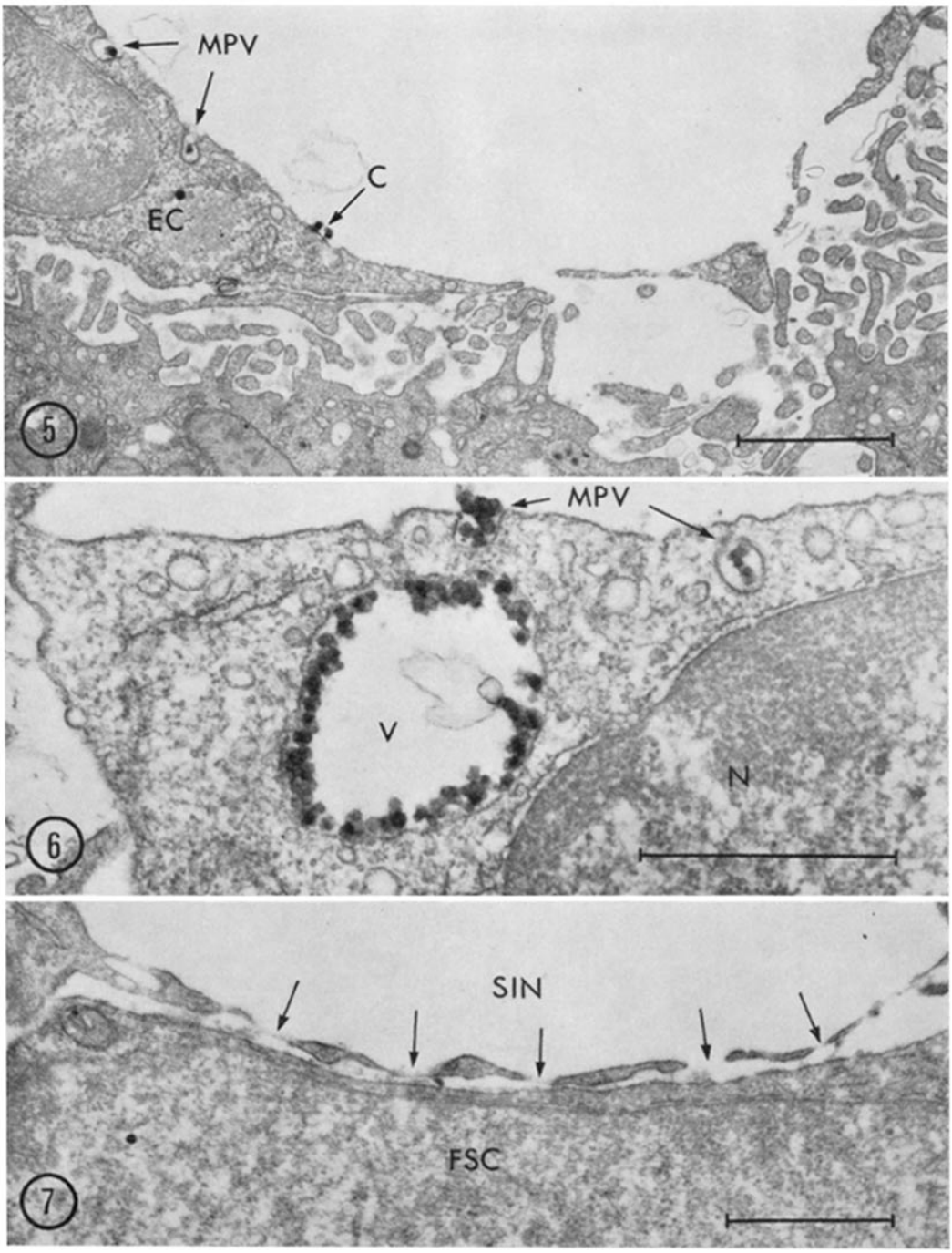


FIGURE 5 Part of a peroxidase-negative endothelial cell (*EC*) 2 min after injection of carbon. A few carbon particles are found on the cell surface (*C*) or in micropinocytic vesicles (*MPV*). × 22,600.

FIGURE 6 High-power electron micrograph of an endothelial cell 5 min after injection of carbon, showing a large carbon-containing cytoplasmic vacuole (*V*). The particles are at the periphery of the vacuole. A few carbon particles are also found in micropinocytic vesicles (*MPV*). nucleus, *N*. × 37,000.

FIGURE 7 High-power view of a "sieve plate" (arrows) (10) in an endothelial-lining cell overlying a "fat-storing cell" (*FSC*). Sinusoidal lumen, *SIN*. × 24,200.

TABLE I

Phagocytosis of Colloidal Carbon by Peroxidase-Positive and Peroxidase-Negative Hepatic Sinusoidal-Lining Cells

	Peroxidase-positive	Peroxidase-negative	Fat-storing cells
No. of cells counted	411	500	143
% with no phagocytosis	33	56	100
% with + carbon	27	39	0
% with ++ carbon	25	5	0
% with +++ carbon	15	0	0

with large phagosomes and phagolysosomes containing carbon particles (3+) are the peroxidase-positive cells. Of 143 fat-storing cells examined, none showed any evidence of ingestion of particles. Also, it is to be emphasized here that the quantitative difference between peroxidase-positive and -negative cells as regards linear carbon sticking (Fig. 2) was absolute. Only peroxidase-positive cells exhibited this phenomenon.

Phagocytosis of Latex Particles

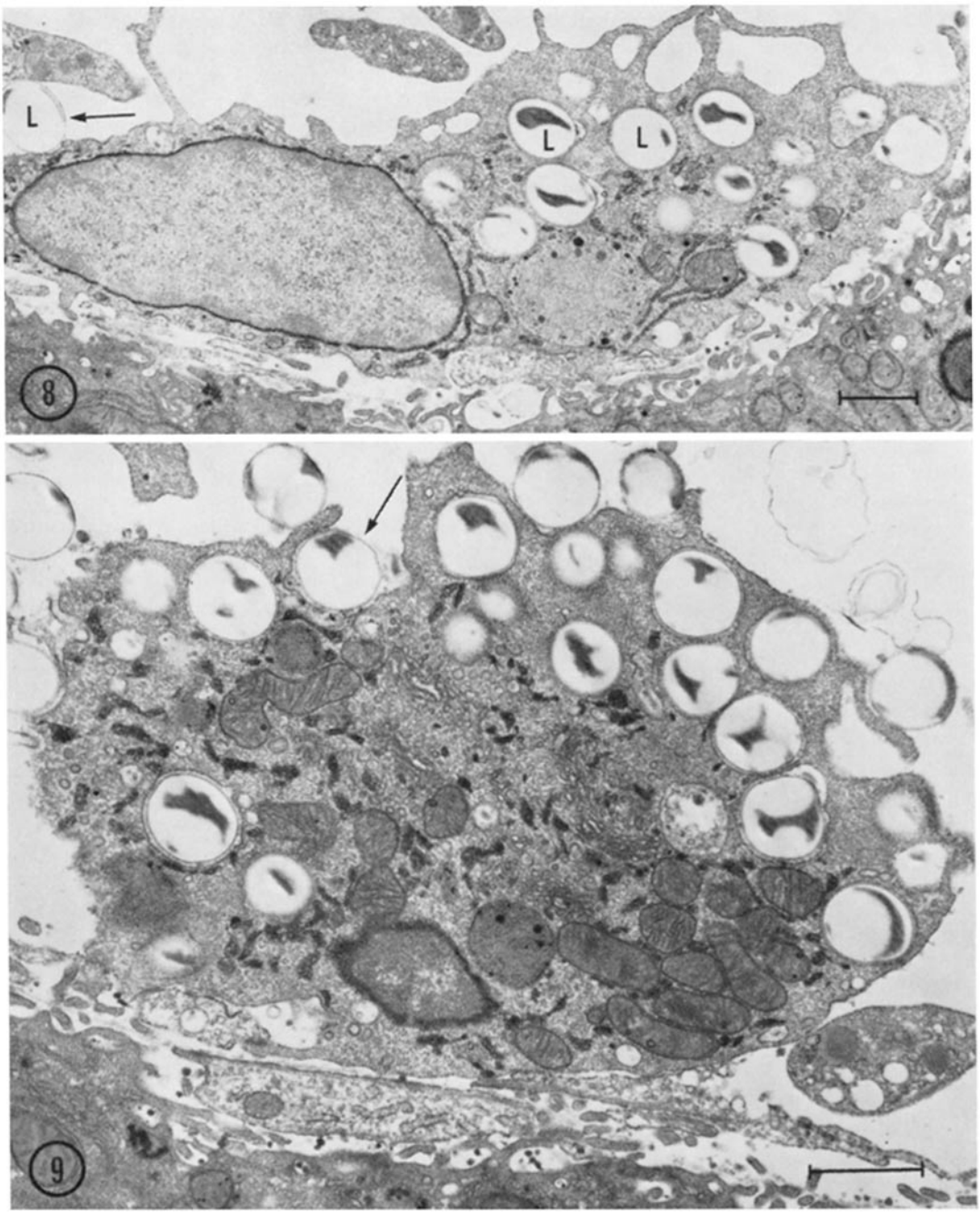
2 min after the injection of latex particles (mean diameter 0.8 μ) into the portal vein, a large number of such particles were seen phagocytized exclusively by peroxidase-positive cells in periportal areas of hepatic lobules. The particles were occasionally noted to be sticking to the cell surface or, more frequently, they were trapped between the pseudopods extending from the surface membrane into the lumen of the hepatic sinusoids (Figs. 8 and 9). In addition, some particles were found in phagosomes inside the peroxidase-positive cells. Of more than 100 peroxidase-negative endothelial-lining cells examined, none showed phagocytosis of latex particles. 5 hr after injection, only peroxidase-positive cells exhibited intracytoplasmic latex.

DISCUSSION

The studies presented in this paper indicate that rat hepatic sinusoidal-lining cells can be differentiated, on the basis of peroxidase reactivity, into two major cell types (excluding "fat-storing cells") with different functional and morphological characteristics: the peroxidase-negative endothelial-lining cell and the peroxidase-positive phagocytic Kupffer cell.

Aschoff in 1924 grouped together several kinds of cells with differing degrees of phagocytosis, such as endothelial cells, fibrocytes, reticular cells of spleen and lymph node, hepatic sinusoidal cells, histiocytes, and monocytes, and designated this grouping the "reticuloendothelial system" (RES) (29). He admitted, however, that vascular endothelial cells and fibrocytes should be separated because of their low phagocytic activity, and he stated that the concept of the "reticuloendothelial system" did not imply that all of the cells were identical. The definition of the RES as used by Aschoff was based on the uptake of vital dyes which he believed were taken into the cells by the process of phagocytosis. Lewis in 1931 described pinocytosis (30) (also called micropinocytosis [31]) as the process of uptake of fluid droplets into the cells, in contrast to phagocytosis which is the ingestion of solid particles. Recent studies suggest that these two processes may have certain common as well as distinct features (32, 33).

In mammals, micropinocytosis is observed in a variety of cell types, including vascular endothelial cells (34, 35), but avid phagocytosis is limited to a small group of specialized cells (36). In a recent comprehensive review (36), the term reticuloendothelial system (RES) has been replaced by mononuclear phagocyte system (MPS), a term which is based on contemporary knowledge of morphological and functional characteristics of these cells and their precursors in bone marrow and peripheral blood (36). The two most important functional criteria that justify inclusion of these cells in a single cell system are: (*a*) avid phagocytosis and (*b*) firm adherence to a glass surface. In this context, our studies with particles of different sizes clearly demonstrate that, whereas the uptake of colloidal carbon occurs in both types of sinusoidal lining cells, it is much greater in the peroxidase-positive cells, which, in turn, are also the only cells capable of phagocytosis of larger latex particles. That regular peripheral vascular endothelium can ingest small amounts of colloidal carbon under some circumstances has been clearly shown by electron microscope studies after carbon overloading (22). The linear sticking of carbon particles to peroxidase-positive cells may reflect the sticky nature of the plasma membrane of these cells. Such stickiness of the surface of some but not all hepatic sinusoidal-lining cells was first observed by light microscopy in 1926 by Pfuhl (37) and was confirmed by electron microscopy in



FIGURES 8 and 9 are from livers of animals injected with latex particles; the livers were fixed 2 min later by portal vein perfusion and processed for peroxidase activity. Sections were counterstained with lead citrate.

FIGURE 8 Electron micrograph of a peroxidase-positive Kupffer cell 2 min after portal injection of latex particles. Latex particles (*L*) are found in phagocytic vacuoles or on the cell surface (arrow). × 11,200.

FIGURE 9 An avidly phagocytizing Kupffer cell, 2 min after injection. Latex particles are seen in phagocytic vacuoles, some on the cell surface where they stick to the luminal cell membrane or appear partially surrounded by pseudopod formations (arrow). × 15,600.

1958 by Parks (38), and more recently by Wisse and Daems (12). The avid phagocytosis and the sticky plasma membrane of peroxidase-positive cells thus indicate that the peroxidase reaction is present exclusively in those hepatic sinusoidal-lining cells that are members of the mononuclear-phagocyte system.

The fine structural characteristics of peroxidase-positive and -negative cells in rat liver, as described in this study, also correspond to the morphological criteria outlined recently by Wisse (10) and Wisse and Daems (11, 12) and other investigators (8 ,9, 26) for distinguishing mononuclear phagocytes (Kupffer cells) from the endothelial cells in sinusoids of rat liver. These morphological criteria, such as the amount of cytoplasm, location of the cell, presence or absence of fenestrations, and number of dense bodies, require that a large representative portion of a given cell be present in a section, and this involves a more or less subjective evaluation of several variable factors. For example, dense bodies may be absent in part of an otherwise typical Kupffer cell, whereas some endothelial cells may show a few dense bodies. In our experience, topography is not an easy criterion to assess; Kupffer cells are often separated from the perisinusoidal space by cytoplasmic processes of wall-forming endothelial cells, but they may also show some focal direct contact with hepatocellular microvilli (Fig. 2) and, in the periportal regions of hepatic lobule, they may be found in the perisinusoidal space itself. Endogenous peroxidase activity appears to be a reliable and easy criterion for distinguishing between Kupffer cells and endothelial cells. Our study shows that a peroxidase-positive cell is characterized by staining of its entire nuclear envelope and endoplasmic reticulum. No intermediate forms of staining were ever encountered. Further, the widely distributed endoplasmic reticulum throughout the cytoplasm provides labeling even if a very small part of a Kupffer cell is present in a section.

The possibility that the endothelial cells are either precursors or mature variants of the peroxidase-positive Kupffer cells cannot be completely excluded from this study. It seems highly unlikely, however, since, as emphasized above, intermediate forms were not encountered. The origin of Kupffer cells is not entirely clear: although derivation from lymphocytes has not been completely excluded, the balance of evidence suggests a bone-marrow monocyte origin (36). In this respect, the similarity of the peroxidase reaction of Kupffer cells to that of peritoneal macrophages (14, 15) is also more in accord with a monocytic ancestry for Kupffer cells.

At present, the functional significance and the exact chemical nature of the peroxidase reaction in Kupffer cells are unknown. A similar pattern of enzyme localization has been observed in peritoneal macrophages of guinea pig (14) and rat (15), in developing granulocytes (48–50), and in epithelial cells of uterus (39), colon (40), and salivary (41), lacrimal (42), and thyroid glands (51). Studies of Klebanoff and others (43–46) indicate that peroxidase from several of these tissues, in association with hydrogen peroxide and a halide, can exert a strong bactericidal, virucidal, and fungicidal activity. It is conceivable that Kupffer cell peroxidase may also participate in a bactericidal mechanism such as the one suggested by Klebanoff for other peroxidases; our observation that the peroxidase reaction is limited to avidly phagocytic Kupffer cells, which are known to play an important role in the clearing of living microorganisms from the blood, would be consistent with such a notion.

Whatever its function, however, the peroxidase reaction in Kupffer cells has clearly documented the existence of two functionally and morphologically different cell types in the lining of the hepatic sinusoids and thus could prove useful as a marker in studies of Kupffer cell kinetics.

The technical assistance of Miss Beatrice Anne Gray and secretarial aid of Miss Tamara Jane Cubi is gratefully acknowledged. This study was supported by grant number 08533 from the National Institute of Neurological Diseases and Stroke, and HE 08251 from the National Heart Institute, National Institutes of Health, Bethesda, Maryland 20014.

Dr. Widmann is a recipient of a fellowship from the World Health Organization. Dr. Cotran and Dr. Fahimi are recipients of Research Career Development Awards from National Institutes of Health, Bethesda, Maryland, 20014.

Received for publication 28 June 1971, and in revised form 30 August 1971.

REFERENCES

1. HOFFMANN, F. A., and F. VON RECKLINGHAUSEN. 1867. Ueber die Herkunft der Eiterkörperchen. *Centralbl. Med. Wissensch.* **5**:481.

2. PONFICK, E. 1869. Studien ueber die Schicksale körniger Farbstoffe im Organismus. *Arch. Pathol. Anat. Physiol. Klin. Med.* (*Virchows*). **48**:1.
3. VON KUPFFER, C. 1876. Ueber Sternzellen der Leber, Briefliche Mittheilung an Prof. Waldeyer. *Arch. Mikrosk. Anat. Entwicklungsmech.* **12**:353.
4. VON KUPFFER, C. 1899. Ueber die sogenannten Sternzellen der Säugethierleber. *Arch. Mikrosk. Anat. Entwicklungsmech.* **54**:254.
5. ATERMAN, K. 1963. The structure of the liver sinusoids and the sinusoidal cells. *In* The Liver, Morphology, Biochemistry, Physiology. C. Rouiller, editor. Academic Press Inc., New York. **1**:61.
6. NICOLESCU, P., and C. ROUILLER. 1967. Beziehungen zwischen den Endothelzellen der Lebersinusoide und den von Kupfferschen Sternzellen. Eleckronenmikroskopische Untersuchung. *Z. Zellforsch. Mikrosk. Anat.* **76**:313.
7. BENACERRAF, B. 1963. Functions of the Kupffer cells. *In* The Liver, Morphology, Biochemistry, Physiology. C. Rouiller, editor. Academic Press Inc., New York. **2**:37.
8. YAMAGISHI, M. 1959. Electronmicroscopic studies on the fine structure of sinusoidal wall and fat storing cells of rabbit livers. *Arch. Histol. Jap.* **18**:223.
9. TANIKAWA, K., K. YOSHIMURA, and S. GOHARA. 1965. Fine structure of the reticuloendothelial cells in the normal rat liver. Morphological classification. *Kurume Med. J.* **12**:139.
10. WISSE, E. 1970. An electron microscopic study of the fenestrated endothelial lining of rat liver sinusoida. *J. Ultrastruct. Res.* **31**:125.
11. WISSE, E., and W. T. DAEMS. 1970. Fine structural study on the sinusoidal lining cells of rat liver. *In* Mononuclear Phagocytes. R. van Furth, editor. F. A. Davis Co., Philadelphia, Pa. 200.
12. WISSE, E., and W. T. DAEMS. 1970. Differences between endothelial and Kupffer cells in rat liver. *Proc. 7th Int. Congr. Electron Microsc.* **3**:57.
13. FAHIMI, H. D. 1970. The fine structural localization of endogenous and exogenous peroxidase activity in Kupffer cells of rat liver. *J. Cell Biol.* **47**:247.
14. COTRAN, R. S., and M. LITT. 1970. Ultrastructural localization of horseradish peroxidase and endogenous peroxidase activity in guinea pig peritoneal macrophages. *J. Immunol.* **105**:1536.
15. ROBBINS, D., R. S. COTRAN, and H. D. FAHIMI. 1971. Fine structural cytochemical localization of peroxidase activity in rat peritoneal cells. Mononuclear cells, eosinophils and mast cells. *J. Histochem. Cytochem.* **19**:571.
16. FAHIMI, H. D. 1967. Perfusion and immersion fixation of rat liver with glutaraldehyde. *Lab. Invest.* **16**:736.
17. FAHIMI, H. D., and P. DROCHMANS. 1965. Essais de standardisation de la fixation au glutaraldéhyde. 1. Purification et détermination de la concentration du glutaraldéhyde. *J. Microsc.* (*Paris*). **4**:725.
18. SMITH, R. E., and M. FARQUHAR. 1965. Preparation of nonfrozen sections for electron microscope cytochemistry. *Sci. Instrum. News.* **10**:13.
19. GRAHAM, R. C., and M. J. KARNOVSKY. 1966. The early stages of absorption of injected horseradish peroxidase in the proximal tubule of the mouse kidney. Ultrastructural cytochemistry by a new technique. *J. Histochem. Cytochem.* **14**:291.
20. CLAUDE, A. 1960. Problems of fixation for electron microscopy. Results of fixation with osmium tetroxide in acid and alkaline media. *Pathol. Biol.* **9**:933.
21. LUFT, J. H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* **9**:409.
22. COTRAN, R. S., E. R. SUTER, and G. MAJNO. 1967. The use of colloidal carbon as a tracer for vascular injury. *Vasc. Dis.* **4**:107.
23. MAJNO, G. 1965. Ultrastructure of the Vascular Membrane. *In* Handbook of Physiology. Williams and Wilkins Co., Baltimore, Md. **3**:2293.
24. ITO, T., and M. NEMOTO. 1952. Uber die Kupfferschen Sternzellen und die "Fettspeicherungzellen" ("fat storing cells") in der Blutkapillarenwand der menschlichen Leber. *Okajimas Folia Anat. Jap.* **24**:243.
25. BRONFENMAJER, S., F. SCHAFFNER, and H. POPPER. 1966. Fat-storing cells (lipocytes) in human liver. *Arch. Pathol.* **82**:447.
26. ITO, T., and S. SHIBASAKI. 1968. Electron microscopic study on the hepatic sinusoidal wall and the fat-storing cells in normal human liver. *Arch. Histol. Jap.* **29**:137.
27. TÖRÖ, I., P. RUSZA, and P. ROHLICH. 1962. Ultrastructure of early phagocytic stages in sinus endothelial and Kupffer cells of the liver. *Exp. Cell Res.* **26**:601.
28. MATTER, A., L. ORCI, W. G. FORSSMANN, and C. ROUILLER. 1968. The stereological analysis of the fine structure of the "Micropinocytosis Vermiformis" in Kupffer cells of the rat. *J. Ultrastruct. Res.* **23**:272.
29. ASCHOFF, L. 1924. Das Reticuloendotheliale System. *Ergeb. Inn. Med. Kinderheilk.* **26**:1.
30. LEWIS, E. H. 1931. Pinocytosis. *Bull. Johns Hopkins Hosp.* **49**:17.

31. FAWCETT, D. W. 1965. Surface specializations of absorbing cells. *J. Histochem. Cytochem.* **13**:75.
32. North, R. J. 1970. Endocytosis. *Seminars Haematol.* **7**:161.
33. RABINOVITCH, M. 1970. Phagocytic recognition. *In* Mononuclear Phagocytes. R. van Furth, editor, F. A. Davis Co., Philadelphia, Pa. 313.
34. BRUNS, R. R., and G. E. PALADE. 1968. Studies on blood capillaries. II. Transport of ferritin molecules across the wall of muscle capillaries. *J. Cell Biol.* **37**:277.
35. KARNOVSKY, M. J. 1967. The ultrastructural basis of capillary permeability studied with peroxidase as a tracer. *J. Cell Biol.* **35**:213.
36. VAN FURTH, R., editor. 1970. Mononuclear phagocytes. F. A. Davis Co., Philadelphia, Pa.
37. PFUHL, W. 1926. Experimentelle Untersuchungen über die Kupfferschen Sternzellen der Leber. *Z. Anat. Entwicklungsgesch.* **81**:90.
38. PARKS, H. F., and A. D. CHIQUOINE. 1956. Observations on early stages of phagocytosis of colloidal particles by hepatic phagocytes of the mouse. *Electron Microsc. Proc. Stockholm Conf.* 154.
39. BRÖKELMANN, J., and D. W. FAWCETT. 1969. The localization of endogenous peroxidase in the rat uterus and its induction by estradiol. *Biol. Reprod.* **1**:59.
40. VENKATACHALAM, M. A., M. H. SOLTANI, and H. D. FAHIMI. 1970. Fine structural localization of peroxidase activity in the epithelium of large intestine of rat. *J. Cell Biol.* **46**:168.
41. STRUM, J. M., and M. J. KARNOVSKY. 1970. Ultrastructural localization of peroxidase in submaxillary acinar cells. *J. Ultrastruct. Res.* **31**:323.
42. ESSNER, E. 1971. Localization of endogenous peroxidase in rat exorbital lacrimal gland. *J. Histochem. Cytochem.* **19**:216.
43. KLEBANOFF, S. J. 1967. Iodination of bacteria: a bactericidal mechanism. *J. Exp. Med.* **126**: 1063.
44. KLEBANOFF, S. J. 1970. Myeloperoxidase-mediated antimicrobial systems and their role in leukocyte function. *In* Biochemistry of the Phagocytic Process. Julius Schultz, editor. American Elsevier Publishing Co., New York. 89.
45. LEHRER, R. I. 1969. Antifungal effects of peroxidase systems. *J. Bacteriol.* **99**:361.
46. BELDING, M. E., S. J. KLEBANOFF, and C. G. RAY. 1970. Peroxidase-mediated virucidal systems. *Science (Washington).* **167**:195.
47. ORCI, L., A. MATTER, and C. ROUILLER. 1971. A comparative study of freeze-etch replicas and thin sections of rat liver. *J. Ultrastruct. Res.* **35**:1.
48. ACKERMAN, G. A., and M. A. CLARK. 1971. Ultrastructural localization of peroxidase activity in normal human bone marrow cells. *Z. Zellforsch. Mikrosk. Anat.* **117**:463.
49. BAINTON, D. F., and M. F. FARQUHAR. 1970. Segregation and packaging of granule enzymes in eosinophilic leukocytes. *J. Cell Biol.* **45**:54.
50. MILLER, F., and V. HERZOG. 1969. Die Lokalisation von Peroxydase und saurer Phosphatase in eosinophilen Leukocyten während der Reifung. Elektronenmikroskopisch-cytochemische Untersuchungen am Knochenmark von Ratte und Kaninchen. *Z. Zellforsch. Mikrosk. Anat.* **97**:84.
51. STRUM, J. M., and M. J. KARNOVSKY. 1970. Cytochemical localization of endogenous peroxidase in thyroid follicular cells. *J. Cell Biol.* **44**:655.