INDUCTION OF PINOCYTOSIS IN RAT HEPATOCYTES BY PARTIAL HEPATECTOMY

MICHIO MORI and ALEX B. NOVIKOFF

From the Department of Pathology, Albert Einstein College of Medicine, Yeshiva University, Bronx, New York 10461. Dr. Mori's present address is Department of Pathology, Sapporo Medical College, Sapporo, 060, Japan.

ABSTRACT

Rat hepatocytes, normally not highly pinocytic cells, become so after partial hepatectomy when about two-thirds of the liver is removed. Droplets, up to 20 μm in diameter, develop, initially by addition to smaller pinocytic structures and later by fusion with lysosomes. The droplets contain a material with an electron microscope periodicity characteristic of fibrin; they are periodic acid Schiff-positive as is plasma. It is therefore reasonable to consider plasma glycoproteins to be major components of the droplets. The droplets are at all times membrane delimited, an observation possible only after perfusion fixation. The droplets are positive for three lysosomal hydrolases identified cytochemically: acid phosphatase, *N*-acetyl-β-glucosaminidase, and β-glucuronidase. From light and electron microscopy it is evident that these activities are acquired by fusion with lysosomes, mostly autophagic vacuoles and residual bodies both of which become very numerous after partial hepatectomy.

Pinocytic structures are seen relatively infrequently in the hepatocytes of normal rats but a great many are present after partial hepatectomy. They are most easily observed if horseradish peroxidase (HRP) is intravenously injected before sacrifice and sections are incubated for HRP cytochemistry. The low dose of HRP employed (10 mg/100 g body weight) does not induce pinocytosis in controls, either untreated rats or rats subjected to laparotomy, including palpation of the liver. However, in partially hepatectomized rats even a much smaller dose of intravenous HRP (3.3 mg/100 g) visualizes the pinocytic structures in hepatocytes (coated vesicles, channels, cuplike bodies, and droplets). Kupffer cells pinocytose much HRP in both control and partially hepatectomized rats.

Some 25 years ago cytoplasmic droplets, 20–30 μm in diameter, were described in the hepatocytes of rat liver after partial hepatectomy (1, 2, 11, 34), but their nature and origin were not established. Doniach and Weinbren (11) showed that the droplets gave staining reactions for protein and thus regarded them as protein droplets. These authors and others (2, 4) observed that the droplets did not appear if partial hepatectomy were performed on fasted rats or on rats fed only dextrose. We will adopt their terminology and refer to the droplets as protein droplets.

Becker and Lane (6), Vorbrodt (39), and Pfeiffer and Bannasch (33) demonstrated that the protein droplets displayed acid phosphatase (AcPase) and other hydrolase activities and could thus be

considered as lysosomes. Becker and Lane (6) suggested that the droplets arise by autophagy. On the other hand, Pfeiffer and Bannasch (33) proposed that plasma membrane damage was induced by partial hepatectomy and Onoé (32) that a permeability change in the plasma membrane followed partial hepatectomy; in both cases these authors considered that plasma from the space of Disse passed, in free form, into the hepatocyte cytoplasm. This conception could be adequately evaluated only in hepatocytes that remained well preserved during fixation and other steps in tissue processing. We have found that with perfusion fixation such preservation is possible. It can then be seen that the protein droplets are at all times delimited by intact membranes and that the droplets have a pinocytic origin.

MATERIALS AND METHODS

40 Sprague-Dawley male rats (Holtzman Co., Madison, Wis.), averaging 61 g, were used in these studies. We were using small rats in other studies where mitotic synchrony was important, and Becker et al. (5) have shown that such synchrony follows partial hepatectomy in small rats to a greater degree than in larger ones. The surgical procedure was that of Higgins and Anderson (16) and involved removal of about 1.7 g of liver. No evidence of shock was observed.

After variable periods from 1 to 48 h after partial hepatectomy, portions of liver were fixed for light and electron microscopy. Controls consisted of untreated rats and rats subjected to laporatomy, including palpation of the liver. Under light ether anesthesia isotonic saline was perfused, via the portal vein, for 1 min, followed by fixative for 2–3 min, both at room temperature, at a flow rate of 2–3.5 ml/min. The fixative was 2.5 glutaraldehyde (Ladd Research Industries, Inc., Burlington, Vt.) and 0.1 M cacodylate buffer, pH 7.2. The liver was then removed and slices, 1–1.5 mm thick, were carefully cut with a Wilkinson razor blade (The Wilkinson Co., Westlake Village, Calif.) and immersed in cold fixative. This fixative (Karnovsky's [19], as modified by Miller and Herzog [27]) consisted of 2.5% glutaraldehyde, 2% formaldehyde (freshly prepared from paraformaldehyde), and 0.025% $CaCl_2$ in 0.09 M cacodylate buffer. The period of immersion fixation was 3 h for light microscopy and 1 h for electron microscopy.

A part of the tissue was prepared for electron microscopy without prior incubation. Osmication and subsequent procedures were done as described previously (23). The remainder of the tissue was kept overnight in cold cacodylate buffer containing 0.2 M sucrose. For light microscopy frozen sections, approx. 10 μm thick, were cut on a Sartorius microtome and placed in cold distilled water. They were incubated for AcPase activity by the lead procedure with CMP as substrate (23) and for three enzyme activities by *p*-rosaniline procedures (AcPase [3], β-glucuronidase [15], and *N*-acetyl β-glucosaminidase [14]). Sections of livers from horseradish peroxidase (HRP)-injected rats were also incubated for peroxidase activity by the 3,3′-diaminobenzidine (DAB) procedure of Graham and Karnovsky (13). For electron microscopy, nonfrozen sections, approx. 25 μm thick, were prepared with the Sorvall TC-2 tissue sectioner (DuPont Instruments, Sorvall Operations, Newton, Conn.), according to the method of Smith and Farquhar (35). The nonfrozen sections were incubated for AcPase activity by the lead procedure (23). In the case of livers from the HRP-injected rats other nonfrozen sections were incubated in the DAB medium (13). Osmication and subsequent procedures were done as with unincubated tissue.

In some experiments, HRP (Type II, Sigma Chemical Co., St. Louis, Mo.), diluted in isotonic saline, was injected slowly into the tail vein of rats, under light ether anesthesia, 10 min before sacrifice of the animal. In all cases 0.1 ml was injected, but the HRP dose was adjusted for partially hepatectomized animals to the approx. two-third reduction in liver mass. Thus the control rats received 10 mg HRP per 100 g body weight and the partially hepatectomized rats 3.3 mg HRP per 100 g body weight. Preliminary experiments showed that 1 h posthepatectomy was best suited for finding HRP activity in the protein droplets. The HRP injections induced signs of cyanosis, i.e., a deeper, more rapid breathing, and a reddening of the ears. In a few experiments, 10-μm frozen sections were cut from both control and regenerating liver after fixation overnight in 10% formaldehyde (prepared from paraformaldehyde); these sections were stained by the periodic acid-Schiff (PAS) procedure (24).

RESULTS

Light Microscopy

The lead procedure for AcPase reveals the lysosomes with great clarity. In control animals, either unoperated or sham-operated rats, the hepatocyte lysosomes are found predominantly along the bile canaliculi, as in the areas indicated by arrows in Fig. 1. They are relatively uniform in size and small, none remotely approaching the large protein droplets in hepatocytes of regenerating liver (Fig. 2).

Confirming previous reports (2, 11, 4) the protein droplets that appear after partial hepatectomy do not form if the rats have been fasted for 24 h before surgery. In normally fed rats the development of droplets is reflected in the AcPase preparations. Soon after partial hepatectomy the pericanalicular concentration of the lysosomes begins to diminish. By 1 h posthepatectomy, AcPase

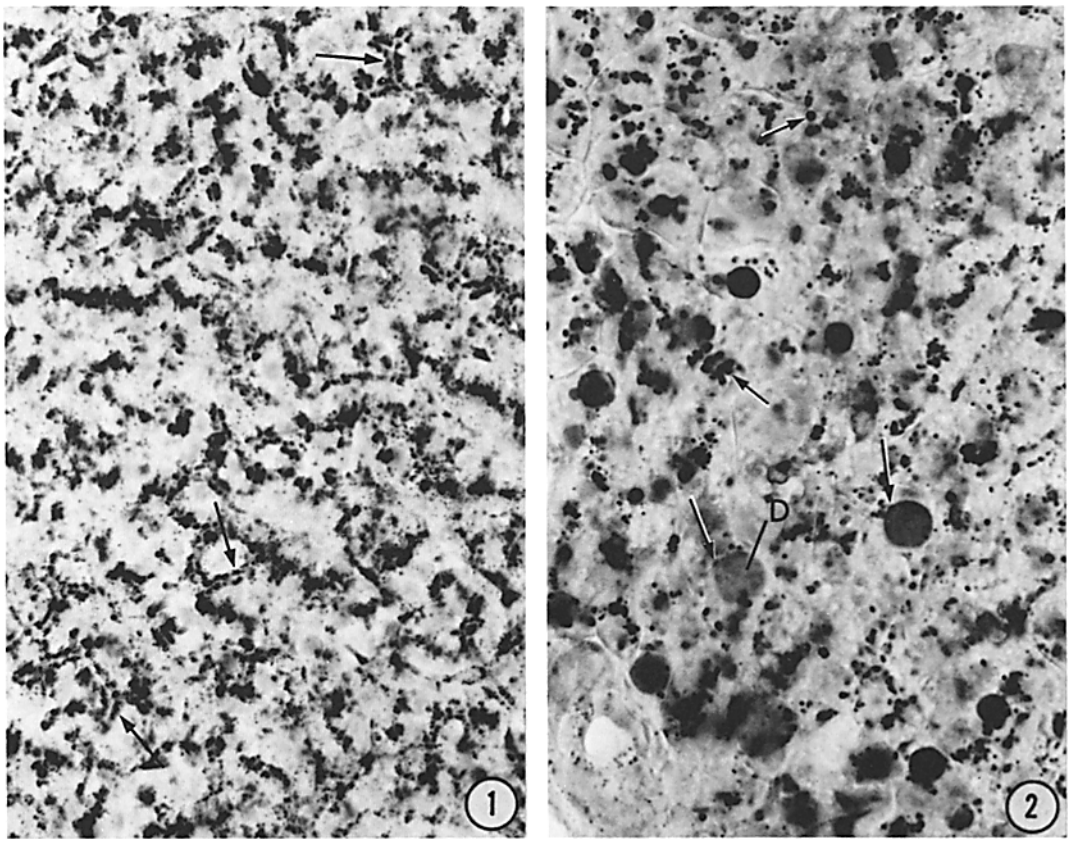
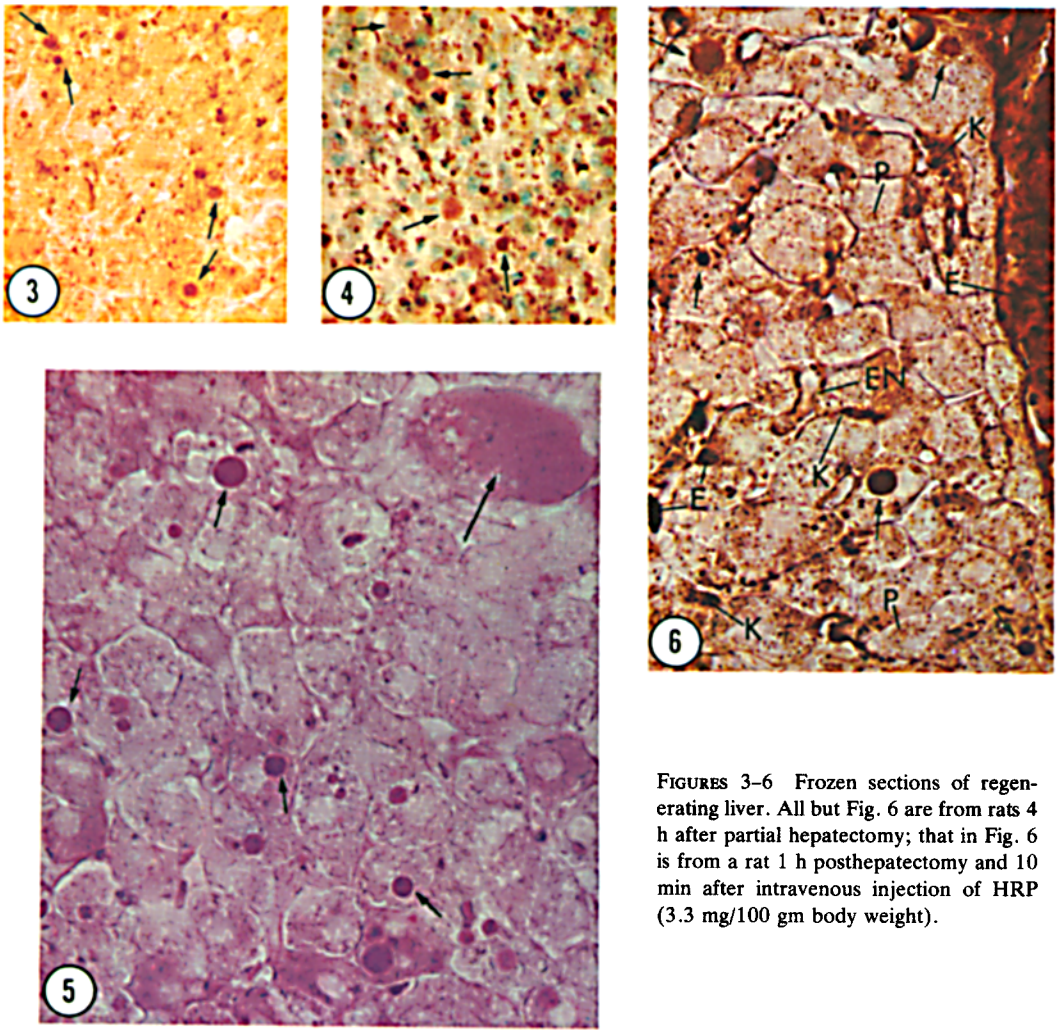


FIGURE 1 Frozen section of liver of control (sham-operated) rat, incubated in CMP medium for 30 min at 37°C. In this relatively thick section (ca. 10 μm) there is considerable overlap of the stained lysosomes. Arrows indicate regions where the pericanalicular distribution of the lysosomes is more evident. × 430.

FIGURE 2 Frozen section of regenerating liver, 4 h after partial hepatectomy, processed as in Fig. 1. The largest intracellular structures evident in the section are the protein droplets. The one indicated by *D* shows little reaction product resulting from acid phosphatase activity, whereas the other large droplets show much reaction product. The long arrows indicate lysosomes near the surfaces of protein droplets. The small arrows indicate lysosomes which, judging by their size, are probably autophagic vacuoles or residual bodies resulting from them; cf. Fig. 7 (see Reference 30). The pericanalicular lysosome distribution (Fig. 1) is no longer evident. This field, like that of Fig. 1, is from the periportal region of the lobule; a bile ductule, with small lysosomes, is at the lower left of the micrograph. × 580.

activity is evident in some large protein droplets. At 4 h posthepatectomy, the lysosomes can be grouped roughly into three classes according to size. Those that remain pericanalicular are the smallest. The largest are the AcPase-positive protein droplets. These may reach 20 μm in diameter, but most are smaller. The intermediate-sized lysosomes (short arrows, Fig. 2) are revealed by electron microscopy (Figs. 7 and 9) to be autophagic vacuoles (see reference 30 for definitions of these and other types of lysosomes). Even at 4 h, a few protein droplets show little AcPase reaction product (droplet *D* in Fig. 2). However, most are highly reactive (Fig. 2). Whether the droplets are reactive or not, lysosomes may be present near their surfaces (Fig. 2, long arrows).

Because the droplets give much less intense staining, the *p*-rosaniline procedures for the three lysosome hydrolases (AcPase, β-glucuronidase [Fig. 4], *N*-acetyl β-glucosaminidase [Fig. 3]) exhibit greater variability in droplet activity than the lead procedure for AcPase. Of the three enzymes, AcPase activity gives the most intense staining and *N*-acetyl β-glucosaminidase the least.



FIGURES 3–6 Frozen sections of regenerating liver. All but Fig. 6 are from rats 4 h after partial hepatectomy; that in Fig. 6 is from a rat 1 h posthepatectomy and 10 min after intravenous injection of HRP (3.3 mg/100 gm body weight).

FIGURE 3 After incubation for *N*-acetyl β-glucosaminidase for 60 min at 37°C. Hepatocyte and Kupffer cell lysosomes are lightly stained; compare deeper staining in Fig. 4. Note variability in staining of protein droplets (arrows). × 200.

FIGURE 4 After incubation for β-glucuronidase for 50 min at 37°C and counterstaining with methyl green. Lysosomes of hepatocytes and Kupffer cells are stained more intensely than in Fig. 3. Note variability in staining of protein droplets (arrows). × 200.

FIGURE 5 After staining by periodic acid-Schiff procedure. Staining is seen in the blood vessel (long arrow) and in the protein droplets, some of which are indicated by the shorter arrows. × 425.

FIGURE 6 After incubation in DAB medium for 60 min at 37°C. Five stained protein droplets are indicated by arrows. Also stained are erythrocytes (*E*) in or near the sinusoids and in a portal blood vessel (upper right), endothelial cells (*EN*), lysosomes of Kupffer cells (*K*) and hepatocyte peroxisomes (*P*). × 425.

Fig. 5 shows that the protein droplets (short arrows) are strongly PAS-positive, as is the blood plasma (long arrow).

DAB preparations of HRP-injected rats show a similar staining of littoral cells in control and in partially hepatectomized rats. The endothelial cells show a uniformly dark staining (*EN*, Fig. 6). The large lysosomes (residual bodies [30]) of the

Kupffer cells are also intensely stained (*K*, Fig. 6). In the hepatocytes the peroxisomes stain (*P*, Fig. 6) despite the suboptimal nature of the incubation mixture for these organelles (see reference 31). In the 1-h regenerating liver some of the droplets show reaction product when the rats are sacrificed 10 min after HRP injection (arrows, Fig. 6).

Electron Microscopy

Perfusion fixation is essential for good preservation of the protein droplets that form in the hepatocytes after partial hepatectomy. These hepatocytes show dramatic increases in heterophagic and autophagic structures. Pinocytic channels (Figs. 11–13), cuplike bodies (Figs. 7 and 12) (see reference 17) and coated vesicles (Fig. 11) are numerous, whereas in hepatocytes of control rats only coated vesicles are seen and they are relatively infrequent. After partial hepatectomy, pinocytic channels may be seen continuing into larger pinocytic structures (Figs. 11 and 15). Apparently, the protein droplets arise by growth of these structures, but merger of pinocytic structures, although not observed, cannot be excluded.

Protein droplets are always membrane delimited (Figs. 7–11). The content of the protein droplets is generally granular and only slightly electron opaque (Fig. 7), but regions of greater opacity may also be seen. Thus, areas of degenerating cytoplasmic organelles are seen within a droplet in Fig. 8 as if fusion of autophagic vacuoles with the droplet had occurred.

In control rats, the number of autophagic vacuoles, identified by the recognizable cytoplasmic organelles they contain (see reference 30), though variable, is always low. After partial hepatectomy the number of autophagic vacuoles increases greatly. Five are seen in Fig. 7, a small portion of a hepatocyte 4 h posthepatectomy. The direct merger of an autophagic vacuole (*A*) with a protein droplet is seen in Fig. 9, showing AcPase reaction product in both vacuole and droplet. The two autophagic vacuoles, in Fig. 7, no. *1*, and in Fig. 8, bottom left (*A*), may reasonably be interpreted as vacuoles close to merger with protein droplets at the time the cells were fixed. We did not observe merger of droplets with small lysosomes. Such lysosomes would include: (*a*) small dense bodies (residual bodies [30]) such as were seen fusing with pinocytosed hemoglobin droplets in earlier studies from our laboratory (12); and (*b*) vesicles, much smaller, that might function as primary lysosomes (30). However, our experiments did not include serial sectioning so that such mergers are not excluded. Often the droplets show a "halo" beneath the delimiting membranes, as in Figs. 7 and 8. These are characteristic of residual bodies (10, 30).

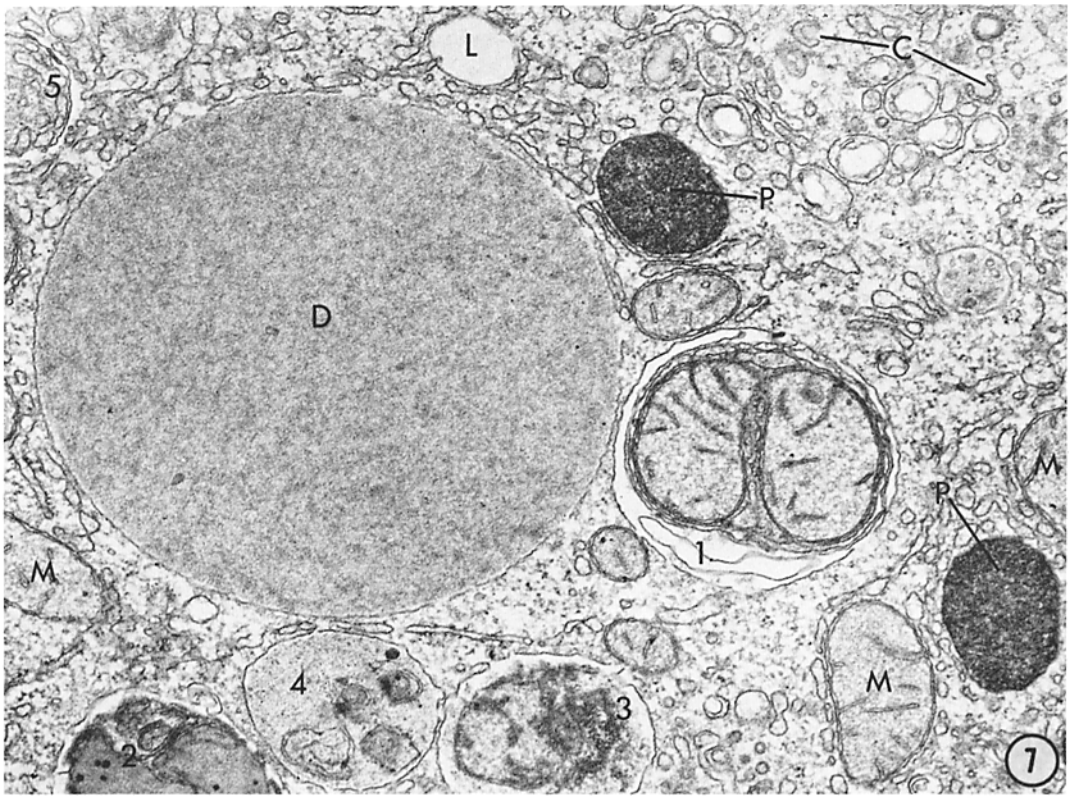
In some droplets, fibrillar strands showing the cross-banding characteristic of fibrin are encountered (Fig. 10), confirming the observations of Pfeiffer and Bannasch (33). In adjacent strands, the cross-bands tend to appear in register.

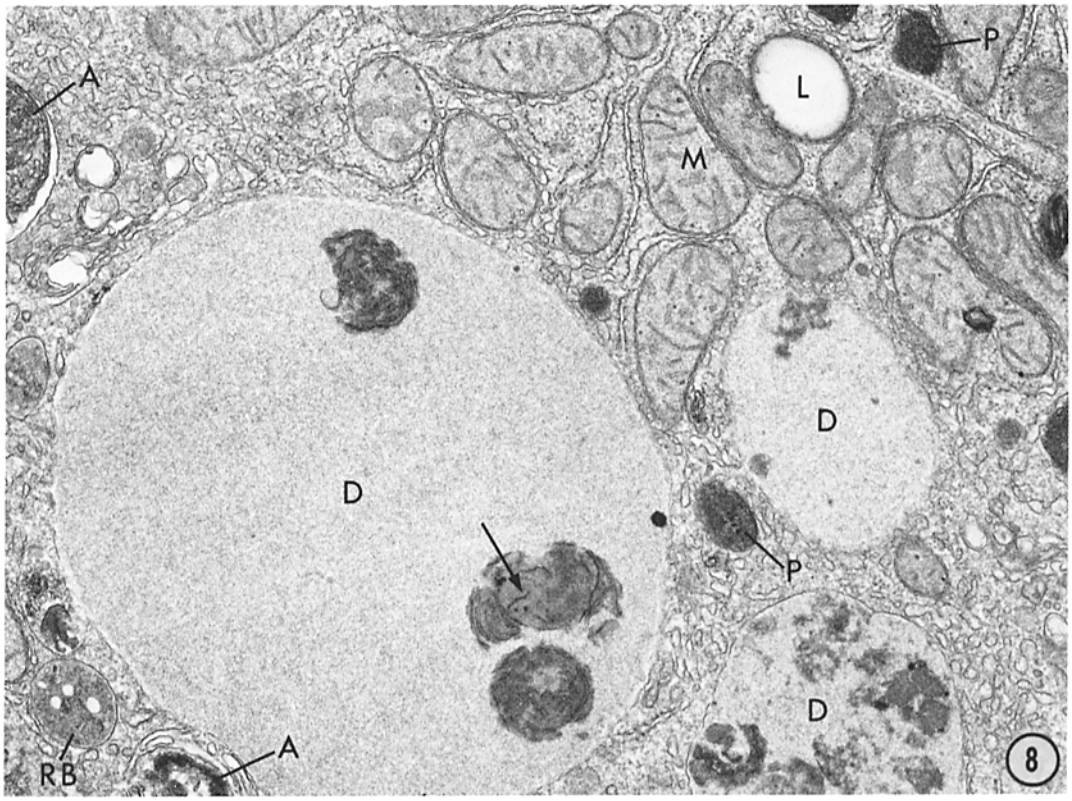
As with light microscopy, electron microscopy shows no differences between untreated controls and sham-operated controls in which the liver was palpated. In control rats injected with 10 mg HRP/100 g body weight, 10 min before sacrifice, the Kupffer cells show many HRP-positive pinocytic vesicles (coated and uncoated), pinocytic channels, cuplike bodies (17), and residual bodies. In contrast, no alterations are apparent in the hepatocytes, including those surrounding the portal tracts and those near the central veins. The coated vesicles that are continuous with the plasma membrane at the space of Disse show no HRP reaction product, and they are still encountered relatively infrequently. Rarely, smaller internal HRP-positive vesicles (without coats) may be seen not far from the space of Disse.

Figs. 11–13 show the numerous pinocytic structures in rats partially hepatectomized 1 h earlier before injection of HRP (3.3 mg/100 g body weight). There are a great many HRP-filled, internalized coated vesicles (Fig. 11), pinocytic channels (Figs. 11–13), cuplike bodies (Fig. 12) and larger structures with which the pinocytic channels are continuous (Fig. 11, arrow). In Fig. 13, a channel is continuous with a circular structure large enough to be considered a small protein droplet. Neither the Golgi apparatus nor structures near it show HRP in the hepatocytes of HRP-injected rats studied in these experiments.

DISCUSSION

Our work confirms the observations of Becker and Lane (6) and Vorbrodt (39) that partial hepatectomy induces the appearance in the rat hepatocyte of many autophagic vacuoles. However, their view that the large protein droplets and autophagic vacuoles are identical is shown to be incorrect. We have shown the two structures: (*a*) to exist as separate entities; (*b*) to be able to fuse with each other; and (*c*) one (the protein droplets) to acquire recognizable contents of the other (the autophagic vacuoles). Also, their modes of origin are differ-


L
C
5
P
D
M
P
1
M
4
3
M
2
7


A
P
L
M
D
D
P
D
RB
A
8

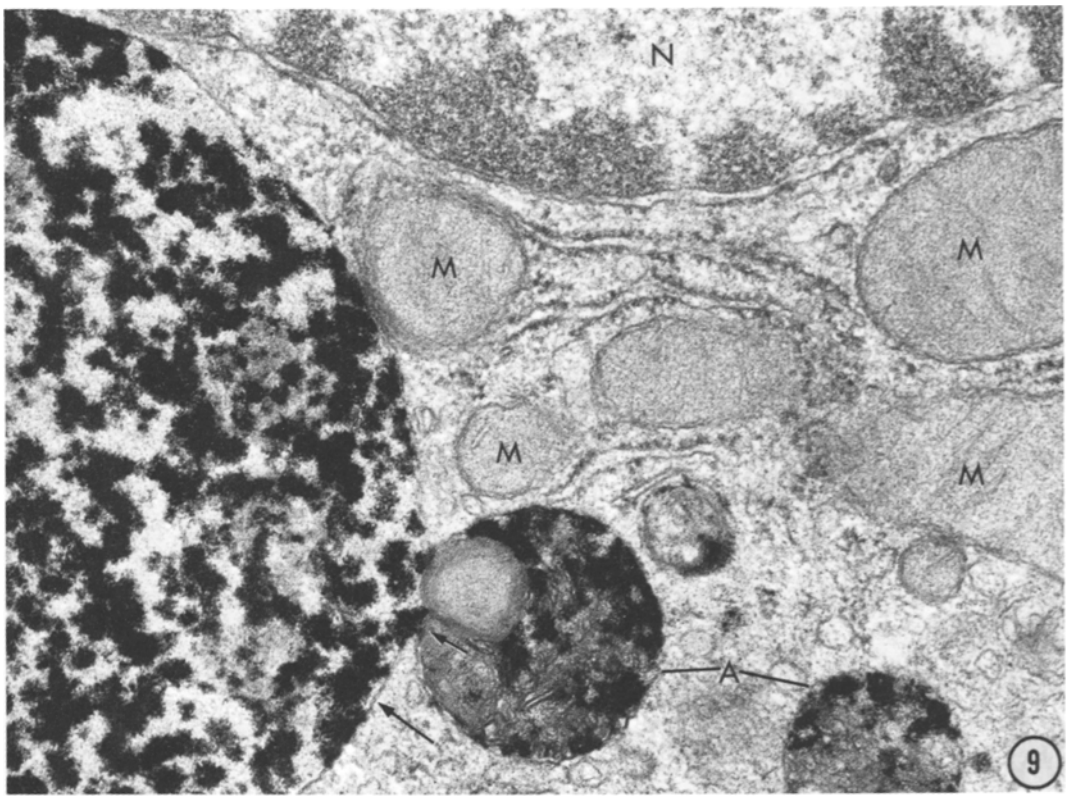


FIGURE 9 Portion of a hepatocyte from regenerating liver, 4 h after partial hepatectomy, incubated in the AcPase (CMP) medium for 30 min at 37°C. Unreactive structures include ER (unlabeled), nucleus (*N*) and mitochondria (*M*). Reaction product is seen in the protein droplet at the left of the Figure and in the autophagic vacuole (*A*, to the left) with which it is apparently fusing. The short arrow is directed to reaction product extending from the autophagic vacuole to the droplet, thus indicating the continuity of the droplet and autophagic vacuole. The long arrow is directed to a region of the droplet where its tripartite membrane is evident. The continuity of this membrane with the membrane at the bottom of the autophagic vacuole is indistinct, either because of accumulated reaction product or because it is improperly oriented in relation to the electron beam. The body indicated by *A*, at the right, is either an autophagic vacuole or residual body (see legend to Fig. 7). × 49,000.

FIGURES 7–8 Portions of hepatocytes from regenerating liver, 4 h after partial hepatectomy, incubated in DAB medium for 60 min at 37°C. In these experiments the peroxidatic activity of catalase within the peroxisomes (*P*) proved to be sufficient to oxidize the DAB (cf. Reference 31). Protein droplets are labeled *D*.

FIGURE 7 Fig. 7 shows autophagic vacuoles numbered *1–5*. Mitochondria are readily recognizable in vacuole *1*, still recognizable in *2* and *3*, and barely, if at all, in *4*. The numeral *5* at upper left indicates an autophagic vacuole containing sequestered ER. Vacuoles 2, 3, and 4 can be considered residual bodies since they show what are probably residua of digestion (see Reference 30). Also labeled are mitochondria (*M*) and cuplike bodies (*C*). × 25,600.

FIGURE 8 Fig. 8 shows three protein droplets within which are seen electron-dense material derived from either autophagic vacuoles or residual bodies. An arrow indicates a still recognizable crista. Also labeled are two autophagic vacuoles (*A*), a residual body (*RB*) and a lipid sphere (*L*) in the cytosol. × 20,000.

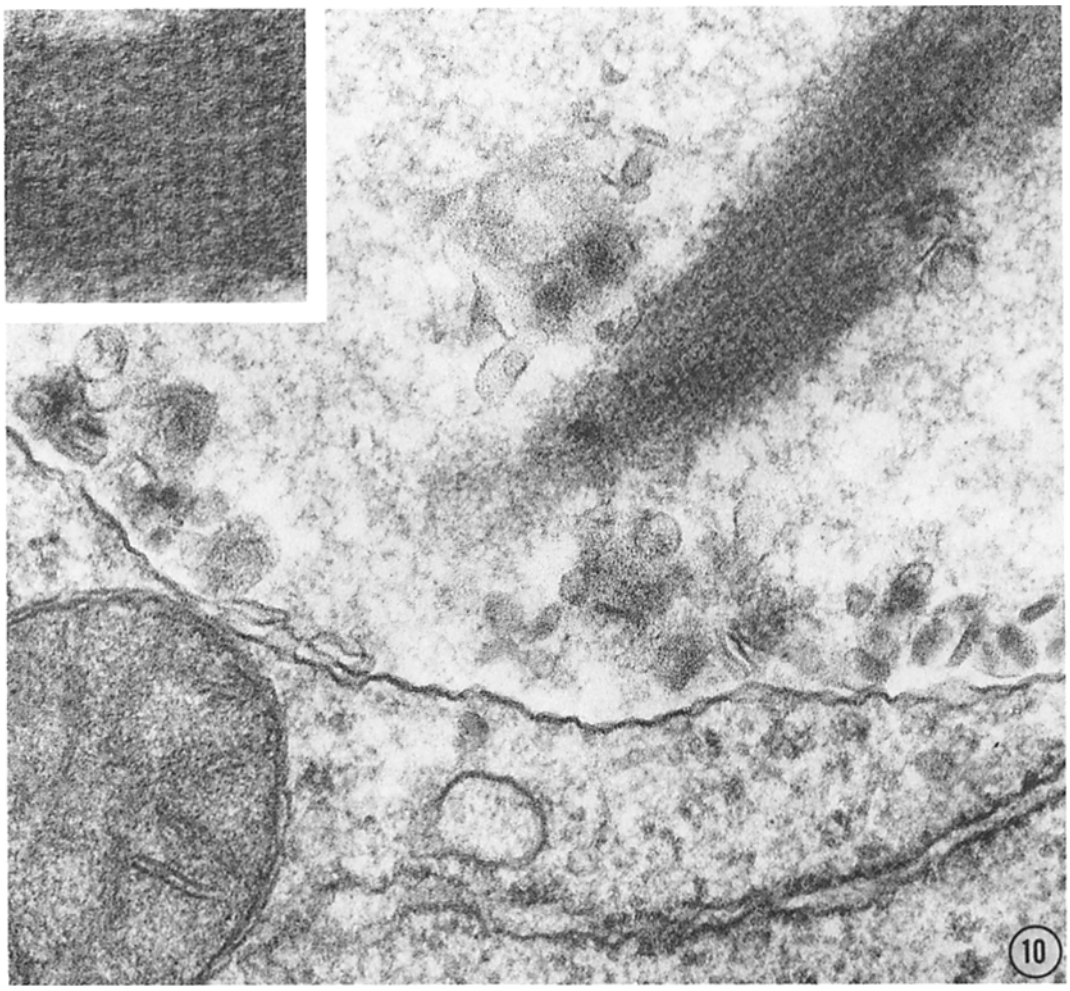


FIGURE 10 A small portion of a hepatocyte from regenerating liver, 4 h posthepatectomy, incubated in the DAB medium for 30 min at 37°C. A part of a protein droplet is seen. Within the droplet is cross-banded material, enlarged in the *inset*. The cross-bands of the two adjacent bundles are in register. The distance between dark bands is 220 Å (*inset*). The delimiting membrane of the droplet is intact although it is not everywhere oriented properly with respect to the electron beam to show its tripartite nature. × 86,000; *inset*, × 135,000.

ent: the droplets form from pinocytic structures, while autophagic vacuoles probably arise by endoplasmic reticulum sequestering portions of cytoplasm (30). The protein droplets acquire acid hydrolase activities when they fuse with autophagic vacuoles and other lysosomes. The hepatocyte of regenerating liver thus constitutes a cell type in which both heterophagy and autophagy are pronounced. Presumably, both the external and internal macromolecules yield building blocks for the growth processes which ensue later and which restore the liver to its initial weight.

Another reported view of protein droplet origin (33, 32) is also shown to be erroneous by our observations. This view holds that gross changes in the plasma membranes facing the space of Disse are induced by partial hepatectomy, permitting plasma, in free form, to move into the hepatocytes. Our experience suggests that the observations in these reports are probably due to inadequate preservation of hepatocyte structure during processing for electron microscopy. In adequately perfused liver the protein droplets are at all times membrane delimited and, as in other actively pi-

nocytic cells, channels and other pinocytic structures lead directly into the droplets. On the other hand, the material pinocytosed by the hepatocytes after partial hepatectomy does indeed appear to originate from plasma since: (*a*) the content of the droplets, like plasma, is PAS-positive; and (*b*) the cross-banded strands present in droplets resemble fibrin. The lysosomal enzymes transferred to protein droplets may be involved in converting soluble plasma fibrinogen into insoluble fibrin-like material. We have explored this hypothesis by adding lysosomes, isolated from rat liver, to fibrinogen in vitro. Indeed, a clot that is presumably fibrin does form, but the clot needs to be examined by electron microscopy.

The possibility that the protein droplets result from anoxia, surgical trauma, or shock appears unlikely since: (*a*) external signs of shock were not evident in our animals and they tolerated the partial hepatectomy very well; (*b*) neither prolonged ether anesthesia (up to 10 min, which is five or more times that used during partial hepatectomy) nor injection of saline without HRP induced droplets; and (*c*) confirming the results of others (2, 11, 4), when partial hepatectomy is performed on fasted rats, droplets do not form.

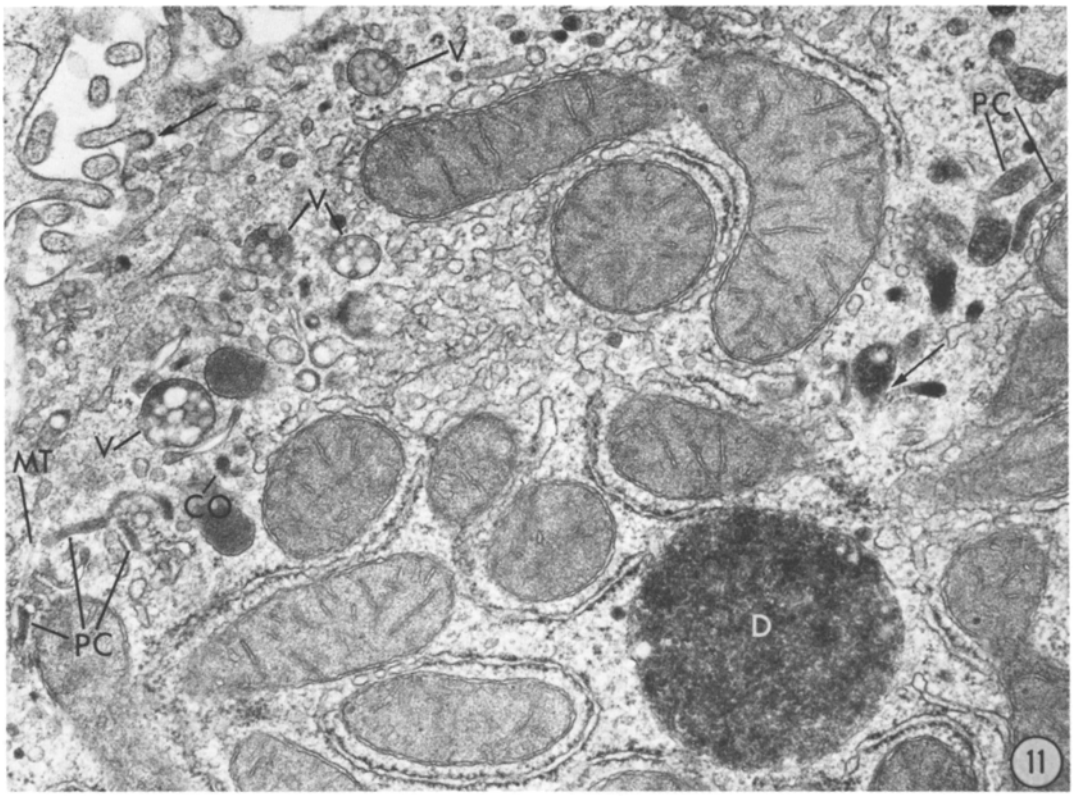
A basic question is raised by our observations, namely, what is it that converts a relatively nonpinocytic hepatocyte into an avidly pinocytic cell after partial hepatectomy? Is it that the hepatocytes in the remnant remaining after removal of two-thirds of the liver are exposed to more plasma in a given time? More than 10 years ago, Benacerraf et al. (8, 7) showed that there is a considerably greater liver blood flow per unit weight of liver in regenerating than in normal rat liver. These authors demonstrated an increased phagocytic activity of the Kupffer cells (8, 7) and Leong et al. (22) showed an increase of bile secretion in the regenerating liver. Goldfischer et al. (12) showed that if low doses of hemoglobin (up to 15 mg/100 g body weight) were injected intravenously into a normal rat, then the protein was pinocytosed only by the Kupffer cells. However, with much higher doses (40–400 mg/100 g body weight), the hemoglobin was pinocytosed by hepatocytes as well, and in the manner described in the current experiments. In the two investigations (26, 9) of which we are aware that report uptake of HRP by normal rat hepatocytes, considerably greater doses of HRP were used than the 10 mg/100 g body weight used in our experiments. Ma et al. (25) studied five mammalian species, including the rat. Only the hepatocytes of the rabbit pinocytosed HRP at low doses as well as at high doses.

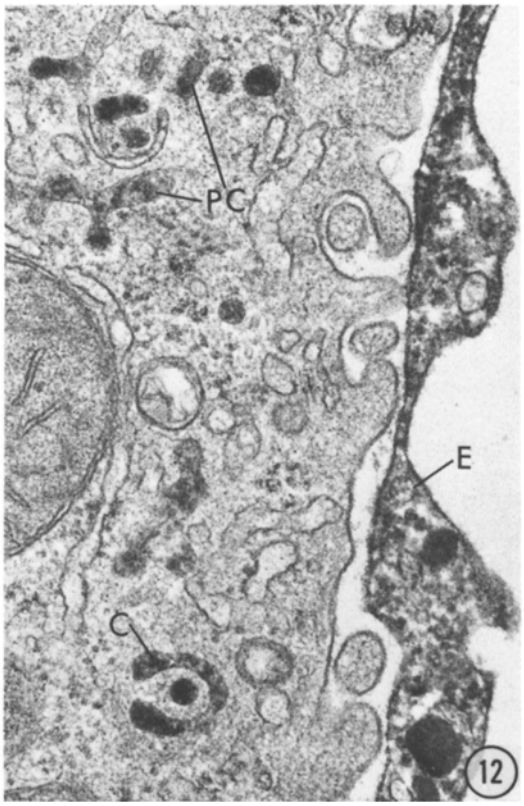
These considerations regarding HRP doses and the fact that the large pinocytic vacuoles, the protein droplets, do not form if the rats are fasted for 24 h before surgery warrant regarding the injected HRP as a marker for the fluid phase in bulk pinocytosis rather than an inducer of pinocytosis. Steinman and Cohn (36) and Steinman et al. (37) demonstrated this to be the case for cultured mouse macrophages and L cells. As in the cells they studied, there is no evidence from cytochemistry that the HRP is adsorbed to the plasma membranes of the hepatocytes.

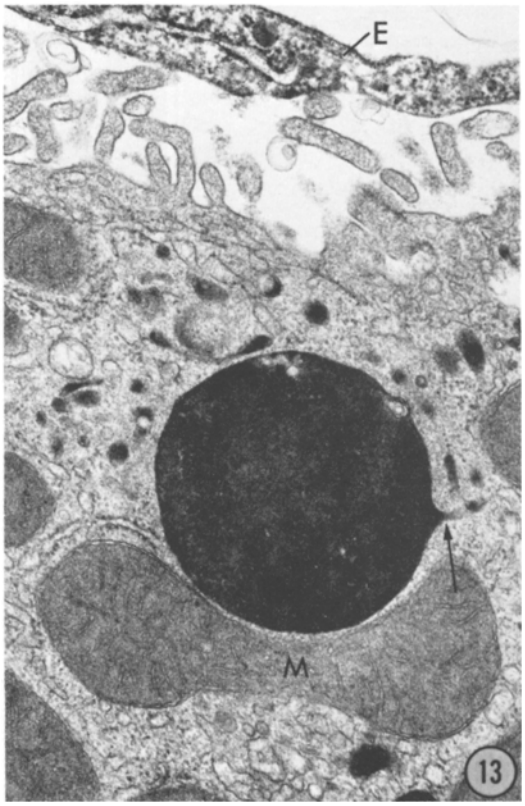
Several possibilities may be raised in considering the mechanisms that lead to the increased pinocytosis after partial hepatectomy. It is most unlikely that synthesis by the liver of "acute phase reactants" (see reference 21 for a review) occurs as a consequence of surgical trauma and that these proteins induce pinocytosis. The synthesis of such proteins does not occur until 3–4 h after an effective stimulus; see Fig. 4.7 in Koj (21). Possibly humoral or other changes in the animal after partial hepatectomy convert receptors on the hepatocyte plasma membranes exposed to the space of Disse. The hepatocyte may be converted to a cell that responds to different plasma constituents.[1] The work of Stockert et al. (38) indicates that hepatocytes have two receptors, one responsive to terminal galactosyl residues and another to terminal *N*-acetyl-glucosaminyl residues, whereas kidney appears to have a receptor responsive to mannosyl-terminating glycoproteins. Another alternative is that partial hepatectomy amplifies the amount of pinocytic inducer present in the portal blood of normal rats during intestinal absorption, although release of new pinocytic inducers from the liver or other organs cannot be excluded. The dramatic hormonal and other molecular changes induced by partial hepatectomy have recently been summarized by Koff and Leffert (20).

In conclusion, it should be emphasized that pinocytosis induction and macromolecular breakdown by heterophagy and autophagy are but two

[1] Dr. Richard J. Stockert assayed the plasma of one control rat and one partially hepatectomized (1 h) rat of our experiments. He found no change in level of any inhibitor of ^{125}I-asialo-orosamucoid binding to hepatic plasma membranes (18), a sensitive procedure for determining the levels of desialydated glycoproteins in plasma.


V
V
V
PC
MT
GO
PC
D
11


PC
E
C
12


E
M
13

of many dramatic changes that follow partial hepatectomy. Marked changes occur in almost all cell organelles, albeit more slowly, as seen in earlier cytochemical studies from this laboratory (29).

The authors gratefully acknowledge the preparation of the final figures by Mr. George Dominguez and the typing of successive versions of the manuscript by Ms. Fay Grad. We are indebted to Dr. R. J. Stockert (Department of Medicine, Albert Einstein College of Medicine) for the assay of the plasma from a control rat and a partially hepatectomized rat.

The research was supported by grants to Dr. Novikoff from the U.S. Public Health Service no. CA 06576 and The John Polachek Foundation for Medical Research. Dr. Michio Mori was supported, in part, by the Albert Einstein College of Medicine Hepatitis Cirrhosis Center grant AM 17702. Dr. Novikoff is the recipient of the U.S. Public Service Research Career Award 5K6 CA 14923 from the National Cancer Institute.

The work has been presented in preliminary form (28).

Received for publication 16 August 1976, and in revised form 9 November 1976.

REFERENCES

1. ATERMAN, K. 1952. Some local factors in the restoration of the rat's liver after partial hepatectomy. I. Glycogen; the Golgi apparatus; sinusoidal cells; the basement membrane of the sinusoids. *A. M. A. Arch. Pathol.* **53**:197–208.
2. ATERMAN, K. 1952. Some local factors in the restoration of the rat's liver after partial hepatectomy. II. "Watery vacuolation:" its relation to the vacuolation of anoxia. *A. M. A. Arch. Pathol.* **53**:209–216.
3. BARKA, T., and P. J. ANDERSON. 1962. Histochemical methods for acid phosphatase using hexazonium pararosanilin as coupler. *J. Histochem. Cytochem.* **10**:741–753.
4. BECKER, F. F. 1972. Acute glycogenolysis: a major stimulus of autophagocytic activity in rat hepatocytes. *Proc. Soc. Exp. Biol. Med.* **140**:1170–1172.
5. BECKER, F. F., R. BASERGA, and J. D. BROOME. 1970. Effect of L-asparaginase on DNA synthesis in regenerating liver and in other dividing tissues. *Cancer Res.* **30**:133–137.
6. BECKER, F. F., and B. P. LANE. 1965. Regeneration of the mammalian liver. I. Auto-phagocytosis during dedifferentiation of the liver cell in preparation for cell division. *Am. J. Pathol.* **47**:783–801.
7. BENACERRAF, B., D. BILBEY, G. BIOZZI, B. N. HALPERN, and C. STIFFEL. 1957. The measurement of liver blood flow in partially hepatectomized rats. *J. Physiol.* **136**:287–293.
8. BENACERRAF, B., G. BIOZZI, A. CUENDET, and B. N. HALPERN. 1955. Influence of portal blood flow and of partial hepatectomy on the granulopectic activity of the reticulo-endothelial system. *J. Physiol.* **128**:1–8.
9. CREEMERS, J., and P. J. JACQUES. 1971. Endocytic uptake and vesicular transport of injected horseradish peroxidase in the vacuolar apparatus of rat liver cells. *Exp. Cell Res.* **67**:188–203.
10. DAEMS, W. T., E. WISSE, and P. BREDEROO. 1969. *In* Lysosomes in Biology and Pathology. J. T. Dingle, and H. B. Fell, editors. Noord-Hollandsche Uitg. Mij., Amsterdam. **1**:64–112.
11. DONIACH, I., and K. WEINBREN. 1952. The development of inclusion bodies in the cells of the rat's liver after partial hepatectomy. *Brit. J. Exp. Pathol.* **33**:499–505.
12. GOLDFISCHER, S., A. B. NOVIKOFF, A. ALBALA, and L. BIEMPICA. 1970. Hemoglobin uptake by rat hepatocytes and its breakdown within lysosomes. *J. Cell Biol.* **44**:513–530.
13. GRAHAM, R. C., and M. J. KARNOVSKY. 1966. The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: ultrastructural cytochemistry by a new technique. *J. Histochem. Cytochem.* **14**:291–302.

FIGURES 11–13 Portions of hepatocytes and endothelial cells from regenerating liver, 1 h posthepatectomy, from two rats injected intravenously with HRP (3.3 mg/100 g body weight) 10 min before sacrifice; nonfrozen sections were incubated in the DAB medium for 30 min at 37°C. Fig. 11 is from one rat and Figs. 12 and 13 from the second. Note the absence of reaction product on the hepatocyte surfaces. The liver of the second rat showed poorly preserved endothelial cells (*E*). Note reaction product on the surfaces of these cells and diffusely in the cytoplasm. The hepatocytes in the three figures possess many pinocytic structures with reaction product. These include an internalized coated vesicle [*CO* in Fig. 11; cf. lack of reaction product in open coated vesicle in continuity with the plasma membrane (arrow at upper left in Fig. (11)], pinocytic channels (*PC* in Figs. 11 and 12 and unlabeled in Fig. 13), and a cuplike body (*C* in Fig. 12). The arrow in Fig. 13 indicates a continuity of a pinocytic channel with a protein droplet. The arrow at the right in Fig. 11 indicates a continuity between a channel and a larger pinocytic structure. Reaction product is seen in the two protein droplets (*D* in Fig. 11 and unlabeled in Fig. 13). Unreactive are mitochondria (one is labeled *M* in Fig. 13), secretory vacuoles (*V* in Fig. 11), microtubules (*MT* in Fig. 11) and other organelles. Fig. 11, × 29,000; Fig. 12, × 54,000; and Fig. 13, × 26,400.

14. HAYASHI, M. 1965. Histochemical demonstration of N-acetyl-β-glucosaminidase employing naphthol AS-BI N-acetyl-β-glucosaminide as substrate. *J. Histochem. Cytochem.* **13**:355–360.
15. HAYASHI, M., Y. NAKAJIMA, and W. H. FISHMAN. 1964. The cytologic demonstration of β-glucuronidase employing naphthol AS-BI glucuronide and hexazonium pararosanilin; a preliminary report. *J. Histochem. Cytochem.* **12**:293–297.
16. HIGGINS, G. M., and R. M. ANDERSON. 1931. Experimental pathology of the liver. I. Restoration of the liver of the white rat following partial surgical removal. *A. M. A. Arch. Pathol.* **12**:186–202.
17. HOLTZMAN, E. 1976. Lysosomes: a Survey. Springer-Verlag New York Inc., New York. 298.
18. HUDGIN, R. L., W. E. PRICE, JR., G. ASHWELL, R. J. STOCKERT, and A. G. MORELL. 1974. The isolation and properties of a rabbit liver binding protein specific for asialoglycoproteins. *J. Biol. Chem.* **249**:5536–5543.
19. KARNOVSKY, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* **27**(2, Pt. 2):137*a* (Abstr.).
20. KOFF, K. S., and H. L. LEFFERT. 1976. Control of hepatic proliferation: a working hypothesis involving hormones, lipoproteins, and novel nucleotides. *Metabolism.* **25**:1419–1422.
21. KOJ, A. 1974. Acute-phase reactants. *In* Structure and Function of Plasma Proteins. A. C. Allison, editor. Plenum Publishing Corporation, New York. **1**:73–131.
22. LEONG, G. F., R. L. PESSOTTI, AND R. W. BRAUER. 1959. Liver function in regenerating rat liver. $CrPO_4$ colloid uptake and bile flow. *Am. J. Physiol.* **197**:880–887.
23. LEUENBERGER, P. M., and A. B. NOVIKOFF. 1975. Studies on microperoxisomes. VII. Pigment epithelial cells and other cell types in the retina of rodents. *J. Cell Biol.* **65**:324–334.
24. LILLIE, R. D. 1954. Histopathologic Technic and Practical Histochemistry. Blakiston Division of the McGraw-Hill Book Co., Inc., New York. 501.
25. MA, M. H., W. A. LAIRD, and H. SCOTT. 1974. Cytopempsis of horseradish peroxidase in the hepatocyte. *J. Histochem. Cytochem.* **22**:160–169.
26. MATTER, A., L. ORCI, and C. ROUILLER. 1969. A study on the permeability barriers between Disse's space and the bile canaliculus. *J. Ultrastruct. Res. Suppl.* **11**:7–71.
27. MILLER, F., and V. HERZOG. 1969. Die Lokalisation von Peroxidase und sauer Phosphatase in eosinophilen Leukocyten wahrend der Reifung. Elektronemikroskopisch-Untersuchungen am Knochenmark von Ratte und Kaninchen. *Z. Zellforsch. Mikrosk. Anat.* **97**:84–110.
28. MORI, M., and A. B. NOVIKOFF. 1975. Lysosomes in rat hepatocytes following partial hepatectomy. *J. Cell Biol.* **67**(2, Pt. 2)(Abstr.):294*a*.
29. MORI, M., A. B. NOVIKOFF, and N. QUINTANA. 1975. Cytochemical studies on regenerating rat liver. *J. Histochem. Cytochem.* **23**:315–316.
30. NOVIKOFF, A. B. 1973. Lysosomes, a personal account. *In* Lysosomes and Storage Diseases. G. Hers and F. Van Hoof, editors. Academic Press, Inc., New York. 1–41.
31. NOVIKOFF, A. B. 1973. Studies on the structure and function of cell organelles: 3,3′-diaminobenzidine cytochemistry. *In* Electron Microscopy and Cytochemistry. E. Wisse, W. Th. Daems, I. Molenaar, and P. Van Duijn, editors. Noord-Hollandsche Uitg. Mij., Amsterdam. 89–109.
32. ONOE, T. 1968. Ultrastructural pathology of the liver. *Tr. Soc. Pathol. Jap.* **57**:3–36. (In Japanese).
33. PFEIFFER, U., and P. BANNASCH. 1968. Zum Problem der "hyalinen Eiweisstropfen" im Cytoplasma der Leberparenchymzellen. *Virchows Arch. Abt. B Zellpathol.* **1**:365–388.
34. PRICE, J. M., and A. K. LAIRD. 1950. A comparison of the intracellular composition of regenerating liver and induced liver tumors. *Cancer Res.* **10**:650–658.
35. SMITH, R. E., and M. G. FARQUHAR. 1965. Preparation of non-frozen sections for electron microscope cytochemistry. *R.C.A. Sci. Instr. News* **10**:13–18.
36. STEINMAN. R. M., and Z. A. COHN. 1972. The interaction of soluble horseradish peroxidase with mouse peritoneal macrophages in vitro. *J. Cell Biol.* **55**:186–204.
37. STEINMAN, R. M., J. M. SILVER, and Z. A. COHN. 1974. Pinocytosis in fibroblasts. *J. Cell Biol.* **63**:949–969.
38. STOCKERT, R. J., A. G. MORELL, and I. H. SCHEINBERG. 1976. The existence of a second route for the transfer of certain glycoproteins from the circulation into the liver. *Biochem. Biophys. Res. Commun.* **68**:988–993.
39. VORBRODT, A. 1967. Cytochemical studies of lysosomal enzymes during early stages in rat liver regeneration. *Folia Histochem. Cytochem.* **5**:231–238.