ELECTRON-OPAQUE BODIES AND FAT DROPLETS IN MOUSE LIVER AFTER FASTING OR GLUCOSE INJECTION

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ABSTRACT

Fasting produces an increased mobilization of lipid from adipose tissue to the liver and a decreased hepatic lipogenesis, but the administration of glucose stimulates lipid synthesis by the liver. After fasting of C3H mice numerous electron-opaque bodies and large lipid droplets were present in the liver. In the liver of untreated controls only a few small electron-opaque bodies and an occasional fat droplet were observed. After glucose injection the number of electron-opaque bodies in the liver was no greater than that observed in livers of saline-injected controls. In the livers of all groups these bodies were located intracellularly within cytoplasmic vesicles; those in extracellular locations were not membrane bounded and were located at indented and thickened hepatocyte plasma membranes or within the space of Disse. In fasted liver the dense bodies were often associated with large fat droplets.

INTRODUCTION

Many electron-opaque bodies may be observed in regenerating mouse liver after partial hepatectomy. These bodies occur in both intracellular and extracellular locations and are thought to contain some lipid component (1, 2). In the present study two sets of experimental conditions known to affect lipid metabolism have been established in order to obtain more information concerning the electron-opaque bodies.

Fasting causes an increased mobilization of lipid from adipose tissue to liver (3 and 4). Williams (5) has observed with the light microscope that the number of fat droplets in the mouse liver is increased after a 1 day fast. In hamster liver an accumulation of lipid also accompanies fasting (6), and in fasted humans there is an increased release of fatty acids from adipose tissue into the blood plasma (4) with a subsequent increase in oxidation of lipid by the liver (7). Since fasting decreases lipid synthesis throughout the body including the liver (8), it is probable that the increase in hepatic lipid content after fasting is due to the entrance into the liver of mobilized lipid. This lipid travels in the blood as free fatty acids, which may be esterified after entering the hepatic cytoplasm.

The administration of glucose results in a nonspecific increase in the synthesis of lipid in the liver as well as in other organs of the mouse (8, 9). The resulting product, primarily fatty acid, does

not remain in the free state but becomes transformed into triglycerides (10), which are visible in the electron microscope. These triglycerides either remain in the parenchymal cells or are destined for export to other organs (10).

MATERIALS AND METHODS

The 26 animals used in this study were 60–90-day-old male mice of strain C3H/StWi. Two experimental groups were established. One group was fasted for various periods (8½, 12½, 24, 36, and 48 hr) before sacrifice; normal mice fed ad libitum served as controls for the fasted animals. The second group received 0.3 cc of 5% glucose intravenously at 3, 5, 10, 30, 60, and 120 min before sacrifice. Controls for this group were given 0.3 cc of physiological saline and sacrificed at intervals of 3, 30, and 120 min after injection.

All mice were sacrificed by decapitation. A piece of the right lateral lobe of the liver was removed for fixation in 2% osmium tetroxide buffered with Veronal-acetate. After this method of fixation, lipid appears fairly electron-opaque, and regions containing glycogen are usually recognizable as mottled light areas. Fixation of glycogen in its particulate form, as with the use of glutaraldehyde or phosphate-buffered osmium tetroxide (11–13), was avoided in order to facilitate identification of the smaller electron-opaque bodies. Samples were dehydrated through a graded series of acetone and embedded in Epon 812. Sections were cut on a Porter-Blum (Sorvall MT-1) microtome with glass or diamond knives (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del. or Ge-Fe-Ri, Frosinone, Italy). Light microscope preparations, 1 μ thick, were stained with 2% toluidine blue buffered with borax to pH 9. Sections for the electron microscope were stained with a saturated aqueous solution of uranyl acetate (14) at a staining temperature of 45°–50°C for 1½ hr. The sections were photographed in an RCA EMU 3G electron microscope.

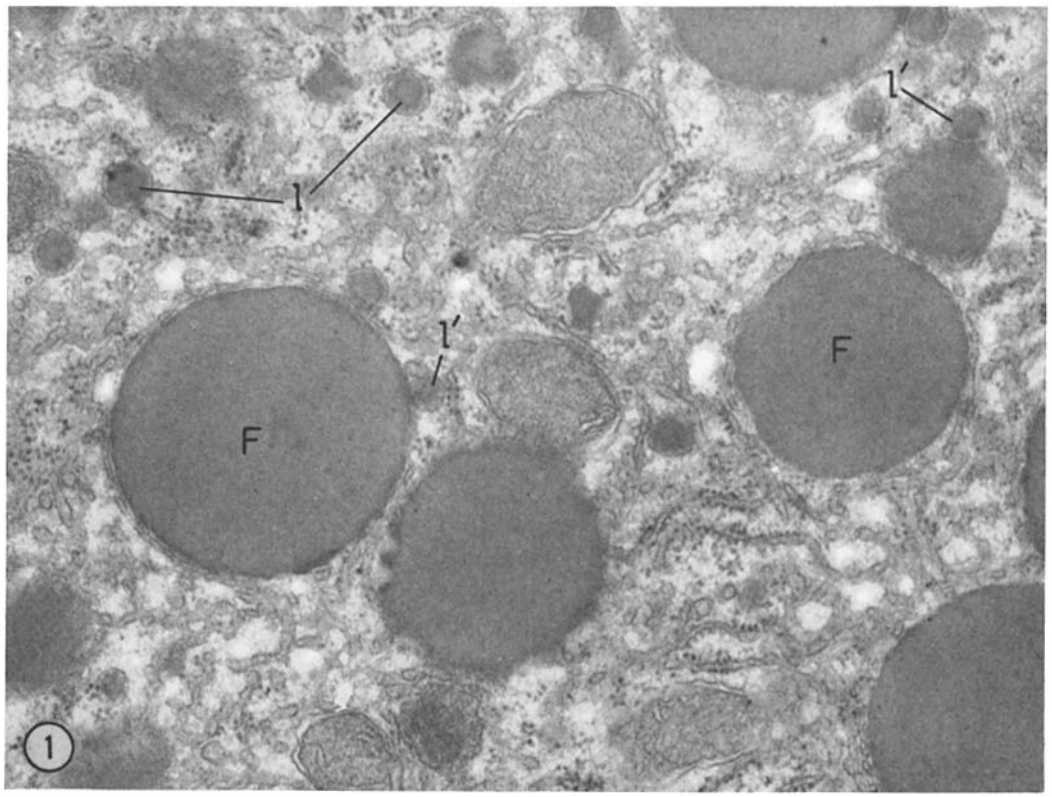

FIGURE 1 C3H mouse liver after a 48 hr fast. Numerous fat droplets (*F*) and membrane-bounded, electron-opaque bodies (*l*) are present. The electron-opaque bodies at *l'* appear separated from the fat droplets only by their surrounding vesicles. × 39,000.

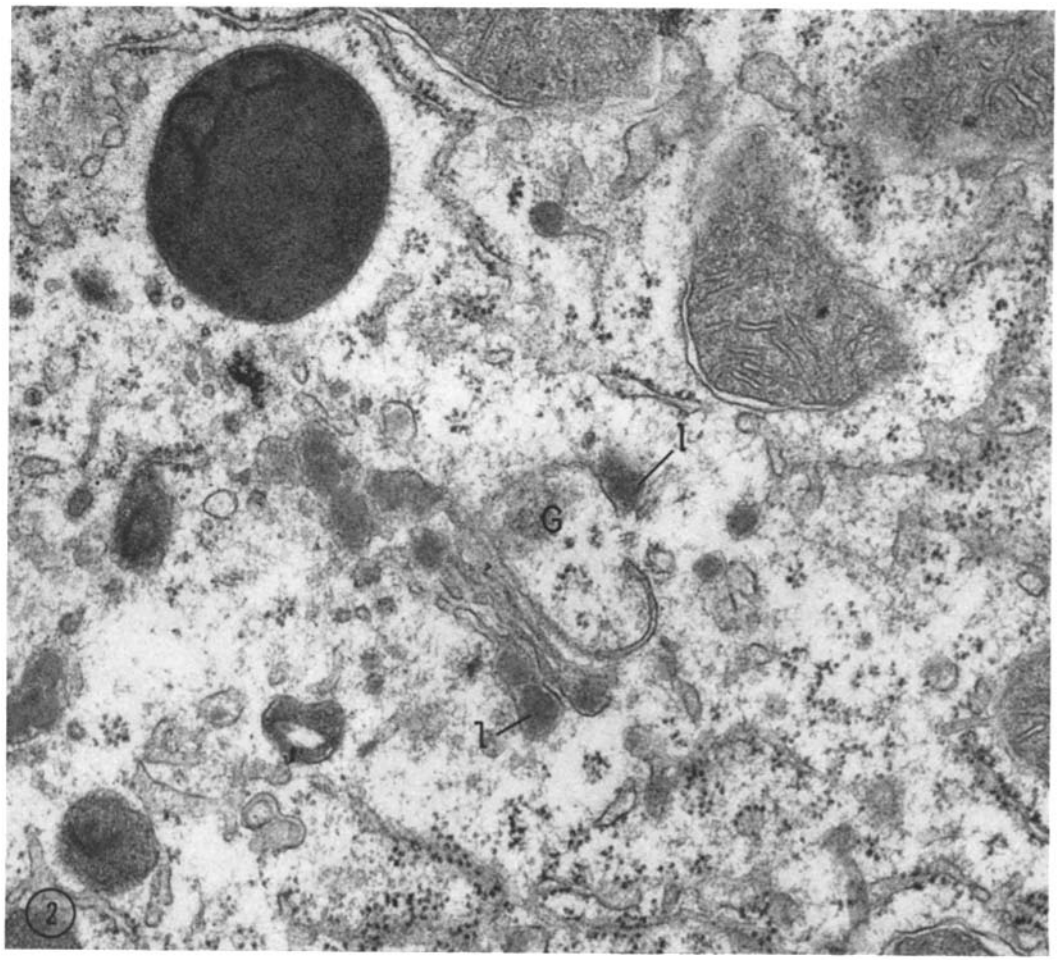

FIGURE 2 Liver 48 hr after the onset of fasting. Electron-opaque bodies (*l*) are located within vacuoles of the Golgi complex (*G*). × 41,000.

OBSERVATIONS

Livers of fasted C3H mice contain numerous small electron-opaque bodies, 300-2400 A in diameter, and numerous large fat droplets scattered throughout the parenchymal cell cytoplasm (Fig. 1). Electron-opaque bodies are often observed in association with the fat droplets (Fig. 1) or within vacuoles of the Golgi complex (Fig. 2). Regardless of their intracytoplasmic location, the electron-opaque bodies are always surrounded by a smooth membrane so that they appear to be enclosed within a vesicle.

The fine structure of normal liver of C3H mice is essentially identical with that of the liver of mice of various other strains (15) and of rats (16–18). Electron-opaque bodies are very rarely seen in normal liver, and only a few scattered fat droplets are present. Glycogen, however, is more abundant than in the liver of the fasted animal and, with the method of fixation employed in this study, appears as an amorphous light area (Fig. 3).

The plasma membrane of hepatic parenchymal cells in both the normal and the fasted animals shows indentations which are sometimes thickened (Figs. 3 and 4). These indentations or pits have also been described by Roth and Porter (19), Fawcett (20), and Tanaka and Pappas (21), who interpret them to be sites of incorporation of various materials. In livers of fasted animals

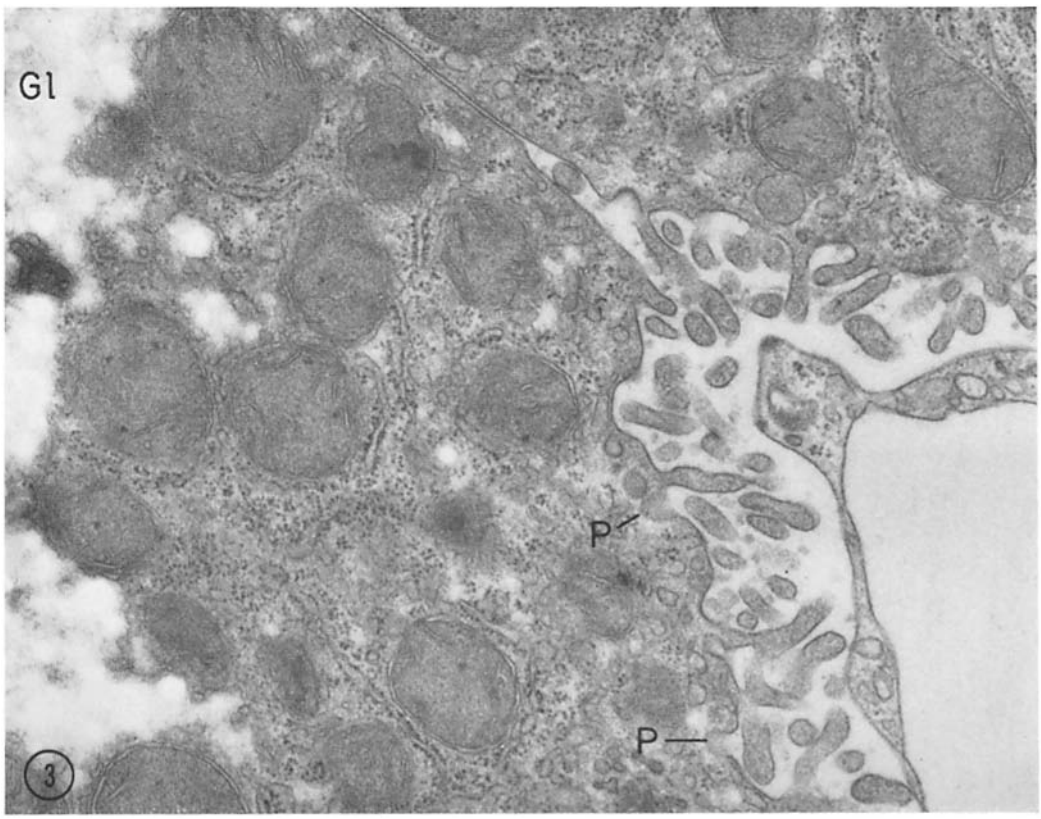

FIGURE 3 Normal liver. Portions of two hepatic cells, an endothelial lining cell, a sinusoid, and the space of Disse. Invaginations of the hepatic cell plasma membrane (*P*) are present. Glycogen appears as an amorphous light area (*Gl*). × 41,000.

electron-opaque bodies are frequently observed within these invaginations (Fig. 4). The electron-opaque bodies present in extracellular locations after fasting are never completely surrounded by a membrane (Figs. 4 and 5). These extracellular dense bodies are most numerous in the space of Disse but may also be found in the intercellular space, the triangular space formed at the junction of the intercellular and Disse spaces, and occasionally in the sinusoids (Fig. 5). The density and size range of extracellular bodies are similar to those of the dense bodies in intracytoplasmic locations.

An accumulation of large fat droplets is also characteristic of fasted mouse liver. As the length of the fast increases, the number of the fat droplets also increases. This is best illustrated in light microscope preparations: compare Fig. 6, liver after 12½ hr of fasting, with Fig. 7, liver sample taken after a 48 hr fast.

After glucose injection (Fig. 8) the typical hepatic parenchymal cell is identical with that of the saline-injected mouse (Fig. 9). A few very small (300–1200 A in diameter) electron-opaque bodies are present within the cytoplasm, at the pits in the plasma membrane, in the space of Disse, and in the intercellular spaces. These liver samples contain more, dense bodies than are present in liver of normal, untreated animals but fewer bodies than are found in fasted liver. In addition, the dense bodies are much smaller than those of fasted liver. An occasional fat droplet is observed after injection of either glucose or saline (Figs. 8 and 9), and glycogen areas are as numerous as in the normal animal (compare with Fig. 3).

DISCUSSION

Biochemical studies have shown that glucose induces the de novo synthesis of lipid (22) in various organs of the mouse including the liver

(8). In order to establish the dosage of glucose necessary to produce lipid synthesis in the liver and to determine the time after injection at which peak activity is reached, we used the experiments of Favarger and Gerlach (8) as a guide. If these experiments were effective and if lipid synthesis were actually occurring in the liver, it is clear that the electron-opaque bodies could not represent newly synthesized lipid since injection of glucose resulted in the same number of opaque bodies as were present after saline injection. In the livers of both the glucose-injected and saline-injected mice, however, the electron-opaque bodies were more numerous than in the livers of normal mice. This suggests that trauma of injection alone may be responsible for the observed increase in the size and frequency of the bodies.

It has been argued that the various electron-opaque bodies in liver contain predominantly albumin (16), lipid (1, 2, 23), or lipoprotein (24–26). It is unlikely that the electron-opaque bodies present in fasted liver represent albumin synthesized for export to the plasma, since it has been demonstrated that fasting results in a decreased synthesis of albumin (27). Fasting has been reported to produce an increased mobilization of lipid and a decreased lipid synthesis by the liver (8). Therefore, the observation of many electron-opaque bodies in liver after fasting strengthens the contention that these bodies represent not newly synthesized lipid but newly mobilized lipid. Lipid is mobilized as free fatty acids (4, 28–33) and is subsequently transported in the blood in loose combination with plasma albumin (28, 31) or low density lipoprotein (34). The thickened pits observed at various sites along the plasma membrane of parenchymal cells might indicate

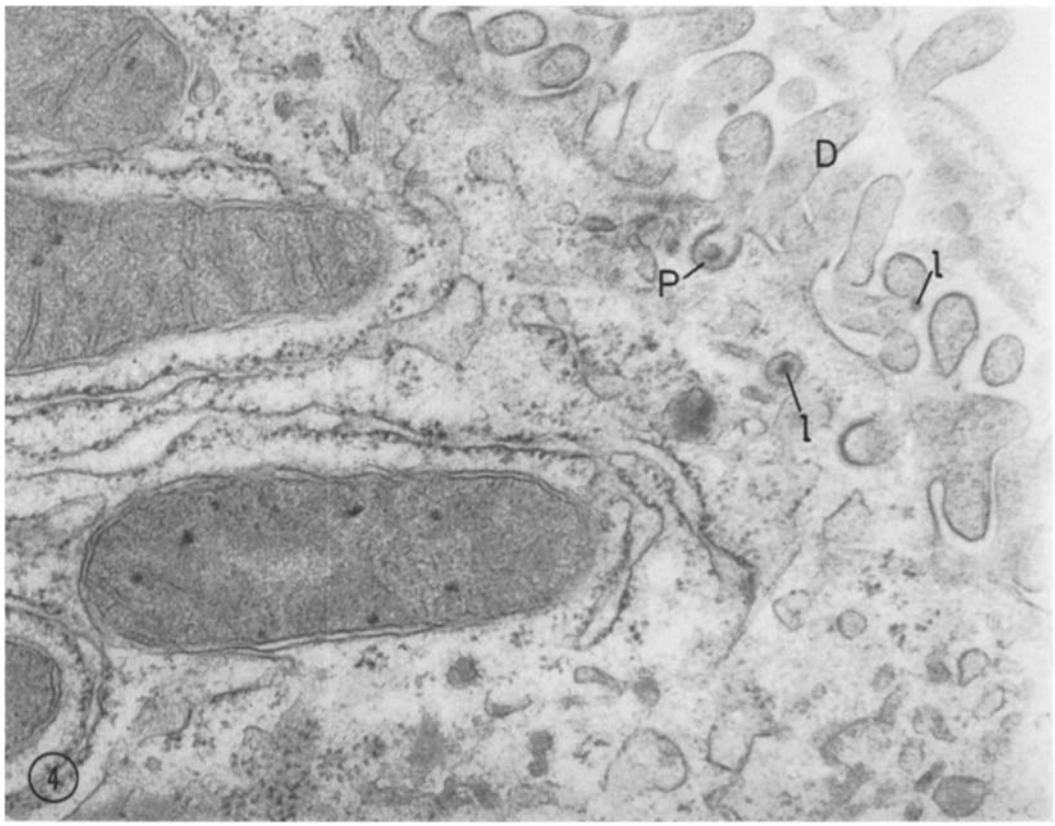

FIGURE 4 Liver 12½ hr after the onset of fasting. Small electron-opaque bodies (*l*) are found within the parenchymal cell cytoplasm, at invaginations of the plasma membrane (*P*), and in the space of Disse (*D*). × 48,000.

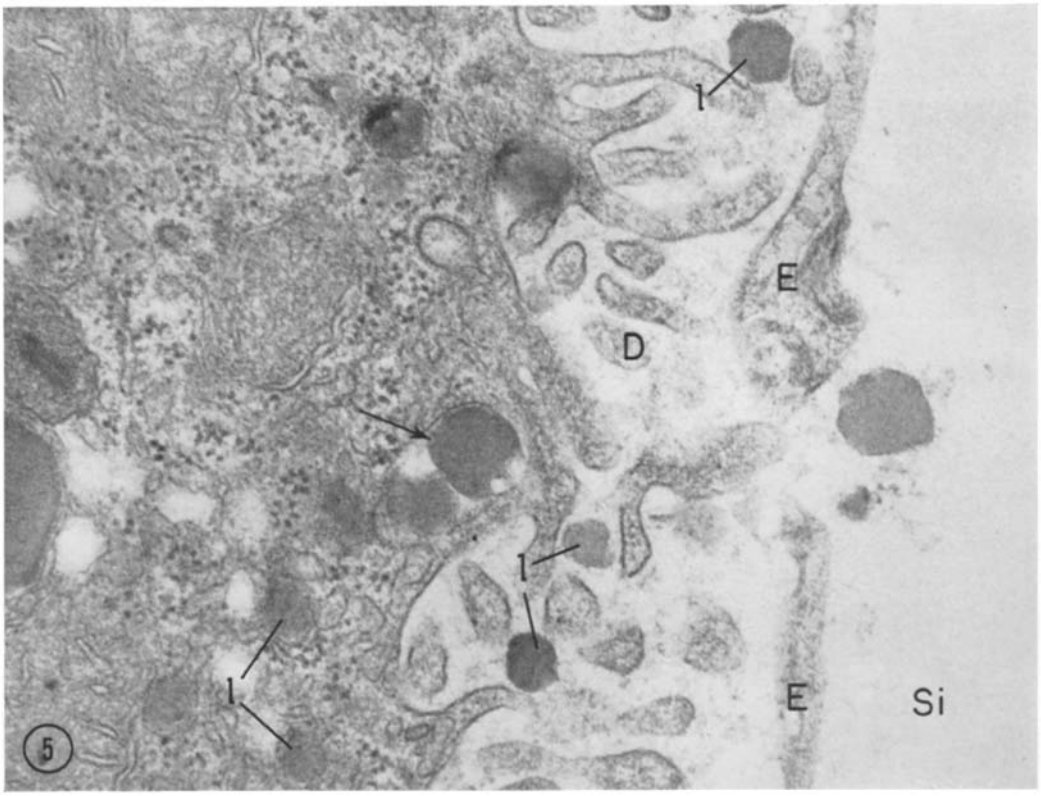

FIGURE 5 Mouse liver after a 48 hr fast. Electron-opaque bodies (*l*) are present within the hepatic parenchyma and in the space of Disse (*D*). Bodies may also be seen in the sinusoid (*Si*) near the junction of two endothelial lining cells (*E*). The dense bodies are often closely associated with the hepatic cell plasma membrane (arrow), but in extracellular locations they are never completely enclosed within a membrane. × 60,000.

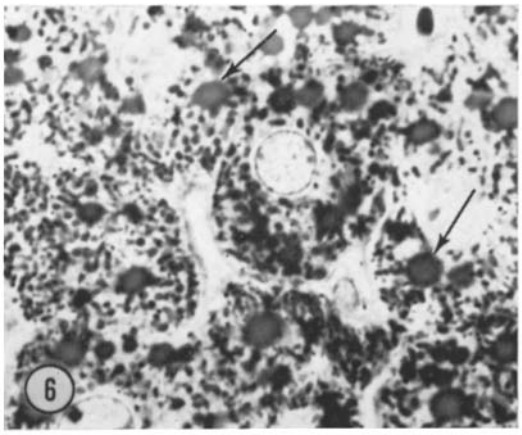

FIGURE 6 Light micrograph of liver 12½ hr after food withdrawal. Note that fat droplets (arrows) have begun to accumulate in the parenchymal cells. × 650.

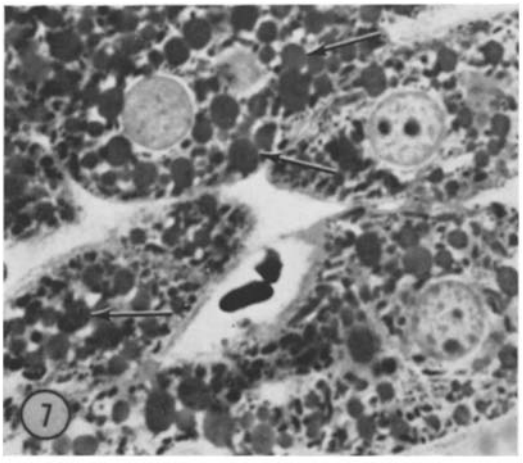

FIGURE 7 Liver after a 48 hr fast. The number of fat droplets (arrows) in hepatic cells is greater than after a 12½ hr fast (Fig. 6). × 650.

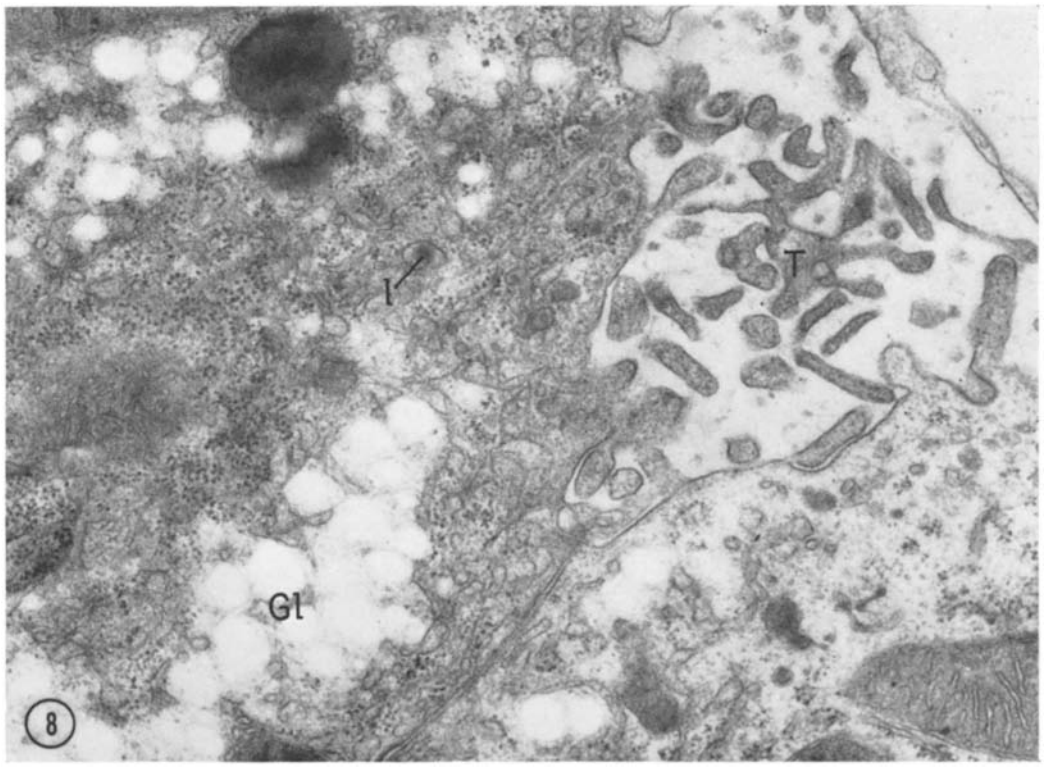

FIGURE 8 Portion of mouse liver 120 min after the intravenous injection of 0.3 cc of 0.5% glucose. A small electron-opaque body (*l*) is present in the parenchymal cell cytoplasm. *T*, triangular space. *Gl*, glycogen area. × 38,000.

sites of incorporation of free fatty acids which are not visible in the electron microscope.

Within the hepatic cells the free fatty acids are available for many roles in lipid metabolism. (*a*) The free fatty acids could become esterified to triglyceride, perhaps inside membranes of the agranular endoplasmic reticulum, and form small electron-opaque bodies which might fuse together to form large fat droplets. Fat droplets could thus be stored temporarily in the liver and later could be oxidized to release the energy needed by the animal during fasting. (*b*) The free fatty acids could leave the liver almost at once to supply other organs with free fatty acids. (*c*) They could be oxidized immediately upon entrance into the liver. (*d*) The fatty acids might be utilized by the liver to synthesize lipoproteins (35, 36). Lipoproteins, according to Bierman et al. (37), are 250–10,000 A in diameter. Since the electron-opaque bodies described here fall within this size range, some of the bodies might represent newly synthesized lipoproteins, and some of the extracellular bodies might be lipoprotein recently exported from the parenchymal cells. Perhaps the lipid moiety of lipoprotein is assembled in the agranular endoplasmic reticulum (38) and is joined to the protein moiety in the Golgi complex in a manner similar to the formation of glycoprotein in goblet cells (39). If a Golgi vacuole could adhere to the plasma membrane and if its contents could be emptied into the sinusoid by a process akin to reversed pinocytosis, perhaps the thickened areas of the plasma membrane could represent sites of export as well as sites of entry.

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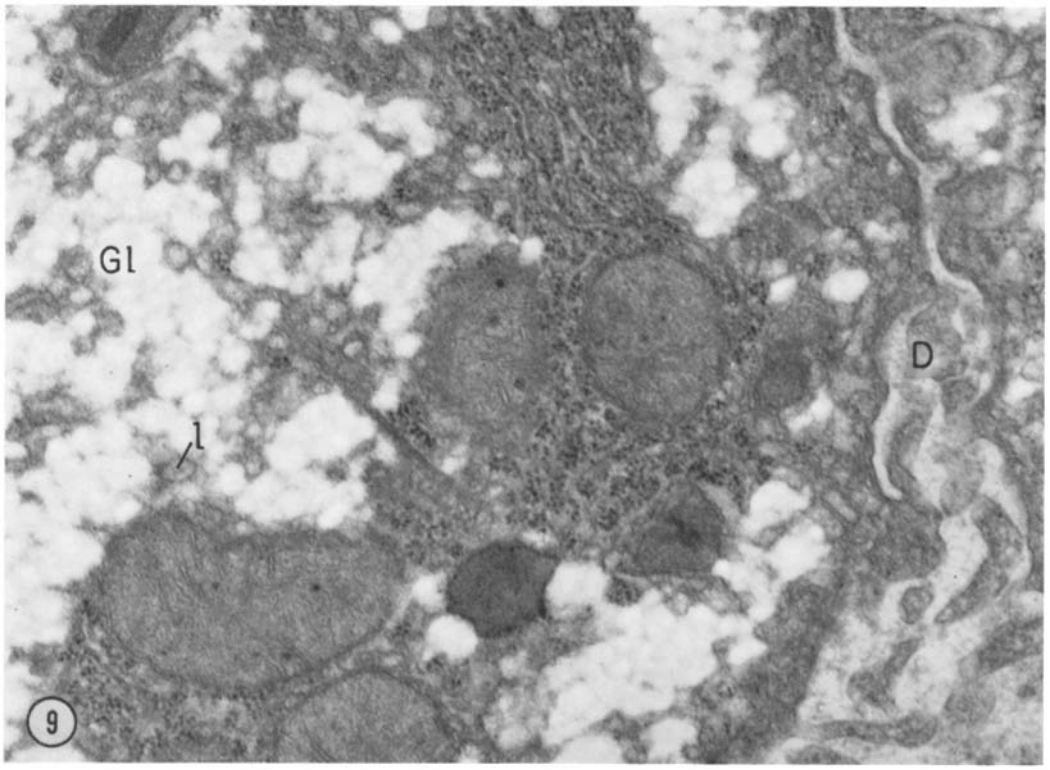

FIGURE 9 Mouse liver 120 min after the intravenous injection of 0.85% saline. Only a few small electron-opaque bodies (*l*) are present in the parenchymal cell cytoplasm. Glycogen areas (*Gl*) are numerous. *D*, space of Disse. ×38,000.

REFERENCES

1. TROTTER, N. L. 1964. A fine structure study of lipid in mouse liver regenerating after partial hepatectomy. *J. Cell Biol.* **21**:233.
2. TROTTER, N. L. 1965. Electron-opaque, lipid-containing bodies in mouse liver at early intervals after partial hepatectomy and sham operation. *J. Cell Biol.* **25**:41.
3. MEAD, J. F. 1963. Lipid metabolism. *Ann. Rev. Biochem.* **32**:241.
4. GORDON, R. S., and A. CHERKES. 1956. Unesterified fatty acid in human blood plasma. *J. Clin. Invest.* **35**:206.
5. WILLIAMS, W. L. 1951. Cytoplasmic changes in hepatic parenchyma of mice during starvation and carbon tetrachloride-induced injury. *Anat. Record.* **111**:629.
6. JONES, A. L., and D. W. FAWCETT. 1966. Hypertrophy of the agranular endoplasmic reticulum in hamster liver induced by phenobarbital (with a review of the functions of this organelle in liver). *J. Histochem. Cytochem.* **14**:215.
7. DOLE, V. P. 1964. Fat as an energy source. *In* Fat as a Tissue. K. Rodahl and B. Issekutz, editors. McGraw-Hill Book Company, New York. 250.
8. FAVARGER, P., and J. GERLACH. 1958. Recherches sur la synthèse des graisses à partir d'acetate ou de glucose IV. Importance de la lipogenèse hépatique; étude expérimentale critique. *Helv. Physiol. Pharmacol. Acta.* **16**:188.
9. COIMBRA, A., and C. P. LEBLOND. 1966. Sites of glycogen synthesis in rat liver cells as shown by electron microscope radioautography after administration of glucose-H^3. *J. Cell Biol.* **30**:151.
10. STEIN, Y., and B. SHAPIRO. 1959. Assimilation and dissimilation of fatty acids by the rat liver. *Am. J. Physiol.* **196**:1238.
11. SABATINI, D. D., K. BENSCH, and R. J. BARRNETT. 1963. Cytochemistry and electron microscopy. The preservation of cellular ultra-

structure and enzymatic activity by aldehyde fixation. *J. Cell Biol.* **17**:19.

12. SABATINI, D. D., F. MILLER, and R. J. BARRNETT. 1964. Aldehyde fixation for morphological and enzyme histochemical studies with the electron microscope. *J. Histochem. Cytochem.* **12**:57.
13. MILLONIG, G., and K. R. PORTER. 1961. Structural elements of rat liver cells involved in glycogen metabolism. *Proc. European Reg. Conf. Electron Microscopy Delft 1960.* **2**:655.
14. WATSON, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. *J. Biophys. Biochem. Cytol.* **4**:475.
15. DAEMS, W. T. 1961. The micro-anatomy of the smallest biliary pathways in mouse liver tissue. *Acta Anat.* **46**:1.
16. BRUNI, C., and K. R. PORTER. 1965. The fine structure of the parenchymal cell of the normal rat liver. I. General observations. *Am. J. Pathol.* **46**:691.
17. FAWCETT, D. W. 1955. Observations on the cytology and electron microscopy of hepatic cells. *J. Natl. Cancer Inst.* **15**:1475.
18. PORTER, K. R., and C. BRUNI. 1959. An electron microscope study of the early effects of 3'-ME DAB on rat liver cells. *Cancer Res.* **19**:997.
19. ROTH, T. F., and K. R. PORTER. 1962. Specialized sites on the cell surface for protein uptake. *In* Electron Microscopy: Fifth International Congress on Electron Microscopy Held in Philadelphia, Pennsylvania, August 29th to September 5th, 1962. S. S. Breese, Jr., editor. Academic Press Inc., New York. **2**:LL-4.
20. FAWCETT, D. W. 1965. Surface specializations of absorbing cells. *J. Histochem. Cytochem.* **13**:75.
21. TANAKA, H., and G. D. PAPPAS. 1964. Electron microscope study of the initial stages of the uptake of iso- and hetero-ferritin and other colloidal marker substances. *J. Cell Biol.* **23**: 94A. (Abstr.)
22. FRITZ, I. B. 1961. Factors influencing the rates of long-chain fatty acid oxidation and synthesis in mammalian systems. *Physiol. Rev.* **41**:52.
23. ASHWORTH, C. T., J. S. LEONARD, E. H. EIGENBRODT, and F. J. WRIGHTSMAN. 1966. Hepatic intracellular osmiophilic droplets. Effect of lipid solvents during tissue preparation. *J. Cell Biol.* **31**:301.
24. BAGLIO, C. M., and E. FARBER. 1965. Reversal by adenine of the ethionine-induced lipid accumulation in the endoplasmic reticulum of the rat liver. A preliminary report. *J. Cell Biol.* **27**:591.
25. HAMILTON, D. W., D. W. FAWCETT, and A. K. CHRISTENSEN. 1966. The liver of the slender salamander *Batrachoseps Attenuatus*. I. The structure of its crystalline inclusions. *Z. Zellforsch. Mikroskop. Anat.* **70**:347.
26. JONES, A. L., N. B. RUDERMAN, and M. G. HERRERA. 1966. An electron microscope investigation of lipoprotein metabolism in isolated perfused rat liver. *J. Cell Biol.* **31**:54A. (Abstr.)
27. MARSH, J. B. 1961. Effects of fasting and alloxan diabetes on albumin synthesis by perfused rat liver. *Am. J. Physiol.* **201**:55.
28. OLSON, R. E., and J. W. VESTER. 1960. Nutrition-endocrine interrelationships in the control of fat transport in man. *Physiol. Rev.* **40**:677.
29. LAURELL, S. 1959. Distribution of C^{14} in rats after intravenous injection of nonesterified palmitic acid-1-C^{14}. *Acta Physiol. Scand.* **46**:97.
30. WILLIAMSON, J. R. 1964. Adipose tissue. Morphological changes associated with lipid mobilization. *J. Cell Biol.* **20**:57.
31. FREDERICKSON, D. S., and R. S. GORDON. 1958. Transport of fatty acids. *Physiol. Rev.* **38**:585.
32. WASSERMANN, F., and R. F. MCDONALD. 1963. Electron microscopic study of adipose tissue (fat organs) with special reference to the transport of lipids between blood and fat cells. *Z. Zellforsch. Mikroskop. Anat.* **59**:326.
33. STEIN, Y., and B. SHAPIRO. 1960. Uptake and metabolism of triglycerid s by the rat liver. *J. Lipid Res.* **1**:326.
34. GOODMAN, D. S., and R. S. GORDON, JR. 1958. The metabolism of plasma unesterified fatty acid. *Am. J. Clin. Nutr.* **6**:669.
35. HAFT, D. E., P. S. ROHEIM, A. WHITE, and H. A. EDER. 1962. Plasma lipoprotein metabolism in perfused rat livers. I. Protein synthesis and entry into the plasma. *J. Clin. Invest.* **41**:842.
36. RADDING, C. M., and D. STEINBERG. 1960. Studies on the synthesis and secretion of serum lipoproteins by rat liver slices. *J. Clin. Invest.* **39**:1560.
37. BIERMAN, E. L., J. L. HAYES, J. N. HAWKINS, A. M. EWING, and F. T. LINDGREN. 1966. Particle-size distribution of very low density plasma lipoproteins during fat absorption in man. *J. Lipid Res.* **7**:65.
38. STEIN, O. and Y. STEIN. 1966. Electronmicroscopic autoradiography of ^{3}H-glycerol labeled lipid in ethanol induced fatty liver. *Exptl. Cell Res.* **42**:198.
39. NEUTRA, M., and C. P. LEBLOND. 1966. Synthesis of the carbohydrate of mucus in the Golgi complex as shown by electron microscope radioautography of goblet cells from rats injected with glucose-H^3. *J. Cell Biol.* **30**:119.