Department of Anatomy (Prof. T. YAMAMOTO), Faculty of Medicine, Kyushu University,
Fukuoka, Japan

Electron Microscopic Observations on the Mouse Liver after Intravenous Administration of Fat Emulsion

Tanenori HATAE

Received June 19, 1974

Summary. Artificial fat emulsion was injected into mice and their uptake by the liver was observed electron microscopically.

At 5 min after the injection, Kupffer cells took up lipid particles by pinocytosis, but no liver cells showed a sign of lipid uptake. At 30 and 60 min after the injection, numerous osmiophilic particles (300–1,000 Å) appeared in the cisternal ends of rough endoplasmic reticulum, smooth endoplasmic reticulum proper and Golgi apparatus of the liver cells. The particles were also found in the space of Disse. At 6 hrs after the injection, the particles were no longer found in the liver cells but many large 0.5–2 μ lipid droplets accumulated in the cell matrix.

It was suggested that the injected lipids were taken into the liver cells by simple diffusion after hydrolysis and incorporated into lipoproteins which were secreted into the circulation.

A large volume of biochemical and physiological literature indicates that the liver plays a significant role in fat metabolism. The fats given to animals as a food stuff, after digestion and absorption in the alimentary canal, appear as chylomicra in the blood plasma and most of them are rapidly taken up by the fat tissue and the liver.

Between 30 and 50% of the uptake occurs in the liver (ROBINSON, 1964). In the next step, it is suggested that the liver produces lipoproteins and discharges them into the circulation. An important problem, however, still remains as to the lipid uptake by the liver cells (FAWCETT, 1964). Some authors have inferred that the liver cells ingest the chylomicra directly by pinocytosis from their surface facing the sinusoid (ASHWORTH, STEMBRIDGE and SANDERS, 1960; PARKS, 1962; OLIVECRONA, 1962a), while others have held that the chylomicra are hydrolyzed into fatty acids and glycerols by the lipoprotein lipase present in the blood plasma and then diffusion into the liver cell occurs (BELFRAGE, 1966; STEIN and STEIN, 1967).

Recently artificial fat emulsions have been used for patients to provide high caloric intake after surgery. It has been shown that fat particles are removed rapidly from the circulation after an intravenous administration of the emulsion and that low density lipoproteins appear in several minutes (KINSELL, MICHAELS and IMAICHI, 1965). However, the detailed mechanism of fat uptake by the liver after artificial fat administration has remained obscure. The present study was carried out to elucidate this problem by means of electron microscopy.

Materials and Methods

Fifteen male mice weighing 15–25 g were used for the present study. The animals were fasted overnight prior to the experiment.

The fat emulsion used was Fatgen, supplied by Dainippon Seiyaku Co., Osaka, containing 20% sesame oil, 0.2% soya lecithins, 0.4% dl-methionine, 5% dextran and 8% sorbitol.

Fig. 1. Sinusoidal portion of the liver 5 min after the injection. Injected fat particles (arrows) are seen in the sinusoidal lumen (*Si*) and in the space of Disse (*DS*). Some of the particles are taken up by the Kupffer cell (*K*). *E* endothelium, *RBC* red blood cell. $\times 11,000$

The fat emulsion, warmed to 40°C, was injected into the tail vein of the mice at a rate of 0.2 ml per minute. The mice were sacrificed 5, 30 and 60 min and 6 hrs after the injection. Small pieces of the liver were removed and fixed for 2 hrs in 1% osmium tetroxide buffered with 0.1 M phosphate at pH 7.4 (MILLONIG, 1962). The materials were dehydrated in a series of graded ethanol and embedded in Epon epoxy resin (LUFT, 1961).

Thin sections were cut with glass knives on a Porter-Blum MT-1 ultramicrotome, stained with 2% uranyl acetate in distilled water for 5 min and with lead citrate (REYNOLDS, 1963) for 15 min and examined in a JEM-7A type electron microscope with an accelerating voltage of 80 kV.

Observations

1. Five minutes after injection of fat emulsion

In the liver sinusoids of the mouse, the endothelial and Kupffer cells do not rest on a well-defined basement membrane. In the former, fenestrations occur through

Fig. 2. Supranuclear portion of the liver cell 5 min after the injection. The cisternal ends of rough endoplasmic reticulum are elongated and contain a flocculent material. *N* nucleus, *M* mitochondria, *Mb* microbody, *G* Golgi apparatus, *BC* bile canaliculus. $\times 11,000$

which non-cellular components of blood can pass freely into the space of Disse (Fig. 1). The liver cells with irregular microvilli face the narrow space of Disse.

Fat particles introduced into the circulation can be readily seen both in the sinusoidal lumen and in the space of Disse (Fig. 1). They are usually round, clear particles varying in diameter between 0.1 and 0.5μ . Considerable amounts of the particles are taken up into the Kupffer cells and endothelial cells by their cytoplasmic projections singly or in clusters and confined to membrane-bounded vacuoles in their cytoplasm (Fig. 1). However, the liver cells do not show any sign of direct uptake of fat. There is no increase in pinocytotic pits containing the fat particles on the surface membrane of liver cells and the surface area contains no vesicle with enclosed fat particles that could be derived from the pits.

Figure 2 shows a part of supranuclear cytoplasm of the liver cell in the same period. Round microbodies which have a dense core or nucleoid, and numerous mitochondria of various shapes are distributed throughout the cytoplasm. Rough endoplasmic reticulum shows parallel arrays of flattened cisternae. Occasionally the ends of cisternae are elongated and contain somewhat flocculent materials in their cavity. Vesicular elements of smooth endoplasmic reticulum appear in the area of the Golgi apparatus. The Golgi apparatus is located in the juxtanuclear portion or near the bile canaliculi. Lysosomes mostly occur near the bile canaliculi or the Golgi apparatus.

Fig. 3. Supranuclear portion of the liver cell 30 min after the injection. Numerous osmiophilic particles (arrows) are seen in the terminal ends of the cisternae of rough endoplasmic reticulum and within the smooth endoplasmic reticulum. *M* mitochondria, *Mb* microbody. $\times 20,000$

2. Thirty and sixty minutes after injection of fat emulsion

These two groups are described together since the changes observed were essentially the same.

The most remarkable change in these stages is marked increase in number of osmiophilic particles in the liver cell cytoplasm (Fig. 3, 4). The vesicular elements of smooth endoplasmic reticulum are increased in number and contain numerous osmiophilic particles. These particles occasionally occur in the smooth surfaced ends of the cisternae of rough endoplasmic reticulum (Fig. 3). Although they are variable in density, their shapes are almost uniform. They measure about 300 to 1,000 Å in diameter. They usually occur singly, not in cluster, in the element of endoplasmic reticulum.

The Golgi apparatus is increased in size and numerous vacuoles of varying sizes derived from its dilated elements are distributed near the bile canaliculi (Fig. 5). These vacuoles also contain many osmiophilic particles of the same sizes as seen in the endoplasmic reticulum but particles of this nature are never noted within the bile capillaries.

The osmiophilic particles are occasionally found in the cytoplasm near the cell surface and also in the space of Disse between the microvilli of the liver cells (Fig. 6, 7).

Large lipid droplets of variable sizes ($0.5-2\mu$) which lack a limiting membrane are frequently found in the cytoplasm of liver cells and they appear to increase moderately both in number and size as the time passed (Fig. 6, 7). However, there is no

Fig. 4. Supranuclear portion of the liver cell 60 min after the injection. Numerous osmiophilic particles (arrows) are seen within the smooth endoplasmic reticulum. *N* nucleus, *M* mitochondria. $\times 20,000$

Fig. 5. Portion of the two liver cells 60 min after the injection. In the region of the Golgi apparatus (G), numerous vacuoles contain osmiophilic particles (300–1000 Å). Note the polarization of the Golgi apparatus toward the bile canaliculus (BC). M mitochondria, Ly lysosome. $\times 18,000$

evidence that the osmiophilic particles are fused into these lipid droplets of large size.

Mitochondria and microbodies do not show any quantitative change, but lysosomes appear to be increased moderately in number in the Golgi area.

3. Six hours after injection of fat emulsion

In the liver cell cytoplasm, the osmiophilic particles are no more found in the endoplasmic reticulum and the space of Disse except for a few remnants in the Golgi area (Fig. 8). However, numerous lipid droplets of large sizes are deposited in the cytoplasm here and there. These droplets are $0.5\text{--}2\mu$ in diameter, usually lack a limiting membrane and occasionally make a close contact with mitochondria (Fig. 8, inset).

In the Kupffer cell cytoplasm, there are large membrane bounded dense bodies near the Golgi apparatus (Fig. 9). The dense bodies are variable in number from cell to cell and the larger ones are usually spherical or oval in shape. Their finely granular matrix exhibits a very dense and dark texture. Sometimes sequestered fat particles are seen in these bodies, where they might be undertaken to become the digesting phagosomes by the lytic process of lysosome.

Fig. 6. Peripheral portion of the liver cell 30 min after the injection. Numerous osmiophilic particles (arrows) are distributed throughout the cytoplasm and they occur also in the space of Disse (DS). Large ($0.5-2\mu$) lipid droplets (LD) are deposited in the cytoplasm. N nucleus, M mitochondria, LD lipid droplet. $\times 18,000$

Fig. 7. Space of Disse (DS) 60 min after the injection. Many osmiophilic particles are seen in the space of Disse (arrows). M mitochondria, LD lipid droplet. $\times 22,000$

Fig. 8. Portion of the liver cell 6 hrs after the injection. Numerous $0.5\text{--}2\mu$ lipid droplets are seen in the cytoplasm. They occasionally make close contact with mitochondria (**inset**). LD lipid droplet, N nucleus, M mitochondria, G Golgi apparatus. $\times 67,000$

Discussion

The mechanism of lipid uptake by various cells has been the concern of a number of investigators.

In the intestinal epithelium, orally ingested lipids are absorbed and appear as triglycerides (chylomicra) in the circulation. Some authors have the opinion that the intestinal epithelium absorbs the lipid by direct uptake (pinocytosis) from the gut lumen (PALAY and KARLIN, 1959; PALAY and REVEL, 1964), whereas others claim that the hydrolysis of lipid occurs in the intestinal lumen by pancreatic lipase and the resulting fatty acids and glycerols diffuse into the mucosal cells where they are re-synthesized to triglycerides (CARDELL, BADENHAUSEN and PORTER, 1967).

Two mechanisms also have been proposed for fat uptake in the liver, i.e., particulate theory and lipolytic theory. Some biochemical studies have suggested that the liver ingests triglycerides as intact molecules (STEIN and SHAPIRO, 1960; OLIVECRONA, 1962ab; OLIVECRONA and BELFRAGE, 1965). Morphologically, ASHWORTH et al. (1960),

Fig. 9. Portion of the Kupffer cell 6 hrs after the injection. Injected fat particles are sequestered within the dense bodies (D) near the Golgi apparatus (G). N nucleus, M mitochondria. $\times 18,000$

PARKS (1962) and TROTTER (1964, 1966) observed an increased number of osmiophilic particles in the liver cell cytoplasm and in the space of Disse after partial hepatectomy or fat meal ingestion and they considered these particles to be lipid being absorbed from the circulation by pinocytosis. These particles, which are in the range of about 300 to 1,000 Å in diameter, have been known to occur in the liver of many species and of certain pathological conditions (BIAVA, 1964; NOVIKOFF, ROHEIM and QUINTANA, 1964; BRUNI and PORTER, 1965; BAGLIO and FARBER, 1965; ASHWORTH, et al., 1965). Several authors inferred that the particles in question were secretory products (lipoproteins) which were synthesized by the liver for export (FAWCETT, 1955; CHANDRA, 1963). Recently, JONES et al. (1967) and HAMILTON et al. (1967) have isolated the particles after fatty acid administration and suggested that they are low density lipoproteins secreted by the liver.

In the present study, although Kupffer cells actively took up the injected lipid by pinocytosis, the liver cells did not show any morphological evidence of direct uptake at the cell surface at any time after the injection. No sooner than 30 min after the injection numerous osmiophilic particles which were similar to those mentioned above appeared in the liver cell cytoplasm and later increased. From these

observations, it seems reasonable to consider that they are formed in the liver cell cytoplasm (lipoproteins) and the injected lipid has been taken into the liver cells by simple diffusion after hydrolysis by lipolytic enzymes.

Recently, FELTS and MAYES (1965), and FELTS (1965) concluded on the basis of their observations in the perfused liver, that the liver plays little or no role in direct uptake and metabolism of triglycerides. From the histochemical studies, some authors have shown that circulating chylomicra are sequestered at the endothelial cell surface in tissue rich in lipolytic enzymes and it appears that their constituent triglycerides are hydrolyzed at this site, then taken up by simple diffusion (MOSKOWITZ and MOSKOWITZ, 1965; BLANCHETTE-MACKIE and SCOW, 1971).

Though some plasma lipoproteins may be synthesized in the intestine, there is general agreement that the liver is the main site of their formation (STEIN and SHAPIRO, 1959; LAURELL, 1959; HAVEL, FELTS and DUYNE, 1962; OLIVECRONA, 1962b). The enzymes necessary for the synthesis of triglycerides in the liver are mostly present in the microsome fraction (WEISS, KENNEDY and KIYASU, 1960; TZUR and SHAPIRO, 1964), which corresponds to the endoplasmic reticulum (PALADE and SIEKEWITZ, 1956). But this biochemical evidence is not valid to determine whether the enzymes are located in the smooth or rough endoplasmic reticulum. It is suggested that the smooth endoplasmic reticulum is the site of synthesis of triglycerides in the intestinal epithelium since they proliferate as fat is absorbed (CARDELL, BADENHAUSEN and PORTER, 1967). In fact, the present study shows 300-1,000 Å osmophilic particles being formed mainly within the vesicles of the smooth endoplasmic reticulum.

However, low but distinct activities of enzymes involved in the triglyceride synthesis have been found in the soluble fraction of the cytoplasm (STEIN and SHAPIRO, 1959) and these enzymes may possibly serve in the formation of the free cytoplasmic lipid droplets which are not enclosed by a membrane. The possibility that a number of the osmophilic particles may coalesce and form the large cytoplasmic droplets as suggested by BAGLIO and FARBER (1965), TROTTER (1964, 1966) and HAMILTON et al. (1967), could not be supported in the present study. It has been also suggested that the enzymes necessary for lipid synthesis might be located on both sides of the membrane of endoplasmic reticulum, thereby allowing fat synthesis to go on simultaneously in the lumen of the endoplasmic reticulum and toward the cytoplasmic matrix (STRAUSS, 1966). These explanations, however, have not been confirmed by any experiment so far.

Whether the endoplasmic reticulum which contains the enzymes necessary for triglyceride synthesis is generated during fat absorption is not clear at present. The apparent increase of smooth endoplasmic reticulum in this observation might suggest the formation of new membrane necessary for triglyceride synthesis, but some authors have considered that the smooth endoplasmic reticulum increases by the loss of ribosomes of the rough endoplasmic reticulum (CARDELL, BADENHAUSEN and PORTER, 1967).

It might be said that the synthesis of the protein moiety of lipoprotein plays an important role as to whether the newly formed triglycerides are incorporated into cytoplasmic lipid droplets or exported into the circulation as lipoprotein.

During the lipoprotein formation, synthesis of the protein components therein

has been shown to increase (RUDERMAN et al., 1968) and if the animals are treated with the inhibitor of protein synthesis, such as puromycin (ROBINSON and SEAKINS, 1962), the formation of lipoproteins is blocked and fatty liver is evoked. Such a protein seems in all likelihood synthesized like other proteins, in the rough endoplasmic reticulum. In the present study, the swelling of rough endoplasmic reticulum and the appearance of flocculent materials in its cavity soon after injection seems to indicate an increased protein synthesis.

Although most of the osmiophilic particles (300–1,000 Å) are observed in the smooth endoplasmic reticulum, they are frequently present at the junction of smooth and rough endoplasmic reticulum, where the protein may conjugate with triglycerides to form lipoprotein particles.

In this study, fatty liver occurred 6 hrs after the injection. This might be caused by the extremely rapid rate of lipid synthesis compared with the rate of synthesis of the protein moiety followed by deposition of triglycerides as large lipid droplets in the liver cell.

Lipoprotein particles have been observed prominently in the Golgi vacuoles of the liver cell 30 min after the injection. CLAUDE (1970) observed that newly synthesized lipoproteins were transported into the Golgi area by the tubulovesicular type of smooth endoplasmic reticulum in the liver cell. The radioautographic study of NEUTRA and LEBLOND (1966) in goblet cells implicated the Golgi apparatus as the site for coupling carbohydrate moieties with other substances. Since the lipoproteins are actually glycolipoproteins (EPSTEIN and BLOCK, 1959; MARSHALL and KUMMEROW,

Fig. 10. Schematic representation of the process of lipid uptake by the liver cell. *FP* fat particle, *FA* fatty acid, *Gly* glycerol, *LP* lipoprotein, *Si* sinusoid, *DS* space of Disse, *K* Kupffer cell, *RBC* red blood cell, *RER* rough endoplasmic reticulum, *SER* smooth endoplasmic reticulum, *G* Golgi apparatus, *BC* bile canaliculus, *M* mitochondria, *N* nucleus.

1962), it seems reasonable that also in the liver cell the carbohydrates are added to the lipoproteins in the Golgi apparatus, thus the recurring passage of lipids through this organelle before export.

Within 30 min after the injection, many osmiophilic particles have been observed in the peripheral cytoplasm of liver cells and the space of Disse. All these particles in the space of Disse are devoid of an enclosing membrane which surround them in the liver cells. Implicated from this is that the enclosing membrane opens to the extracellular space through the plasma membrane, and thereby discharges the lipoproteins to the space of Disse.

The role of the Kupffer cells in the lipid metabolism has been debated. Several investigators have suggested that the Kupffer cells may have a significant role in the uptake and metabolism of lipid from the circulation (MURRAY and FREEMAN, 1951). However, the present study showed that the lipid which was taken up by the Kupffer cells was segregated by a limiting membrane and processed to the cytolysosome, showing no feature suggesting an active role in lipid metabolism.

In an attempt to explain the observations, a schematic representation is proposed on the process of lipid uptake in the liver after administration of fat emulsion (Fig. 10).

The lipid particles introduced into the circulation are hydrolysed to fatty acids and glycerols by the lipolytic enzymes in the blood plasma or on the endothelial surface of the sinusoids. Then they are taken up by the liver cells by simple diffusion. On the contrary, the Kupffer cells directly take up the lipid particles by pinocytosis. In the liver cell cytoplasm, triglycerides are synthesized and released into the cavity of smooth endoplasmic reticulum in association with proteins which are formed by rough endoplasmic reticulum. These complexes (lipoproteins) are transported to the vacuoles of the Golgi apparatus. The Golgi vacuoles containing these complexes migrate to the surface of the liver cells and release their contents into the space of Disse.

Acknowledgment. Sincere thanks are due to Dr. Shigeki MINAKAMI, Professor of Biochemistry, Kyushu University, for his helpful advice on the manuscript.

脂肪乳剤投与後のマウス肝臓の電子顕微鏡的観察

波 多 江 種 宣

人工脂肪乳剤（ファトゲン）をマウスの尾静脈から投与し、それらの肝臓における取り込みを電子顕微鏡で観察した。

投与 5 分後、クプファー細胞は脂肪球をパイノサイトーシスで取り込んだ像が観察されたが、肝細胞ではそのような過程はみられなかった。投与 30 分から 60 分後、たくさんの $300-1,000 \text{ \AA}$ の好オスミウム性の顆粒が肝細胞の粗面小胞体の末端、滑面小胞体およびゴルジ装置に出現した。これらの顆粒はディッセ腔にもまた観察された。投与 6 時間後、これらの顆粒はもはや肝細胞内にはみられなかったが、たくさんの $0.5-2\mu$ の脂肪滴が細胞基質中に蓄積した。

これらの結果より、肝臓における脂肪の取り込みの機構が論じられた。

References

- Ashworth, C. T., V. T. Stembridge and E. Sanders:** Lipid absorption, transport and hepatic assimilation studied with electron microscopy. *Amer. J. Physiol.* 198: 1326-1328 (1960).
- Ashworth, C. T., F. Wrightsman, B. Cooper and N. R. DiLuzio:** Cellular aspects of ethanol-induced fatty liver. A correlated ultrastructural and chemical study. *J. Lipid Res.* 6: 258-267 (1965).
- Baglio, C. M. and E. Farber:** Reversal by adenine of the ethionine-induced lipid accumulation in the endoplasmic reticulum of the rat liver. A preliminary report. *J. Cell Biol.* 27: 591-602 (1965).
- Belfrage, P.:** Metabolism of chyle triglycerides in the liver. 1. Studies of the mechanism for liver uptake of intravenously injected, glycerol- and fatty acids-labelled chyle in the carbohydrate-fed rat. *Biochim. biophys. Acta* 125: 474-484 (1966).
- Biava, C. G.:** Studies of cholestasis. A re-evaluation of the fine structure of normal human bile canaliculi. *Lab. Invest.* 13: 840-864 (1964).
- Blanchette-Mackie E. J. and R. O. Scow:** Sites of lipoprotein lipase activity in adipose tissue perfused with chylomicrons. Electron microscope cytochemical study. *J. Cell Biol.* 51: 1-25 (1971).
- Bruni, C. and K. R. Porter:** The fine structure of the parenchymal cell of the normal rat liver. *Amer. J. Pathol.* 46: 691-754 (1965).
- Cardell, R. R. Jr., S. Badenhausen and K. R. Porter:** Intestinal triglyceride absorption in the rat. An electron microscopical study. *J. Cell Biol.* 34: 123-156 (1967).
- Chandra, S.:** Electron microscopy of hamster liver. 1. Morphology of secretion. *J. Microsc.* 2: 297-308 (1963).
- Claude, A.:** Growth and differentiation of cytoplasmic membrane in the course of lipoprotein granule synthesis in hepatic cell. 1. Elaboration of elements of Golgi complex. *J. Cell Biol.* 47: 745-766 (1970).
- Epstein, F. H. and W. D. Block:** Glycoprotein content of serum lipoproteins. *Proc. Soc. Exp. Biol. Med.* 101: 740-742 (1959).
- Fawcett, D. W.:** Observation on the cytology and electron microscopy of hepatic cells. *J. Nat. Cancer Inst.* 15: 1475-1502 (1955).
- : Morphological consideration of lipid transport in the liver. In: (ed. by) H. Meng: *Proceedings of an intestinal symposium on lipid transport.* Springfield, Charles C. Thomas, 1964. (P. 210-222).
- Felts, J. M.:** The mechanism of triglyceride fatty acids by perfused rat livers and by intact rats. *Ann. New York Acad. Sci.* 131: 24-33 (1965).
- Felts, J. M. and P. A. Mayes:** Lack of uptake and oxidation of chylomicron triglyceride to carbon dioxide and ketone bodies by perfused rat liver. *Nature* 206: 195-196 (1965).
- Hamilton, R. L., D. M. Regen, M. E. Gray and V. S. Le-Quire:** Lipid transport in the liver. 1. Electron microscopic identification of very low density lipoproteins in perfused rat liver. *Lab. Invest.* 16: 305-319 (1967).
- Havel, R. J., J. M. Felts and C. M. Duyne:** Formation and fate of endogenous triglycerides in blood plasma of rabbits. *J. Lipid Res.* 3: 297-308 (1962).
- Jones, A. L., N. B. Ruderman and M. G. Herrera:** Electron microscopic and biochemical study of lipoprotein synthesis in the isolated perfused rat liver. *J. Lipid Res.* 8: 429-446 (1967).
- Kinsell, L. W., G. D. Michaels and K. Imaichi:** Studies with fat emulsion. Metabolism of intravenously administered C¹⁴-tripalmitin. *Amer. J. clin. Nut.* 16: 97-100 (1965).
- Laurell, S.:** Recycling of intravenously injected palmitic acid-1-C¹⁴ as esterified fatty acid in the plasma of rats and turnover rate of plasma triglycerides. *Acta physiol. scand.* 47: 218-232 (1959).

- Luft, J. H.:** Improvements in epoxy resin embedding methods. *J. biophys. biochem. Cytol.* 9: 409-414 (1961).
- Marshall, W. E. and F. A. Kummerow.:** The carbohydrate constituents of human serum β -lipoprotein: galactose, mannose, glucosamine and sialic acid. *Arch. Biochem. Biophys.* 98: 271-273 (1962).
- Millonig, G.:** Further observations on a phosphate buffer for osmium solutions in fixation. In: *Electron microscopy (Proc. 5th Int. Congr. Electron Microscopy)*. New York, Academic Press, 1962 (Vol. 2, P. 8).
- Moskowitz, M. S. and A. A. Moskowitz:** Lipase. Localization in adipose tissue. *Science* 149: 72-73 (1965).
- Murray, R. G. and S. Freeman:** The morphologic distribution of intravenously injected fatty chyle and artificial fat emulsion in rats and dogs. *J. Lab. clin. Med.* 38: 56-69 (1951).
- Neutra, M. and C. P. Leblond:** 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: 119-136 (1966).
- Novikoff, A. B., P. S. Roheim and N. Quintana:** Lipid in the liver cells of rats fed orotic acid and adenine. *Fed. Proc.* 23: 126 (1964).
- Olivecrona, T.:** Metabolism of chylomicrons labelled with C^{14} -glycerol- H^3 -palmitic acid in the rat. *J. Lipid Res.* 3: 439-444 (1962a).
- : The metabolism of 1- C^{14} -palmitic acid in the rat. *Acta physiol. scand.* 54: 295-305 (1962b).
- Olivecrona, T. and P. Belfrage:** Mechanism for removal of chyle triglyceride from the circulating blood as studied with (C) glycerol and (H) palmitic-acid-labelled chyle. *Biochem. biophys. Acta* 98: 81-93 (1965).
- Palade, G. E. and P. Siekewitz:** Liver microsome. An integrated morphological and biochemical study. *J. biophys. biochem. Cytol.* 2: 171-200 (1956).
- Palay, S. L. and L. J. Karlin:** An electron microscopic study of the intestinal villus. 2. The pathway of fat absorption. *J. biophys. biochem. Cytol.* 5: 373-384 (1959).
- Palay, S. L. and J. P. Revel:** The morphology of fat absorption. In: (ed. by) H. Meng: *Proceedings of an intestinal symposium on lipid transport*. Springfield, Charles C. Thomas, 1964. (P. 33-43).
- Parks, H. F.:** On the uptake of chylomicrons by hepatic cells of mice. *Anat. Rec.* 142: 320 (1962).
- Reynolds, E. S.:** The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 17: 208-212 (1963).
- Robinson, D. S.:** The uptake and release of lipids by the liver. In: (ed. by) H. Meng: *Proceedings of an intestinal symposium on lipid transport*. Springfield, Charles C. Thomas, 1964. (p. 194-201).
- Robinson, D. S. and A. Seakins:** The development in the rat of fatty livers associated with reduced plasma-lipoprotein synthesis. *Biochim. biophys. Acta.* 62: 163-165 (1962).
- Ruderman, N. B., K. C. Richards, V. Valles de Bourges and A. L. Jones:** Regulation of production and release of lipoprotein by the perfused rat liver. *J. Lipid Res.* 9: 613-619 (1968).
- Stein, O. and Y. Stein:** The role of the liver in the metabolism of chylomicrons, studied by electron microscopic autoradiography. *Lab. Invest.* 17: 436-446 (1967).
- Stein, Y. and B. Shapiro:** Assimilation and dissimilation of fatty acids by the rat liver. *Amer. J. Physiol.* 196: 1328-1241 (1959).
- : Uptake and metabolism of triglycerides by the rat liver. *J. Lipid Res.* 1: 326-331 (1960).
- Strauss, E. W.:** Electron microscopic study of intestinal fat absorption in vitro, from mixed micelles containing linolenic acid, monoolein, and bile salt. *J. Lipid Res.* 7: 307-323 (1966).
- Trotter, N. L.:** A fine structure study of lipid in mouse liver regenerating after partial hepatectomy. *J. Cell Biol.* 21: 233-244 (1964).
- : A fine structure study of mouse liver after partial hepatectomy and fasting. In: (ed. by)

R. Uyeda: Electron microscopy 1966 (6th Int. Congr. Electron Microscopy), Tokyo, Maruzen Co., 1966. (Vol. II, P. 627-628).

Tzur, R. and B. Shapiro: Dependence of microsomal lipid synthesis on added protein. J. Lipid Res. 5: 542-547 (1964).

Weiss, S. B., E. P. Kennedy and J. Y. Kiyasu: The enzymatic synthesis of triglycerides. J. Biol. Chem. 235: 40-44 (1960).

波多江 種 宣
〒812 福岡市東区堅粕1276
九州大学医学部
解剖学第1教室

Dr. Tanenori HATAE
Department of Anatomy
Kyushu University Faculty of Medicine
Fukuoka, 812 Japan