HISTOCHEMICAL STUDIES ON FAT METABOLISM BY INTRAVENOUS ADMINISTRATION OF FATTY CHYLE

by

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I. INTRODUCTION

Previously, Asada and Izukura in our laboratory had carried out histochemical studies on the metabolic process of fat with a fat emulsion, which was produced in our laboratory and could be safely given intravenously. The fat emulsion was infused intravenously into cats, mice and rabbits, and it was established that the infused neutral fat globules were first phagocytized by the reticuloendothelial cells in the lung, liver and spleen and then entered into the hepatic parenchymatous cells, where they were oxidized, in the form of phospholipid after lipoidization of neutral fat by the former cells. Furthermore, from the fact that the intravenously infused fat emulsion showed morphologically analogous characteristics to those of the orally administered fat or of those in the hunger state, Asada emphasized that the intravenous infusion of fat in emulsified form such as we have done was really a valid method of parenteral nutrition of fat.

Thereupon, the author attempted the following experiments for the purpose of studying the problem of whether or not the fat infused intravenously in an emulsified form would be handled by the body in exactly the same manner as the fat of chyle absorbed physiologically from the intestinal tube.

II. MATERIALS AND METHODS

(A) Materials

1) Fat by Oral Administration: In the present investigation, 15 per cent cod liver oil emulsion and 15 per cent sesame oil emulsion which had been produced in our laboratory were used. These fat emulsions contained 7 per cent glucose and small quantities of stabilizers. Their principal fat component was neutral fat.

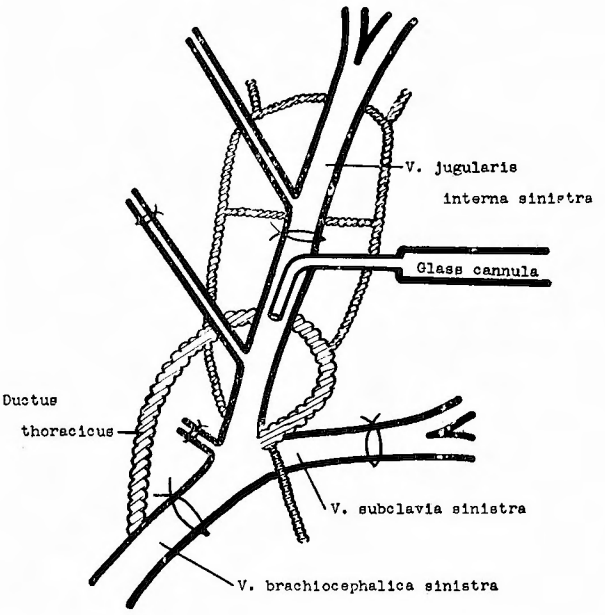
2) Experimental Animals: Adult cats representing carnivores whose fat absorption and fat disposal are vigorous and each weighing approximately 3 kg, were used.

(B) Methods

1) Method of Collecting Chyle: The adult cats in the post-absorptive state received the fat emulsion by stomach tube at a rate of about 4 g of fat per kg of body weight. An hour and a half after oral administration, chyle was collected under local anesthesia (about 3 cc of 0.05 per cent nupercain solution) by the indirect

method shown in Fig. 1. Namely, a 4 cm long incision was made in the left supraclavicular fossa, and the left jugular vein, the left subclavian vein, the superior vena cava and several veinlets emptying into them were isolated and ligated. Then, a glass cannula was inserted into the left jugular vein at the central portion from its ligated point. If the thoracic duct of the cat were as large as that of the dog, a polyethylene tube could be directly inserted into the thoracic duct. In cats of about 3 kg of body weight, however, such a direct cannulation of the thoracic duct is often difficult. Also, on account of various variations in the anatomical arrangement of the thoracic duct, chyle was usually collected by the indirect method as above mentioned.

Fig. 1 Method of Collecting Chyle



The cats were maintained on 100,000 units of oil penicillin injected subcutaneously before operation. In order to keep the collected chyle sterilized, the glass cannulae and the collecting flasks were sterilized beforehand and about 1,000 units of crystalline penicillin per 10 cc of chyle were added to the collecting flasks. These flasks were placed immediately in the icebox after one hour's flow of chyle had been collected. Within several hours after filtration through gauze, the chyle could never coagulate without the addition of heparin. Recently, it has been advocated that heparin has the cleaning action of lipemia. Consequently, chyle was used without heparin after it had been gently expressed from the clot through several layers of gauze into a beaker. It took about two to three hours to collect a necessary dose of chyle. The cats were set at liberty except during the operating and collecting periods, so as to enable them to return to the normal state as soon as possible.

2) Fat Components of Chyle: The average amount of each fat component in cat chyle following oral administration of the fat emulsion is shown in Table I.

The quantitative determination of each fat component was made by the fol-

Table I Fat Components in Cat Chyle Following Oral Administration of Fat Emulsions

Fat fed		Total lipids	Glyceride	Phospholipid	Cholesterol
Cod liver oil	mg/dl	5847	4465	873	509
	%	100	76.4	14.9	8.7
Sesame oil	mg/dl	6615	5083	949	583
	%	100	77.8	14.3	8.8

lowing methods; the method of VAN DE KAMER A and B for total fatty acid and glyceride, the method of BLOOR for phospholipid and the method of RAPPAPORT for cholesterol.

3) Method of Injection and Dose of Chyle: Essentially, the fat content of each organ is closely connected with the sort of food and the time of administration. Especially, when the same cats are used for the collection and intravenous injection of chyle, as in the present investigation, the effect of ingested fatty food and starvation must be thoroughly considered. Accordingly, after the cats had been fed with a definite diet for a week or more prior to examination, they were given the fat emulsion orally in the post-absorptive state. The thoracic duct was left alone after collection of chyle, lest the fat absorbed through the thoracic duct should flow into the blood stream. A definite quantity of the collected chyle was reinjected intravenously into the same cats from which the chyle had been collected and the cats were sacrificed 24 hours after oral administration. From the preliminary examinations, we found that at 24 hours after oral administration of the fat emulsion, the fat content of each organ was hardly affected by the oral administration.

The chyle was allowed to come to room temperature before injection and was administered through the subcutaneous vein of the hind leg. The chyle was analyzed for total fatty acids by the method of VAN DE KAMER before injection and the dose injected was equivalent to 0.5 g of fat per kg of body weight.

4) Preparation of Histological Specimens: After injection, the cats were sacrificed successively by bleeding at definite intervals. The tissues of the lung, liver and spleen for sectioning were placed at once in 10 per cent formalin neutralized with $CaCO_3$ or BAKER's solution for a week or more. In all cases carbowax-embedding was employed. The thickness of the sections was defined as 12μ in the lung and 6μ in the liver and spleen.

5) Staining Methods: In the present study, GOLDMANN's Sudan III method (ASADA's modification), Oil red O method and SMITH-DIETRICH's method were employed. In case of necessity BAKER's method was used

III. OBSERVATIONS

In the descriptions that follow, the term "chyle A" always refers to the fatty chyle collected after the oral administration of cod liver oil emulsion; the term "chyle B" refers to the fatty chyle collected after the oral administration of sesame oil emulsion, the preparations of which will be described briefly hereafter.

(A) Intravenous Administration of Chyle A (Table II).

The chyle was injected at a rate of 0.5 g of fat per kg of body weight and the cats were sacrificed successively by bleeding at definite intervals (10, 30 minutes, 1, 2, 3, 6, 24 hours).

1) The lung

Ten minutes after injection, the fat globules of the injected chyle were almost absent in the capillaries of the lung. These 10 minute cases showed that the fat globules were phagocytized by numerous so-called "alveolar phagocytes" (Fig. 2).

Table II Changes of Fat Content in Each Organ Following Intravenous Injection of Chyle A.

Intervals after injection	Lung	Liver		Spleen	
		Kupffer's cells	Hepatic cells	Macrophages	Ellipsoids
10 minutes	++	++	++	++	++
30 minutes	++	++	+	+	+
1 hour	+	+	−	±	−
2 hours	±	±	−	±	−
3 hours	±	±	+	−	+
6 hours	−	−	−	−	−
24 hours	−	−	−	−	−

However, the number of the alveolar phagocytes which had taken the fat globules was somewhat less than the case of the artificial fat emulsion injected in similar quantity and manner. In the 30 minute cases, the alveolar phagocytes taking the fat globules were slightly less in number than in the 10 minute cases, decreasing gradually in number, and almost entirely disappeared in the 3 hours cases. Most of these alveolar phagocytes existed in the interalveolar septa, but some of them, either singly or in a group of 5 to 10 cells, were found in the alveolar spaces separating from the alveolar wall up to 24 hours after injection.

In the 10 minute cases, the phagocytized fat globules were found to fill up the cytoplasma of the hypertrophied alveolar phagocytes (Fig. 3). These fat globules were somewhat gross and produced a red colour when stained with Oil red O or Sudan III, and they clearly showed the characteristics of glycerides. Afterwards, the phagocytized fat globules not only increased gradually their yellow tone as a result of the Oil red O or Sudan III stain, but they also fined away and came to indicate a positive reaction to Smith-Dietrich's lipoid stain with the passage of time (Fig. 4). In other words, these fat globules came to indicate histochemically the character of phospholipid. It appeared that the glyceride of the injected chyle changed to phospholipid in the alveolar phagocytes.

2) The liver

Fat globules of the injected chyle were hardly found in the hepatic sinusoids in the 10 minute cases. In the liver, the chyle fat appeared in the Kupffer's stellate cells and the hepatic parenchymatous cells.

a) The Kupffer's stellate cells

In the 10 minute cases, the fat globules of the injected chyle were phagocytized by numerous Kupffer's cells, especially, at the peripheries of the hepatic lobules (Fig. 5). The number of the Kupffer's cells which had eaten the fat globules was somewhat smaller than that of the alveolar phagocytes previously mentioned, but was nearly equivalent to that in the cases of the intravenous infusion of artificial fat emulsion. The Kupffer's cells eating the fat globules decreased slightly in number 30 minutes after injection, diminishing gradually in number thereafter, and after 3 hours almost disappeared entirely.

In the 10 minute cases, the cytoplasma of the Kupffer's cells was full of nume-

rous larger fat globules. Accordingly, the KUPFFER's cells were hypertrophied and the nuclei were excentrically pressed (Fig. 6). In the 10 minute cases, the phagocytized fat globules turned red by staining with Oil red O or Sudan III and showed glyceride characteristics. Thereafter, these fat globules not only gradually increased their yellow tone resulting from the Oil red O or Sudan III stain, but also became fine and indicated a positive reaction to SMITH-DIETRICH's lipoid stain with the passage of time (Fig. 7). Namely, these fat globules came to indicate histochemically a phospholipid character in the KUPFFER's cells as they had in the alveolar phagocytes. Therefore, we could not help thinking that the glyceride of the injected chyle changed to phospholipid within the KUPFFER's cells as well. The lipoidization of glyceride was evident in the margins of the cytoplasma of the KUPFFER's cells.

b) The hepatic parenchymatous cells

Already in the 10 minute cases, the substance indicating a positive reaction to the SMITH-DIETRICH's lipoid stain could be diffusely recognized within the hepatic parenchymatous cells, especially, at the peripheries of the hepatic lobules (Fig. 8). These hepatic cells also appeared reddish yellow in colour as a result of the Oil red O or Sudan III stain. Namely, these facts indicate that a large amount of phospholipids had entered diffusely into the hepatic parenchymatous cells.

In the 30 minute cases, the phospholipid which appeared diffusely in the hepatic cells decreased fairly much in quantity (Fig. 9) and once entirely disappeared in the 1 and 2 hour cases. In the 3 hour cases, the phospholipid appeared again diffusely in these cells, though it was less than that in the 10 and 30 minute cases (Fig. 10). In cases of 6 hours or more, the phospholipid was not recognized in the above mentioned cells.

After an intravenous injection of the chyle, the glyceride of the chyle could not be found to infiltrate directly into the hepatic parenchymatous cells. As mentioned above, however, the phospholipid appeared biphasicly in these cells. This fact was a peculiar point of difference from the experimental results of the relation between the chyle and the artificial fat emulsion.

3) The spleen

The fat globules of the injected chyle were almost absent in the splenic sinusoids and the so-called "marginal zone" (this zone is the region of the red pulp immediately adjacent to the white pulp, the Knötchenrandzone of WEIDENREICH) already in the 30 minute cases. In the spleen, the injected chyle fat appeared in the macrophages of the red pulp and in the ellipsoids (sheathed arteries).

a) The macrophages of the red pulp

In the 10 minute cases, the fat globules of the injected chyle were eaten by numerous macrophages (Fig. 11). The number of the macrophages eating the fat globules was nearly equivalent to that of the cases of the intravenous infusion of the artificial fat emulsion. Thereafter, these macrophages diminished gradually in number, and in the 3 hour cases, they almost disappeared.

Like in the alveolar phagocytes and in the KUPFFER's stellate cells, it could be found that the phagocytized glyceride of the chyle had changed to phospholipid in

the macrophages mentioned above.

b) The ellipsoids (sheathed arteries)

In the 10 and 30 minute cases, the fine fat granules which showed a reddish yellow colour on staining with Oil red O or Sudan III, and they also indicated a positive reaction to SMITH-DIETRICH's lipoid stain, phospholipids could be recognized in large amounts in the reticular cells of the ellipsoids (Fig. 12). Though the phospholipid in the ellipsoids disappeared completely in the 1 and 2 hour cases, it appeared again to a slight degree in the 3 hour cases (Fig. 13) and disappeared again in the cases of 6 hours or more.

As in the hepatic parenchymatous cells, the same phospholipid appeared biphasicly in the reticular cells of the ellipsoids; the rise and fall of the phospholipid in the course of time being coincident with that in the hepatic cells.

4) The leucocytosis

After injection of the chyle, there appeared a transitory but remarkable leucocytosis in the blood vessels in the said organs, as in the cases of the intravenous infusion of the artificial fat emulsion. The increase in the number of the leucocytes commenced 30 minutes after injection, and this became more and more conspicuous until 3 hours after, while the leucocyte count gradually returned to normal within 6 hours.

(B) Intravenous Administration of Chyle B (Table III).

Table III Changes of Fat Content in Each Organ Following Intravenous Injection of Chyle B.

Intervals after injection	Lung	Liver		Spleen	
		KUPFFER'S cells	Hepatic cells	Macrophages	Ellipsoids
10 minutes	++	++	+	++	+
30 minutes	++	++	±	+	±
1 hour	+	+	−	±	−
2 hours	±	±	−	±	−
3 hours	±	±	±	−	±
6 hours	−	−	−	−	−
24 hours	−	−	−	−	−

Previously, IZUKURA had compared the histochemical findings in cases of the intravenous infusion of sesame oil emulsion with those of cod liver oil emulsion and he found that there was an obvious difference in metabolic process between them. Furthermore, he has clarified that the above difference is due to the sort of fatty acids which are contained within these emulsions, and that the sesame oil emulsion containing only long chain fatty acids without highly unsaturated fatty acids can also be utilized far more rationally in the body than the cod liver oil emulsion containing large quantities of highly unsaturated fatty acids. Thereupon, the author performed similar experiments on chyle B for the purpose of clarifying the problem of whether the same difference can be shown or not, when the chyle collected by oral administration of these both fat emulsions is intravenously injected.

1) The fat globules of the injected chyle in the blood vessels

Ten minutes after injection, the fat globules of the injected chyle were scarcely found in the pulmonary capillaries, hepatic sinusoids, splenic sinusoids and the marginal zone of the splenic nodules in a free state, which fact would suggest that they rapidly disappeared from the blood stream.

2) The reticuloendothelial cells

The fat globules of the injected chyle were obviously phagocytized by the reticuloendothelial cells in the lung, liver and spleen; the so-called "alveolar phagocytes", the KUPFFER's cells and the macrophages in the spleen. The number and manner of these reticuloendothelial cells taking the fat globules were exactly similar to those in cases of the intravenous infusion of chyle A. The time necessary for the disappearance of the fat from these cells was also about 3 hours after injection. Namely, after the phagocytized glyceride of the injected chyle gradually changed to phospholipid in these cells with the passage of time, it disappeared from these cells.

3) The hepatic parenchymatous cells

The rise and fall in the course of time of the phospholipid which appeared in the hepatic parenchymatous cells was biphasic as in cases of the intravenous injection of chyle A. But the quantity of the phospholipid in these cells was far less as compared with that in cases of the intravenous injection of chyle A. Namely, 10 minutes after, the phospholipid could be diffusely recognized within the hepatic cells at the peripherics of the hepatic lobules (Fig. 14). This decreased fairly much 30 minutes after and once disappeared entirely after 1 and 2 hours. Then 3 hours after, the phospholipid appeared again in the same cells, though to a slight degree (Fig. 15).

4) The ellipsoids

As in the hepatic parenchymatous cells, the rise and fall in the course of time of the phospholipid which appeared in the reticular cells of the ellipsoids was biphasic and also the phospholipid in these cells was far smaller in quantity as compared with that in cases of the intravenous injection of chyle A.

5) The leucocytosis

Leucocytosis was transitory proved after injection of chyle B. The degree and the rise and fall in the course of time of the leucocytosis were exactly similar to those in cases of the intravenous injection of chyle A.

(C) Simultaneous Injection of Chyle A with Methionine (Table IV).

Table IV Changes of Fat Content in Each Organ Following Simultaneous Injection of Chyle A with Methionine.

Intervals after injection	Lung	Liver		Spleen	
		KUPFFER's cells	Hepatic cells	Macrophages	Ellipsoids
10 minutes	++	++	±	+	±
30 minutes	+	+	−	+	−
1 hour	±	±	−	±	−
3 hours	−	−	−	−	−

Recently, since the so-called "lipotropic action" of methionine came to be noticed,

ASADA and others in our laboratory have demonstrated histo- and biochemically the facts that methionine accelerates lipoidization of neutral fat by reticuloendothelial cells and secondarily expedites fatty acid oxidation in tissues. Thereupon, the author examined the problem as to whether such actions of methionine could be seen or not in regard to chyle. Chyle A was intravenously injected at a rate of 0.5 g of fat per kg body weight in combination with *l*-methionine, 5 mg per 0.5 g of fat. The cats were sacrificed successively by bleeding at definite intervals (10, 30 minutes, 1, 3 hours).

Similar to the above cases without methionine, it was recognized that the fat globules of the injected chyle were phagocytized by the reticuloendothelial cells and changed to phospholipid in these cells. The lipoidization of glyceride in these cells, however, was found in that the greater part of the phagocytized fat globules had already changed to phospholipid 10 to 30 minutes after injection. Accordingly, the phospholipid in these cells disappeared almost 1 hour later. The phospholipid which appeared in the hepatic parenchymatous cells and ellipsoids was barely recognized in a small quantity only in the 10 minute cases.

IV. DISCUSSION

Nowadays, the former interpretation on the mechanism of fat absorption has entirely been revised. Namely, it is universally believed now that; fat is absorbed from intestinal mucous membrane, after it changes to various mixtures (tri-, di-, monoglycerides and free fatty acids) by the action of intestinal lipase and takes on a completely emulsive form with the help of the bile components. Also, for the absorption of fat, complete hydrolysis is not indispensable, but perfect emulsifying is requisite. The absorbed long chain fatty acids enter mostly into the blood stream through the thoracic duct in the form of triglyceride, while the absorbed short chain fatty acids with under 10 carbon atoms are chiefly conveyed through the portal vein to the liver to be oxidized.

Under the impression that from the above-mentioned point of view, it is ideal to infuse fat in the form of emulsion intravenously in order to achieve the purpose of parenteral nutrition with fat, we have been endeavouring to produce a fat emulsion which will suit to this purpose up to this day. In the first place, we should solve the problem of whether the artificial fat emulsion produced in our laboratory would surely be utilized in the body by quite the same metabolic process as the chyle which is physiologically absorbed through the intestine, in other words, whether the fat emulsion is really utilized by way of the physiological metabolic process or not. However, there have been very few studies on the destiny of the chyle fat.

In regard to the histochemical studies of the chyle intravenously injected, there are none except for some experiments by MURRAY and FREEMAN on rats and dogs in 1951. According to their research, the animals into which chyle was injected were different from the dogs from which chyle was collected in their experiments, and hence we find it difficult to say that their experimental results were quite physiological.

We injected the cat chyle into the same cats from which it was collected. In our experiments, the fat globules of the injected chyle could hardly be found in the pulmonary capillaries, hepatic sinusoids, splenic sinusoids and the marginal zone of the splenic nodules already 10 minutes after injection. As compared with the experimental results of ASADA and IZUKURA in which the artificial fat emulsion was intravenously infused in similar quantity and manner, there was a tendency that the fat globules injected in the form of chyle was cleared from the blood stream more rapidly than the synthetic emulsion.

The fat globules in the injected chyle were obviously phagocytized by the reticuloendothelial cells; the so-called "alveolar phagocytes" in the lung, the KUPFFER's cells in the liver and the macrophages in the spleen. As will be stated later, however, the glyceride of the chyle could never be found to infiltrate directly into the hepatic parenchymatous cells. The fat globules phagocytized by the former cells, in the 10 minute cases, were somewhat gross and produced a red colour after staining with Oil red O or Sudan III, and so all the globules indicated clearly the character of the glyceride, glycerinester of fatty acids. Thereafter, the phagocytized fat globules came to indicate gradually the character of the phospholipid with the passage of time and at 3 hours after injection, the phospholipid disappeared entirely from these cells. Comparing with ASADA's and IZUKURA's experimental results which were obtained by the intravenous infusion of an equivalent quantity of the artificially emulsified fat, the number of the reticuloendothelial cells, especially the alveolar phagocytes, which had taken the fat globules, was somewhat smaller. In this connection, though conceivably the possibility that a part of triglycerides of long chain fatty acids can be stored directly in fat depots, as established by LERNER and GEYER etc., this is perhaps based on the facts that the majority of the fat contained in our fat emulsion is glyceride, whereas the glyceride contained in the chyle is about 75 per cent.

In the present experiments on the intravenous injection of the chyle, the evidence that the glyceride contained in the chyle infiltrated directly into the hepatic parenchymatous cells was never found. In the 10 minute cases, however, large amounts of phospholipid could be diffusely recognized within the hepatic parenchymatous cells, especially, at the peripheries of the hepatic lobules. In the 30 minute cases, the phospholipid which appeared diffusely in the hepatic cells decreased fairly much in quantity and once entirely disappeared in the 1 and 2 hour cases. In the 3 hour cases, the phospholipid appeared again in the same cells, though it was less than that of the 10 minute cases. In the cases of 6 or more hours, the phospholipid was never recognized in the above cells. Namely, when the chyle was intravenously injected, the phospholipid appeared biphasicly in the hepatic cells. This fact was a peculiar point which differs from the experimental results of the intravenous infusion of the fat emulsion.

Regarding the phospholipid which appeared in the early intervals such as the 10 or 30 minute cases, it can be presumed from the viewpoint of the fact that the chyle contains about 15 per cent phospholipid, that the phospholipid of the chyle

infiltrated directly into the hepatic parenchymatous cells in large quantities immediately after injection. In recent years, FISHLER and his co-workers have established that after the intravenous injection of P^{32}- and C^{14}- labeled phospholipid, the greater part of the phospholipid is taken by the hepatic cells and a part is taken by the spleen, kidney and intestine etc. in this form. In the present study, not only in the hepatic cells, but also in some part of the red pulp of the spleen, especially in the ellipsoids, the phospholipid contained in the chyle appeared.

On the contrary, as regards the phospholipid which appeared in the hepatic cells 3 hours after injection, from the viewpoint of the time of its appearance, we can not help considering that the glyceride of the chyle entered into the hepatic parenchymatous cells in the form of phospholipid after lipoidization of the glyceride by the above-mentioned reticuloendothelial cells and then the movement of the phospholipid to the hepatic cells reached the climax 3 hours after injection. These findings were quite identical with those in cases of the intravenous infusion of the artificial fat emulsion. It is not too much to say that it could be demonstrated that the fat contained in our fat emulsion was utilized in the body through the same metabolic process as the glyceride contained in the chyle which was physiologically absorbed. MURRAY reported, on his study of the intravenous injection of chyle, that the injected chyle fat never appeared in the reticuloendothelial cells and thus all chyle fat entered into the hepatic parenchymatous cells directly and rapidly not in the form of phospholipid but in that of glyceride. Because in his study, only Oil red O stain was employed and the same dog was repeatedly biopsied at the immediate, 3, 6 and 24 hours intervals after injection of chyle, it seems that only the fat globules appeared in the hepatic cells at the earliest intervals were found and so the other fat was passed over. On account of the facts that the fat globules appeared in the above cells at the early interval indicated obviously the character of a phospholipid histochemically, though they showed a reddish yellow colour from the Oil red O stain, and that for the most part, at least, the glycerides contained in the chyle were eaten by the reticuloendothelial cells. It is quite unthinkable that all chyle fat appears directly in the hepatic parenchymatous cells as MURRAY had mentioned.

The findings described above were found after intravenous injection of either chyle A or chyle B. But the point of difference between both chyles is that any phospholipid appeared in the hepatic parenchymatous cells at the 10, 30 minute, and 3 hour intervals after injection of chyle B was far less in quantity as compared with that after the injection of chyle A. The reason why such a difference occurred, should be presumed to be due to the point of difference that while the cod liver oil emulsion contains comparatively large quantities of highly unsaturated fatty acids, the sesame oil emulsion does not contain such highly unsaturated fatty acids, but contains only triglycerides of long chain fatty acids, such as linoleic, oleic, stearic, palmitic and a small quantity of myristic acid etc.. Namely, while all highly unsaturated fatty acids enter at once into the hepatic parenchymatous cells in the form of phospholipid and can be oxidized in these cells, long chain fatty acids not only

enter the liver but also the extrahepatic tissues in the form of phospholipid and can be oxidized in these cells. Conversely, from the fact that the phospholipid appeared in the hepatic parenchymatous cells after the intravenous injection of chyle A was always far more in quantity as compared with that in the cases of chyle B. We can not help but realize that highly unsaturated fatty acids contained in such fat as cod liver oil can be absorbed in the thoracic duct, as well.

From the above findings, it is also plain that the sesame oil emulsion containing only triglycerides of long chain fatty acids without highly unsaturated fatty acids can be utilized far more logically in the body than the cod liver oil emulsion containing comparatively large amounts of highly unsaturated fatty acids. From the viewpoint not only of adverse reaction, as ascertained by OTANI, but also of oxidation, it is needless to say that in order to achieve the purpose of parenteral nutrition with fat by infusing fat intravenously in the emulsified form, a fat containing only triglycerides of long chain fatty acids, such as sesame oil, should be used as its raw material, as asserted by IZUKURA and OSA.

In spite of the striking lipoidization of glyceride in the reticuloendothelial cells, the phospholipid produced from glyceride in these cells could hardly be recognized as entering the hepatic parenchymatous cells, in the cases of the simultaneous infusion with the chyle and methionine. Furthermore, at the 10 or 30 minute intervals after injection, when the phospholipid contained in the chyle seemed to have entered rapidly and directly into the hepatic parenchymatous cells, the phospholipid which appeared in these cells was very small in quantity as compared with that in the cases of the intravenous infusion of chyle A alone. From these observations, as demonstrated by researchers of our laboratory, I can not help thinking that methionine accelerates not only the lipoidization of glyceride in the reticuloendothelial cells, but also the oxidation process of fatty acids in tissues.

V. SUMMARY AND CONCLUSIONS

The author intravenously injected cats with chyle collected from the same cats at certain intervals after the oral administration of fat emulsion produced in our laboratory, and examined histochemically the metabolic process of the fat of the injected cat chyle, and reached the following conclusions:

1) The cat chyle contains relatively large quantities (about 15 per cent) of phospholipid. The phospholipid contained in the injected chyle can enter into the hepatic parenchymatous cells and the ellipsoids of the spleen directly and rapidly.

2) The glyceride contained in the injected chyle is firstly phagocytized by the reticuloendothelial cells and enters gradually into the hepatic parenchymatous cells and the ellipsoids of the spleen in the form of phospholipid after lipoidization of glyceride by the former cells, and then the movement of the phospholipid to the latter cells reaches its climax at an interval of 3 hours after injection.

3) Accordingly, when the chyle is intravenously injected, the phospholipid appears biphasicly in the hepatic parenchymatous cells and the ellipsoids.

4) It seems that while highly unsaturated fatty acids enter only into the liver in the form of phospholipid to be oxidized, long chain fatty acids without highly unsaturated fatty acids not only enter the liver but also the extrahepatic tissues in the same form to be oxidized.

5) It can be surmised even from these histochemical findings that also highly unsaturated fatty acids can undoubtedly be absorbed in the thoracic duct.

6) Methionine accelerates lipoidization of glyceride by the reticuloendothelial cells and secondarily expedites fatty acid oxidation, at least, in the hepatic parenchymatous cells.

7) From the above histochemical findings, the author has reached the following conclusions: The fat contained in our artificial fat emulsion is utilized through the same metabolic process as the glyceride contained in the chyle which is physiologically absorbed. In order to achieve the purpose of parenteral nutrition with fat by infusing fat intravenously in the emulsified form, a fat containing only triglycerides of long chain fatty acids without highly unsaturated fatty acids, such as sesame oil, should be used as its raw material.

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Figures

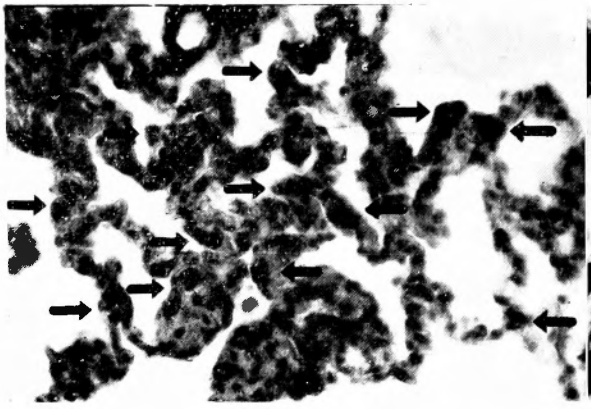

Fig. 2. Lung 10 minutes after injection of chyle A. Many alveolar phagocytes are seen to have taken the fat globules. (Oil red O stain, × 400)

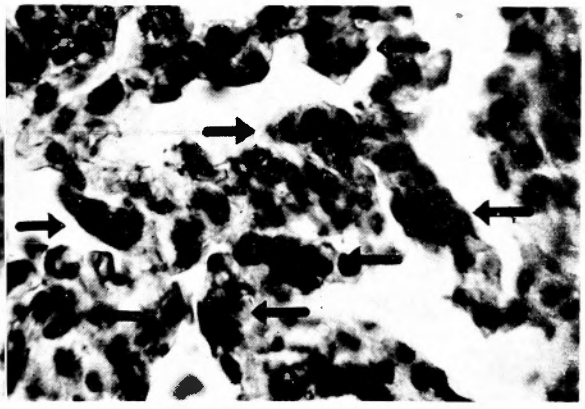

Fig. 3. The magnification of Fig. 2. The alveolar phagocytes are filled with larger fat globules. (Oil red O stain, × 900)

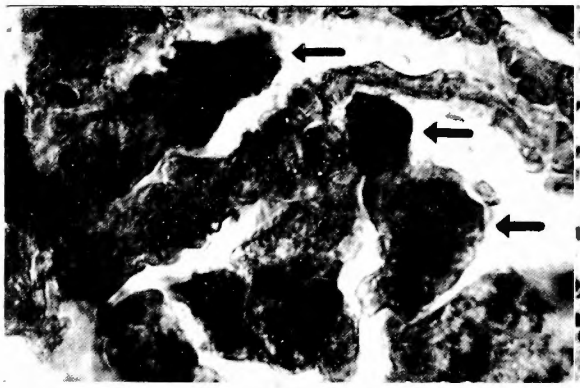

Fig. 4. Lung 2 hours after injection of chyle A. Phospholipd is visible in the alveolar phagocytes. (SMITH-DIETRICH's stain, × 900)

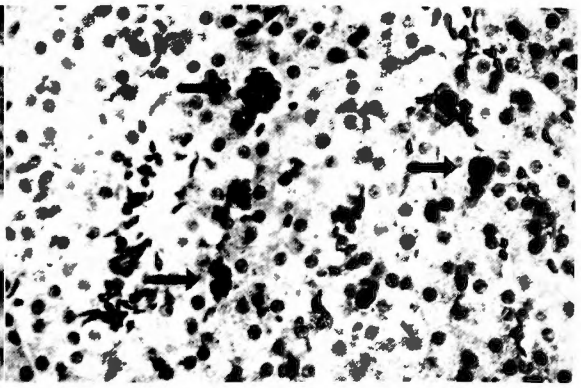

Fig. 5. Liver 10 minutes after injection of chyle A. Note the KUPFFER's stellate cells containing the fat globules. (Oil red O stain, × 400)

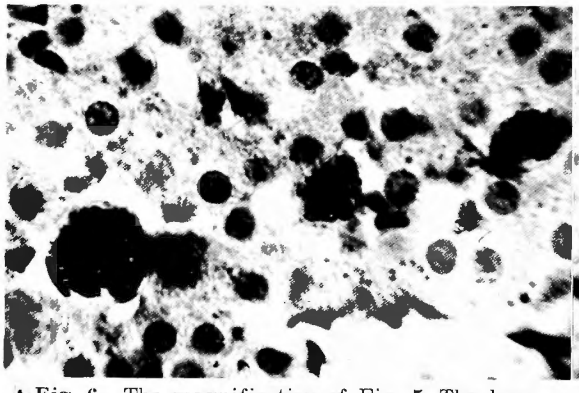

Fig. 6. The magnification of Fig. 5. The hypertrophied KUPFFER's cells are filled with larger fat globules. (Oil red O stain, × 900)

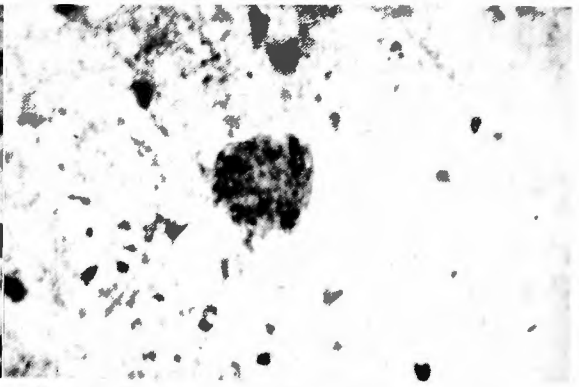

Fig. 7. Liver 1 hour after injection of chyle A. Phospholipid in the KUPFFER's cells. (SMITH-DIETRICH's stain, × 900)

Fig. 8. Liver 10 minutes after injection of chyle A. Appearance of phospholipid in the hepatic parenchymatous cells, especially at the peripheries of the hepatic lobules. (SMITH-DIETRICH's stain, × 40)

Fig. 9. Liver 30 minutes after injection of chyle A. Phospholipid is visible in relatively large amounts in the hepatic parenchymatous cells. (SMITH-DIETRICH's stain, × 40)

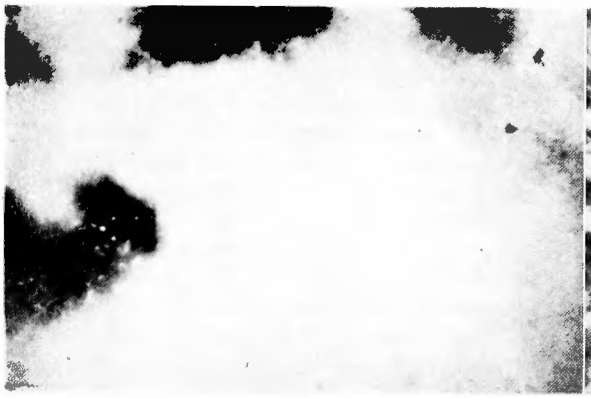

Fig. 10. Liver 3 hours after injection of chyle A. Phospholipid is seen in moderate amounts in the hepatic parenchymatous cells. (SMITH-DIETRICH's stain, × 40)

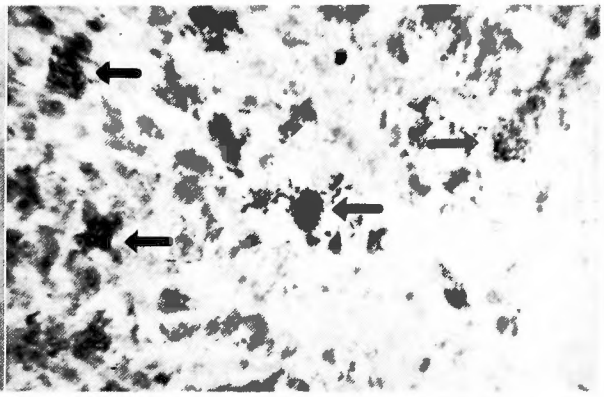

Fig. 11. Spleen 10 minutes after injection of chyle A. The fat globules are phagocytized by the macrophages of the red pulp. (Oil red O stain, × 900)

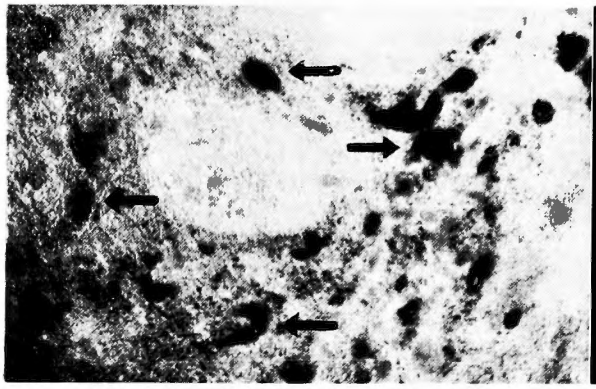

Fig. 12. Spleen 10 minutes after injection of chyle A. Appearance of phospholipid in the ellipsoids. (SMITH-DIETRICH's stain, × 100)

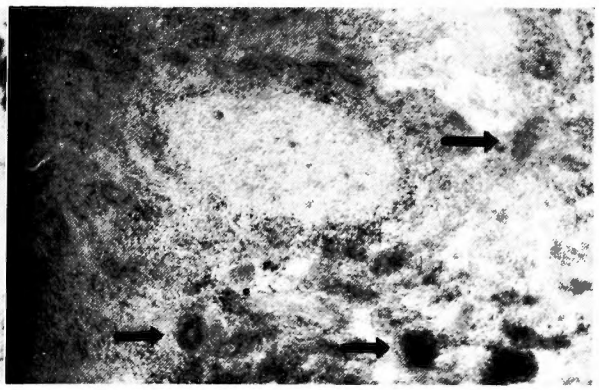

Fig. 13. Spleen 3 hours after injection of chyle A. Phospholipid is seen present in the ellipsoids, though to a slight degree. (SMITH-DIETRICH's stain, × 100)

Fig. 14. Liver 10 minutes after injection of chyle B. Phospholipid is visible in moderate amounts in the hepatic parenchymatous cells. (SMITH-DIETRICH's stain × 40)

Fig. 15. Liver 3 hours after injection of chyle B. Phospholipid is seen in very limited amounts in the hepatic parenchymatous cells at the peripheries of the hepatic lobules. (SMITH-DIETRICH's stain, × 40)

REFERENCES

1) Artom, C.: Role of choline in the oxidation of fatty acids by the liver. J. Biol. Chem., **205**; 101, 1953. 2) Asada, S.: Histochemical studies on the intravenously infused fat emulsion. Arch. Jap. Chir., 22, 77, 1953. **22**; 217, 1953. Acta Med. Univ. Kioto, **31**; 171, 1951. 3) Bauersfeld, E. H.: Traumatic chylothorax from ruptured thoracic duct treated by intravenous injection of aspirated chyle. J.A.M.A. **109**; 16, 1937. 4) Berry, I.M. & Ivy, A.C.: The tolerance of dogs to intravenously administered fatty chyle and synthetic fat emulsion. Federation Proc., **7**; 7, 1948. 5) Bloom, B., Chaikoff, I. L., Reinhardt, W.O., Entenman, C. & Dauben, W. G.: The quantitative significance of the lymphatic pathway in transport of absorbed fatty acids. J. Biol. Chem., **184**; 1, 1950. 6) Bloom, B., Chaikoff, I. L., Reinhardt, W.O. & Dauben, W.G.: Participation of phospholipides in lymphatic transport of absorbed fatty acids. J. Biol. Chem., **189**; 261, 1951. 7) Bloom, B., Chaikoff, I. L. & Reinhardt, W. O.: Intestinal lymph as pathway for transport of absorbed fatty acids of different chain lengths. Am. J. Physiol., **166**; 451, 1951. 8) Bloor, W. R.: The oxidative determination of phospholipid (Lecithine and Cephalin) in blood and tissues. J. Biol. Chem., **82**; 273, 1929. 9) Cantarow, A. & Trumper, D.: Lipid metabolism. Clin. Biochem., 131, 1949. 10) Collins, H.S., Kraft, L.M., Kinnet, T.D., Davidson, C.S., Young, J. & Stare, F.J.: Studies on the tolerance of dogs to intravenous administration of fat emulsion. J. Lab. & Clin. Med., **33**; 143, 1948. 11) Dermann, G. L. & Leites, S.: Experimentell-morphologische Studien über die Rolle der Lunge, Leber u. Milz im Fett-u. Lipoidstoffwechsel. Virchow's Arch., **268**; 440, 1928. 12) Frazer, A.C.: Fat absorption and its relationship to fat metabolism. Physiol. Rev., **20**; 561, 1940. 13) Geyer, R.P., Chipman, J. & Stare, F.J.: Oxidation in vivo of emulsified radioactive trilaurin administered intravenously. J. Biol. Chem., **176**; 1469, 1948. 14) Geyer, R.P., Matthews, L. W. & Stare, F. J.: Metabolism of emulsified trilaurin ($-C^{14}OO-$) and octanoic acid ($-C^{14}OO-$) by rat tissue slices. J. Biol. Chem., **180**; 1037, 1949. 15) Goldman, D.S., Chaikoff, I.L., Reinhardt, W.O., Entenman, C. & Dauben, W.G.: The oxidation of palmitic acid-1-C^{14} by extrahepatic tissues of the dog. J. Biol. Chem., **184**; 719, 1950. 16) Goldmann, J.: Über die Lipoidfärbung mit Sudan-α- Naphtol. Zentralbl. Pathol. u. pathol. Anat., **46**; 289, 1929. 17) Gorens, S.W., Geyer, R.P., Matthews, L.W. & Stare, F.J.: Observations on the use of a fat emulsion for intravenous nutrition in man. J. Lab. & Clin. Med., **34**; 1627, 1949. 18) Groen, J.: Absorption and metabolism of Lipoidol after oral administration. Am. J. Med., **4**; 814, 1948. 19) Hashino, H.: Experimental studies on fat metabolism from the view-point of ketone body formation. Arch. Jap. Chir., 24; 488, 1955. 20) Healey, J.E.: Experimental thoracic duct fistula. Surg. Gyne. & Obstr., **89**; 200, 1949. 21) (a) Hikasa, Y., Ishigami, K., Asada, S., Zaitsu, A., Tsukada, A. & Nakata, K.: Studies on the intravenous administration of fat emulsion. J. Jap. Surg. Soc., 52; 298, 1951. (b) Hikasa, Y., Asada, S., Zaitsu, A., Tsukada, A. & Nakata, K.,: Studies on the intravenous administration of fat emulsion. Arch. Jap. Chir., **21**; 1, 1952. (c) Hikasa, Y.: Studies on the intravenous administration of fat emulsion prepared by high pressure jet. Rev. Phys. Chem. Jap., **12**; 83, 1952. (d) Hikasa, Y., Osa, H., Hashino, H. & Takeda, S.: Nippon-Rinsho, **13**; 1255, 1955. 22) Hirose, S.: Cytological study on the fat liver caused by feeding high fat diet. Arch. hist. jap., **7**; 513, 1955. 23) Hoffman, M. C.: Radioactive iodine-labeled fat. J. Lab. & Clin. Med., **41**; 521, 1953. 24) Ikeda, H.: unpublished 25) Izukura, T.: unpublished 26) Jaffe, R.H., & Berman, S. L.: Relations between Kupffer cells and liver cells; Functional studies. Arch. Path., **5**; 1020, 1928. 27) Kennedy, E. P. & Lehninger, A.L.. The products of oxidation of fatty acids by isolated rat liver mitochondria. J. Biol. Chem., **185**; 275, 1950. 28) Kimura, S.: Histologische Untersuchung über das Schicksal intravenös infundierten Fettes im Organismus. Tohoku J. Exp. Med., **30**; 315, 1937. 29) Kosaki, N.: Studies on fat metabolism after total pancreatectomy. Experiments in dogs. Arch. Jap. Chir., **25**; 138, 1956. 30) Lerner, S.R., Chaikoff, I.L., Entenman, C.& Dauben, W. G.: Oxidation of parenterally administered C^{14}-labeled tripalmitin emulsion. Science, **109**; 13, 1949. 31) Lerner, S. R., Chaikoff, I. L., Entenman, C. & Dauben, W.G.: The fate of C^{14}-labeled palmitic acid administered intravenously as a tripalmitin emulsion. Proc. Soc. Exper. Biol. & Med., **70**; 384, 1949. 32) Lillie, R.D.: Various oil-soluble dyes as fat stains in supersaturated isopropanol technique. Stain Technol., **19**; 55, 1941. 33) Little, J. M., Harrison, C. & Blalock, A.: Chylothorax and Chyloperitoneum. Effects of

reintroduction of aspirated chyle. Surgery. **11**; 392, 1942. 34) Mann, G.V., Geyer, R.P., Watkin, D.M., Smythe, R. L., Dju, D., Zamcheck, N. & Stare, F.J.: Metabolic studies on puppies infused with fat emulsions. J. Lab. & Clin. Med., **33**; 1503, 1948. 35) Mann, G. V., Geyer, R. P., Watkin, D.M. & Satre, F.J.: Fat emulsions for intravenous nutrition in man. J. Lab. & Clin. Med., **34**; 669, 1949. 36) McKibbin, J.M., Pope, A., Thayer, S., Ferry, R. M. & Stare, F. J.: Studies on fat emulsions for intravenous administration. J. Lab. & Clin. Med., **30**; 488, 1945. 37) McKibben, J.M., Ferry, R.M., & Stare, F. J.: The utilization of emulsified fats given intravenously. J. Clin. Investigation, **25**; 679, 1946. 38) Meng, H.C. & Freeman, S.,: Experimental studies on the intravenous injection of a fat emulsion into dogs. J. Lab. & Clin. Med., **33**; 689, 1948. 39) Murray, R. G. & Freeman, S.: The morphologic distribution of intravenously injected fatty chyle and artificial fat emulsion in rats and dogs. J. Lab. & Clin. Med., **38**; 56, 1951. 40) Nakata, K.: Experimental studies on fat metabolism in the lung. Arch. Jap. Chir., **23**; 445, 1954. 41) Nishino, T.: Laboratory studies on the intravenous administration of the fat emulsion in the light of tissue metabolism. Arch. Jap. Chir., **23**; 607, 1954. 42) Oeken: Ein Fall von Zerreissung des Ductus thoracicus infolge Brustquetschung. München. med. Wchnschr., 55; 1182, 1908. 43) Ōno, K.: Fat metabolism. Saishin-Igaku, **10**; 52, 1955. 44) Osa, H.: Experimental studies on the intravenous administration of a fat emulsion for nutritional purposes. Arch. Jap. Chir., **25**; 154, 1956. 45) Otani, S.: On mechanism of adverse reactions caused by intravenous administration of fat emulsion. Arch. Jap. Chir., **25**; 172, 1956. 46) Rappaport, E. & Engelberg, H.: Eine Mikromethode der Cholesterinbestimmung. Klin. Wchnschr., **10**; 700, 1931. 47) Reiser, R., Bryson, M. J., Carr, M. J. & Kuiken, K. A.: The intestinal absorption of triglycerides. J. Biol. Chem., **194**; 131, 1952. 48) Rutenberg, A. M., Seligman, A. M. & Fine, J.: Studies with radioactive iodized fat; II. Tissue distribution. J. Clin. Investigation, **28**; 1110, 1949. 49) Seno, A.: A study of the fat metabolism in the isolated perfused liver. Arch. Jap. Chir., **24**; 179, 1955. 50) Shafiroff, B.G.P., Mulholland, J. H., Roth, E., & Baron, H.C.: Intravenous infusions of a combined fat emulsion into human subjects. Proc. Soc. Exper. Biol. & Med., **70**; 343, 1949. 51) Stanley, M.M. & Thannhauser, S.J.: The absorption and disposition of orally administered I^{131}-labeled neutral fat in man. J. Lab. & Clin. Med., **34**; 1634, 1949. 52) Takahashi, K.: Experimental studies on the absorption of fat from the intestine. Tohoku-Igaku, **33**; 239, 1943. 53) Takeda, S.: Experimental studies on the effect of riboflavin following the intravenous administration of fat emulsion. Arch. Jap. Chir., **25**; 221, 1956. 54) Tan, N.: unpublished. 55) Tatsumi, W.: unpublished. 56) Tsukada, A.: Studies on the intravenous administration of the fat emulsion in the light of protein metabolism. Arch. Jap. Chir., **23**; 215, 1954. 57) Van de Kamer, J.H.: Rapid method for the determination of the fat in feces. J. Biol. Chem., **177**; 347, 1949. 58) Weidenreich, F.: Das Gefässsystem der menschlichen Milz. Arch. f. mikr. Anat., **58**; 247, 1901. 59) Weinhouse, S., Millington, R.H., & Volk, M.E.: Oxidation of isotopic palmitic acid in animal tissues. J. Biol. Chem., **185**; 191, 1950. 60) Woerner, C.A.: Cytological distribution of fat injected intravenously into guinea pigs. Anat. Rec., **104**; 61, 1949. 61) Yasuda, M.: Lipid metabolism (1) J. Biol. Chem. Jap., **17**; 1, 1942. Lipid metabolism (2) Nisshin-Igaku, **32**; 501, 1943. 62) Zilversmit, D. B., Chaikoff, I. L., & Entenman, C.: Are phospholipids obligatory participants in fat transport across the intestinal wall? J. Biol. Chem., **172**; 637, 1948. 63) Zilversmit, D. B., Entenman, C. & Chaikoff, I. L.: The measurement of turnover of the various phospholipides in liver and plasma of the dog and its application to the mechanism of action of choline. J. Biol. Chem., **176**; 193, 1948.

和文抄録

胸管乳糜靜脉內注入法による脂肪代謝の組織顕微化学的研究

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生体内脂肪代謝過程を究明する目的で，脂肪処理能力の旺盛な肉食動物である猫に多量の脂肪を経口的に投与せしめた後，当該猫より採取し得た脂肪含有率の高い胸管乳糜を一定時間後に同一猫の静脈内に注入し，その胸管乳糜中に含有される脂肪の処理過程を逐時的に組織顕微化学的に検討し，次の結論に到達した．

(1) 胸管乳糜中には比較的多量（15%内外）のPhospholipidを含有し，そのPhospholipidは早期に而も直接的に肝実質細胞内及び脾臓のEllipsoids内に移行し得る．

(2) 胸管乳糜中のGlycerideは凡て網内系細胞群に摂取され，そこでPhospholipid化された後血中に放出され，徐々に肝実質細胞内及び脾臓のEllipsoids内へ移行し，之等のPhospholipidの移行量は乳糜注入後3時間目に最高潮に達する．

(3) 従つて乳糜注入時には，肝実質細胞内及びEllipsoids内に出現するPhospholipidの態度は2相性である．

(4) 高度の不飽和脂肪酸は専ら肝臓にPhospholipidとして移行処理されるのに反して，高度の不飽和脂肪酸を除く長鎖脂肪酸はPhospholipidとして肝臓のみならず肝外組織へも移行処理されるものと思われる．

(5) 高度の不飽和脂肪酸も明らかに胸管内へ吸収され得ることが斯る組織顕微化学的検索によつても推察され得る．

(6) Methionineは，網内系細胞群によるGlycerideのPhospholipid化機転を促進すると共に，少くとも肝実質細胞内に於ける脂肪酸酸化機転をも促進する作用を有する．

(7) 以上の組織顕微化学的所見から，我々の人為的脂肪乳剤中の含有脂肪体は，生理的に吸収された胸管乳糜中のGlycerideと同様の処理を受け，利用されて行くということ及び脂肪を乳化態として静脈内へ注入し，脂肪の非経口的栄養補給を行うためには，高度の不飽和脂肪酸を含有しない長鎖脂肪酸のTriglycerideのみからなるゴマ油のような脂肪体をその原料として使用することが望ましいとの結論を得た．