PARENTERAL ADMINISTRATION OF FATS
I. FAT METABOLISM IN VIVO, STUDIED WITH FAT EMULSION

by

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I. INTRODUCTION

Attempts to prepare a fat emulsion which could be given intravenously have been made for the following purposes: one was to clarify, for the most part, processes of fat metabolism, and the other was to utilize the fat emulsion as a parenteral nutritional supplement.

Independent of Geyer and Shafiroff's studies[1)-17)], in 1949, we succeeded in producing the fat emulsion[18),19)]. Since then we have continued this experimental study and the clinical observation of the same. The present report concerns the problem of the fat metabolism in vivo after the intravenous administration of the fat emulsion.

II. MATERIALS AND METHODS

For experimental animals, adult cats and dogs as carnivora, mice and rats as omnivora and albino rabbits as herbivora in the postabsorptive state were used.

In the case of microscopic examination, the animals were sacrificed by bleeding without anesthesia at definite intervals after the injection of fat emulsions, and tissues for sectioning were placed at once in a 10 per cent neutral formalin solution or in Baker's solution. Carbowax-embedding was mainly used, but the freezing method was employed if deemed necessary. The methods used for staining the section were Goldman's[20)], Oil red O stain, Ciaccio's, Baker's, Smith-Dietrich's, moreover the Nile-blue stain. When needed, the hematoxilin-eosin and Best's glycogen staining methods were also employed.

Paper chromatography was carried out by Hirayama and Noda's method[22)-25)] with the Toyo filter paper No. 2.

Urinary nitrogen excretion was determined by micro-Kjeldahl analysis and urinary creatinine excretion was estimated by Jaffe's method[26)].

The electrophoretic analysis of serum was performed by the use of Hitachi's HT-B type of electrophoretic apparatus with veronal buffer solution of pH 8.6 and an ionic strength of 0.1 μ.

To determine the serum lipoprotein, Kobayashi's horizontal type of paper electrophoretic apparatus with the Toyo filter paper No. 51 (vernal buffer solution of pH 8.6 and an ionic strength of 0.05 μ) was used. The protein and lipid, stained by Amidoschwarz 10 B and Oil red O stain, on the filter paper were analysed by

densitometer.

Physiological saline-soluble or insoluble protein of tissues was determined by FISHMANN and VEEN's method[27)-28)].

The phospholipids in organs were quantified from the extract of the organ from which the water-soluble inorganic phosphate was removed by the method of FAWAZ-LIEB-ZACHERL[29)].

The determination of ketone body levels was made by GREENBERG and LESTER's method[30)] and blood sugar levels were measured by SOMOGYI's[31)] method.

In the present investigation, riboflavin as riboflavin-5'-phosphate, vitamin C as *l*-ascorbic acid, nicotinic acid as niacin amide, pantothenic acid as calcium pantothenate, vitamin B_1 as thiamin hydrochloride and methionine as *l*-methionine were used in the formation of the solution.

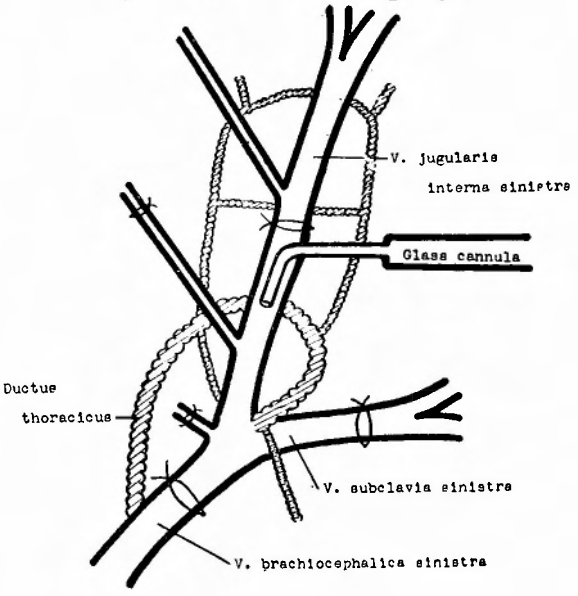
III. RESULTS AND DISCUSSION

1) Mechanism of fat absorption after oral administration

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. However, the study on the postabsorptive fate of fat was not conclusive. In order to solve this problem, the classification of fatty acid in the chyle and the portal blood after the oral administration of various fats was performed by paper chromatography.

In this investigation, various natural and synthetized fats were infused by stomach tube into dogs, and the chyle was directly led to a polyethylene tube, so as to intercept entrance to the blood stream, as shown in Fig. 1. After that, the absorbed fatty acids in the chyle and the portal blood were analyzed by paper chromatography and compared with those contained in the administered fats.

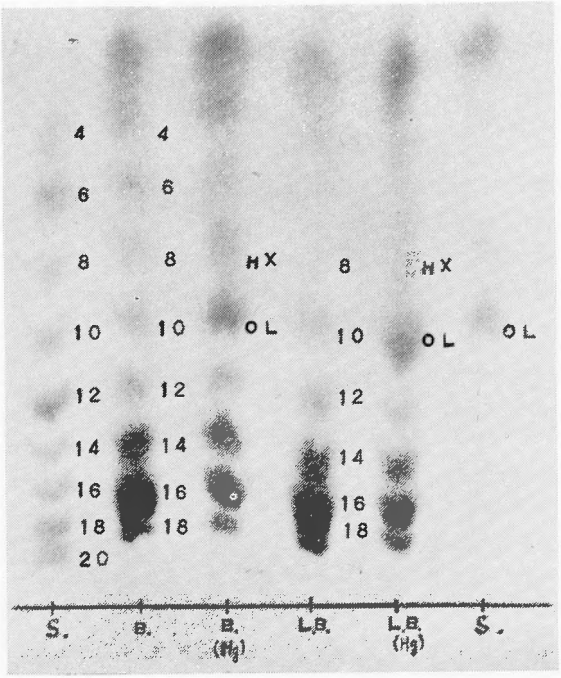
Fig. 1 Method of Collecting Chyle



According to the results obtained by TAN[32)], higher fatty acids entered mostly into the chyle, in the form of triglyceride. On the other hand, the absorption coefficient of lower fatty acids into the chyle decreased markedly and were scarcely to be found in the case of caproic acid having 6 carbon atoms (Figs. 2 and 3).

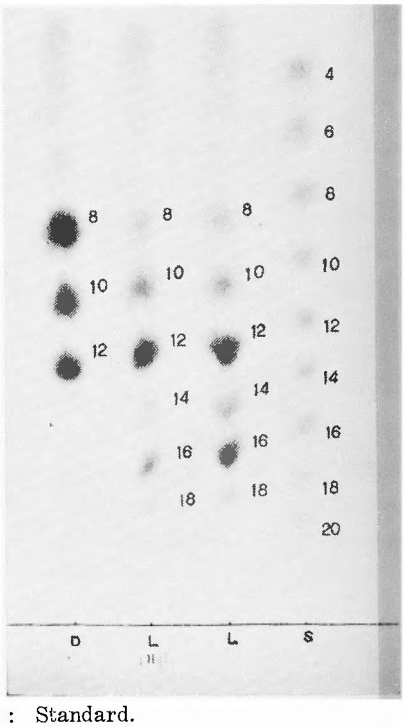
However, it may be impossible to conclude here that lower

Fig. 2 Paper Chromatographical Analysis of Fatty Acids in the Chyle Collected after Oral Administration of Butter Fat into Dog.



S : Standard.
B : Fatty Acids in the Butter Fat.
L.B. : Fatty Acids in the Chyle Collected after Oral Administration of Butter Fat.

Fig. 3 Paper Chromatographical Analysis of Fatty Acids in the Chyle Collected after Oral Administration of Synthesized Simple Glyceride Mixture (Dog).

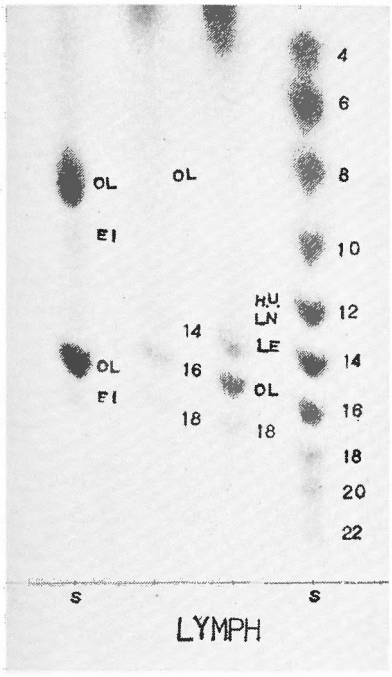


S : Standard.
D : Fatty Acids in the Mixture of Simple Glycerides (Equal Amount of Trilaurin, Tricaprin and Tricaprylin).
L : Fatty Acids in the Chyle Collected after Oral Administration of Simple Glyceride Mixture.

fatty acids more than caproic acid can not enter into the chyle, because butter fat has only very small quantities of lower fatty acids. To study a postabsorptive fate of lower fatty acids, various simple glycerides of lower fatty acids, such as Tricaprylin and Tricaproin were prepared in our laboratory, and were administered to dogs by the above mentioned method. Although in normal dogs in the postabsorptive state lower fatty acids were found neither in the portal blood nor in the chyle (Figs. 4 and 5), lower fatty acids, such as caproic (C_6) and caprylic (C_8) acids were observed in the portal blood and also in the chyle after the oral administration of these simple lipids even when the chyle was intercepted from entering into the blood stream. Furthermore, caproic and caprylic acids were recognized in the portal blood in larger quantity than those in the chyle (Figs. 6 and 7).

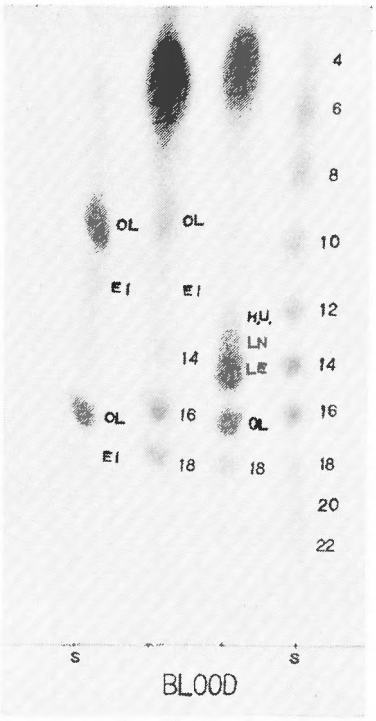
From these findings, it is evident that lower fatty acids mostly enter into the portal blood, while a portion of them enter into the chyle. After all, these results agreed with the opinion of BLOOM[33)-35)] that lower fatty acids are chiefly conveyed through the portal vein from the intestinal mucous membrane to the liver.

Fig. 4. Paper Chromatographical Analysis of Fatty Acids in the Chyle Collected in the Postabsorptive State (Dog).



S: Standard

Fig. 5 Paper Chromatographical Analysis of Fatty Acids in the Portal Blood Collected in the Postabsorptive State (Dog).



S: Standard

2) Studies on the fat metabolism in vivo by the administration of fat emulsion

Several kinds of the fat emulsions, containing triglycerides of various fatty acids and having the fat globules of less than 0.5μ size were prepared in our laboratory (Table 1).

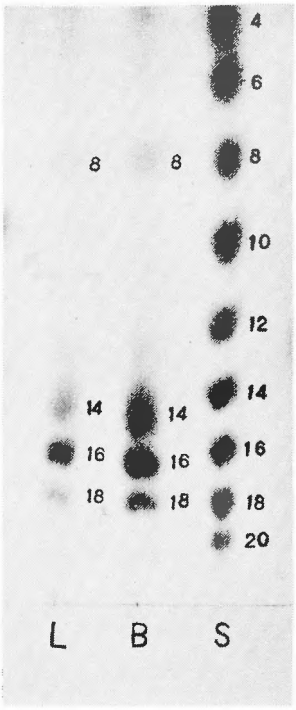
After the intravenous administration of these fat emulsions, the oxygen consumption of the liver, kidney, lung, spleen, cardiac muscle and skeletal muscle was markedly increased and each respiratory quotient (R. Q) showed around 0.5～0.7.[36)-38)] From this fact, it is supposed that infused fats were oxidized in the body.

(A) Fate of infused fat.

ASADA and IZUKURA, in our laboratory, had carried out histochemical studies on the metabolic processes of fat with fat emulsions, and it was found that the intravenously infused fat globules were first phagocytized by the reticuloendothelial cells in the lung, liver and spleen, and then changed gradually into phospholipids. The fat globules in these cells appeared in small quantity when the fat emulsions, consisting of large amounts of such fatty acids as in fat depôts[32)], were administered (Table 2).

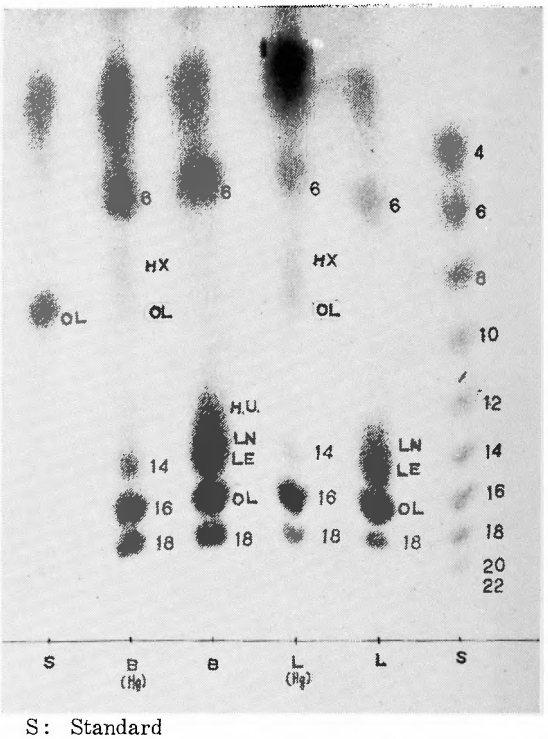
The phospholipids in the hepatic parenchymatous cells were found in the 30 minute or more postinfusion cases, and increasing gradually, could be observed di-

Fig. 6 Paper Chromatographical Analysis of Fatty Acids in the Chyle and Portal Blood Collected after Oral Administration of Synthesized Tricaprylin into Dog.



S : Standard
L : Fatty Acids in the Chyle Collected after Oral Administration of Tricaprylin.
B : Fatty Acids in the Portal Blood Collected after Oral Administration of Tricaprylin.

Fig. 7 Paper Chromatographical Analysis of Fatty Acids in the Chyle and Portal Blood Collected after Oral Administration of Synthesized Tricaproin into Dog.



S : Standard
L : Fatty Acids in the Chyle Collected after Oral Administration of Tricaproin
B : Fatty Acids in the Portal Blood after Oral Administration of Tricaproin

Table 1 Fatty Acids in Various Fats

		Butter	Sesame Oil	Cod Liver Oil	Olive Oil
Saturated Fatty Acids	Butyric Acid	+	−	−	−
	Caproic Acid	+	−	−	−
	Caprylic Acid	+	−	−	−
	Capric Acid	+	−	−	−
	Lauric Acid	+	−	−	−
	Myristic Acid	+	+	+	+
	Palmitic Acid	++	+	+	++
	Stearic Acid	+	+	+	+
	Arachidic Acid	±	−	−	±
Unsaturated Fatty Acids	Oleic Acid	+++	+++	++	+++
	Linoleic Acid	−	+++	+	++
	Linolenic Acid	−	+	+	−
	Docosenoic Acid	−	−	+	−
	Eicosenoic Acid	−	−	+	±
	Hexadecenoic Acid	+	−	−	+
	Highly Unsaturated Fatty Acids	−	−	++	−

Table 2 Changes of Fat Content in Each Reticuloendothelial Cells of Lung, Liver and Spleen Following Intravenous Administration of Various Fat Emulsions into Cats.

Time after Infusion	Infusion of Cod Liver Oil Emulsion			Infusion of Sesame Oil Emulsion			Infusion of Triolein Emulsion		
	Lung	Liver	Spleen	Lung	Liver	Spleen	Lung	Liver	Spleen
10 min	###	##	##	##	##	##	##	##	+
30 min	##	##	+	##	##	##	##	##	+
1 hr	+	+	±	+	+	+	+	+	+
2 hrs	+	±	±	+	±	+	+	±	+
3 hrs	±	±	−	±	±	±	±	±	±
4 hrs	−	−	−	±	±	±	±	±	±
6 hrs	−	−	−	−	−	−	−	−	−
24 hrs	−	−	−	−	−	−	−	−	−

ffusely in the peripheries of the hepatic lobules at 3 hours after infusion. However, there was no evidence that infused fat globules infiltrated directly into the parenchymatous cells in the form of glyceride. Furthermore, the phospholipids appeared in the hepatic parenchymatous cells in far greater quantities in the case of the administration of cod liver oil emulsion, which contained many highly unsaturated fatty acids, than the case of the administration of the sesame oil and triolein emulsion, not containing them (Table 3).

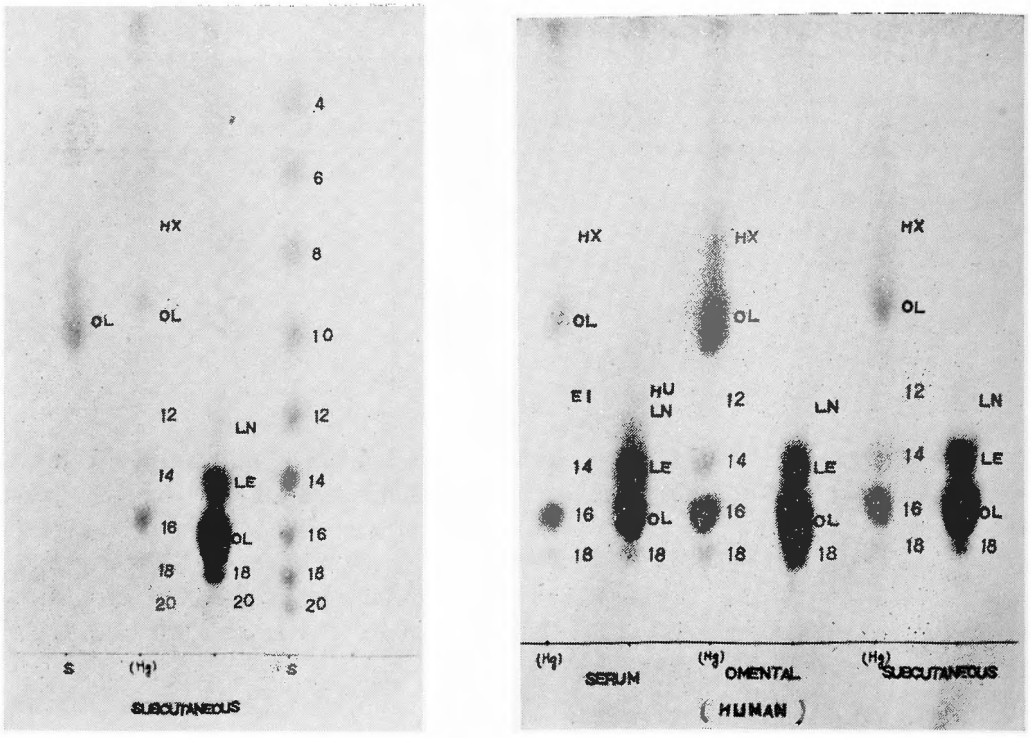
Table 3 Phospholipid Content in Hepatic Parenchymatous Cells Following Intravenous Administration of Various Fat Emulsions into Cats.

	10 min	30 min	1 hr	3 hrs	4 hrs	24 hrs
Cod Liver Oil Emulsion	−	−	±	###	##	±
Sesame Oil Emulsion	−	±	±	+	±	−
Triolein Emulsion	−	±	±	±	±	−

From the above facts, and the results in the biochemical determination of the phospholipids in various organs as shown in Fig. 9, it is understood that highly unsaturated fatty acids were shifted chiefly into the hepatic parenchymatous cells as phospholipid, but the phospholipids, being changed from glyceride of higher fatty acids other than highly unsaturated fatty acids, enter not only into the hepatic parenchymatous cells but also into the extrahepatic tissues to be oxidized. According to our experimental results of paper chromatographical determination of various organs[32], all extrahepatic tissues always contained highly unsaturated fatty acids. Accordingly, we can not help considering that even highly unsaturated fatty acids can enter into the extrahepatic tissues in small quantities and very slowly.

The amounts of phospholipids appeared in the hepatic parenchymatous cells in the case of the oral administration of cod liver oil and butter fat were far greater than the case of the administration of olive oil and sesame oil, like the retults

Fig. 8 Paper Chromatographical Analysis of Fatty Acids in Storage Fat



(Dog)
S: Standard

4……Butyric Acid, 6……Caproic Acid, 8……Caprylic Acid,
10……Capric Acid, 12……Lauric Acid, 14……Myristic Acid,
16……Palmitic Acid, 18……Stearic Acid, 20……Arachidic Acid,
OL……Oleic Acid, LE……Linoleic Acid, LN……Linolenic Acid,
DO……Docosenoic Acid, EI……Eicosenoic Acid, HX……Hexadecenoic Acid,
HU……Highly Unsaturated Fatty Acids

obtained in the experiments on their intravenous administration[39)-41)].

Cats were injected intravenously with the chyle collected from the same cats at certain intervals after the oral administration of fat emulsions. And the metabolic processes of the fat in the cat chyle were histochemically examined[42)].

The fat globules in the injected chyle were obviously first phagocytized by the reticuloendothelial cells as well as in the case of the intravenous administration of fat emulsion (Table 4), but phospholipids appeared biphasicly in the hepatic parenchymatous cells (Table 5).

Namely, in the 10 minute cases, large amounts of phospholipids could be diffusely recognized within the hepatic parenchymatous cells, especially at the peripheries of the hepatic lobules. In the 30 minute cases, the phospholpids in the hepatic cells decreased fairly much in quantity and once entirely disappeared in 1 and 2 hour cases. In the 3 hour cases, the phospholipids appeared again in the same cells, though they were less than those of the 10 minute cases.

Table 4 Changes of Fat Content in Each Reticuloendothelial Cells of Lung, Liver and Spleen Following Intravenous Administration of Chyle A* and Chyle B** into Cats.

Time after Infusion	Infusion of Chyle A			Infusion of Chyle B		
	Lung	Liver	Spleen	Lung	Liver	Spleen
10 min	++	++	++	++	++	++
30 min	++	++	+	++	++	+
1 hr	+	+	±	+	+	±
2 hrs	±	±	±	±	±	±
3 hrs	±	±	−	±	±	−
6 hrs	−	−	−	−	−	−
24 hrs	−	−	−	−	−	−
Remarks	*Chyle A: The Chyle Collected after the Oral Administration of Cod Liver Oil. **Chyle B: The Chyle Collected after the Oral Administration of Sesame Oil.					

Table 5 Phospholipid Content in Hepatic Parenchymatous Cells Following Intravenous Administration of Chyle A* and Chyle B** into Cats.

Time after infusion	Infusion of Chyle A	Infusion of Chyle B
10 min	++	+
30 min	+	±
1 hr	−	−
2 hrs	−	−
3 hrs	+	±
6 hrs	−	−
24 hrs	−	−
Remarks	*Chyle A: The Chyle Collected after Oral Administration of Cod Liver Oil. **Chyle B: The Chyle Collected after Oral Administration of Sesame Oil	

Regarding the phospholipid which appeared in the early intervals such as the 10 or 30 minute cases, it can be presumed from the point of view that the chyle contains approximately 15 per cent phospholipid, which infiltrates directly into the hepatic parenchymatous cells in large quantities immediately after injection. On the other hand, as regards the phospholipid which appeared in the hepatic cells in the 3 hour cases, from the time of its appearance, we can not help considering that the glyceride of the chyle entered into the hepatic parenchymatous cells after changing to phospholipid from glyceride by the above mentioned reticuloendothelial cells and then the movement of the phospholipid to the hepatic parenchymatous cells reached the climax 3 hours after injection.

It is not too much to say that it could be demonstrated that the glyceride 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.

Comparing the fat-phagocytizing abilities in cats with those in mice and rabbits, it has been established that carnivora are strongest in the ability to dispose

of fat, next omnivora, and herbivora being the weakest (Table 6).

Table 6 Changes of Fat Content in Each Reticuloendothelial Cells of Lung, Liver and Spleen Following Intravenous Administration of Cod Liver Oil Emulsion into Various Animals.

	Lung			Liver and Spleen		
	Fat Content	Climax of Fat Content after Infusion	Time of Disappearance of Fat Globules after Infusion	Fat Content	Climax of Fat Content after Infusion	Time of Disappearance of Fat Globules after Infusion
Cats	###	30 min	3 hrs	+	1 hr	4 hrs
Mice	##	30 min	3 hrs	###	15 min to 90 min	24 hrs
Rabbits	+	30 min	3 hrs	###	90 min to 4 hrs	48 hrs

Recently, TAKAHASHI[13] and co-workers demonstrated that in rabbits only 0.3 to 0.4 g per hour of fat entered into the blood stream from the thoracic duct, while in dogs 12 g per hour could be absorbed in the similar manner. The results cited above are well in accord with the ones obtained by us.

In our laboratory, it has been observed histochemically and biochemically that the fat-phagocytizing abilities of the alveolar phagocytes were stronger than those of the KUPFFER's cells and the reticuloendothelial cells of the spleen. Such an important role of the lung in fat metabolism is understood by the anatomical and physiological fact that it is the first parenchymatous organ which takes the chyle from the blood stream, just like the liver takes the portal blood. In recent years, CHAIKOFF[44] has established that the greater part of the phospholipids is produced in the liver. From the present study, however, not only in the liver, but also in the spleen and lung, especially in the latter of carnivora, the great ability of changing to phospholipid from glyceride should be recognized.

The perfusion experiments of the isolated lung and liver, by NAKATA[45] and SENO[46], and the experimental studies with a blocked reticuloendothelial system by IKEDA[47] ascertained that the infused fat were first phagocytized by the cells of the reticuloendothelial system and must pass through the phospholipid stage in order to be utilized in the body.

The elevation of the blood lipid levels, which diffused into the circulating blood, caused the increase in α-and β-globulin fraction by the increase in α-and β-lipoprotein in the serum. After these began decreasing gradually, the total proteins, the ratio of physiological saline-soluble to insoluble proteins, the α- plus β-globulin fractions in the physiological saline-soluble protein and the phospholipids in the liver began to increase (Fig. 9 and Tables 7 and 8).

These findings suggested that the phospholipids which were produced from the infused fats were transported to the hepatic parenchymatous cells or extrahepatic tissues in the form of lipoprotein, not in the isolated form[48]. In fact, the phospholipids which were produced from glycerides in the alveolar phagocytes, KUPFFER's cells and splenic reticuloendothelial cells were well stained by BAKER's method, which

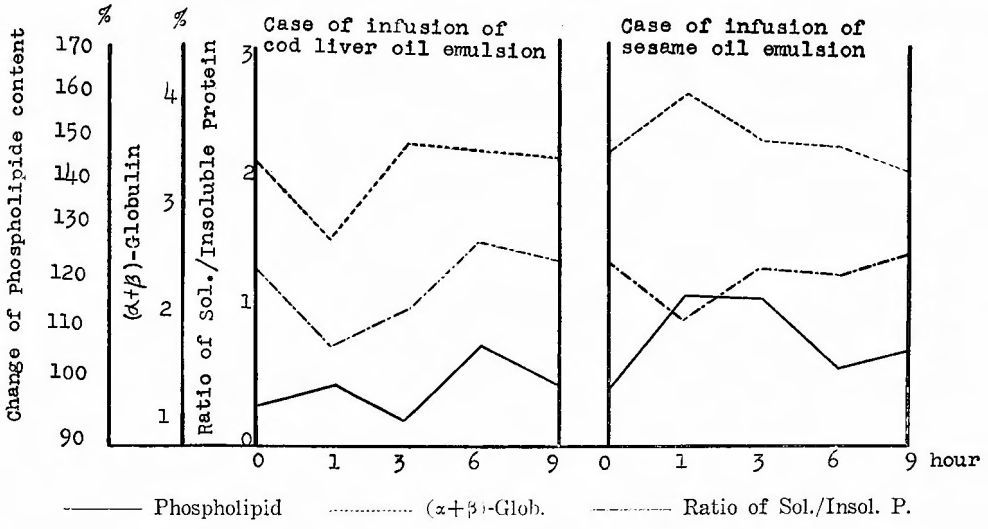
is specific for the dyeing of phospholipid, but the phospholipids in the hepatic parenchymatous cells could be stained only by SMITH-DIETRICH's and CIACCIO's methods, which are not specific for the dyeing of phospholipid.

(B) The oxidation process in vivo of infused fat

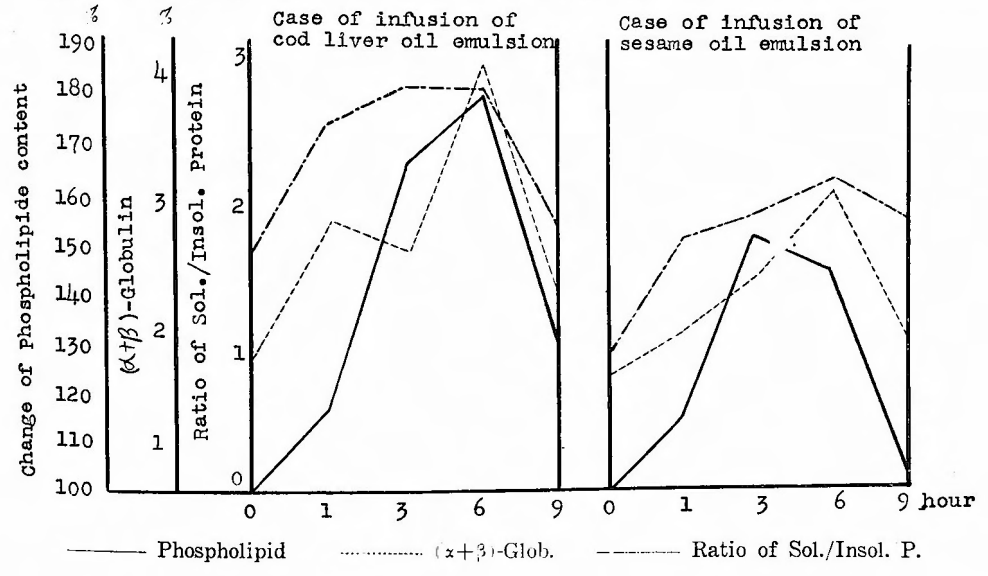
In the case of the intravenous administration and the perfusion of the isolated liver, ketone body levels in the blood and the circulating fluid in the perfusion increase after the infusion of fat[49]. According to the determination of acetoacetic acids with tissue slices, ketone bodies are produced in greater quantity in the case

Fig. 9

A: Changes of phospholipid content in the muscles following single administration of fat emulsions.



B: Changes of phospholipid content in the liver following single administration of fat emulsions.



C: Changes of phospholipid content in the kidneys following single administration of fat emulsions.

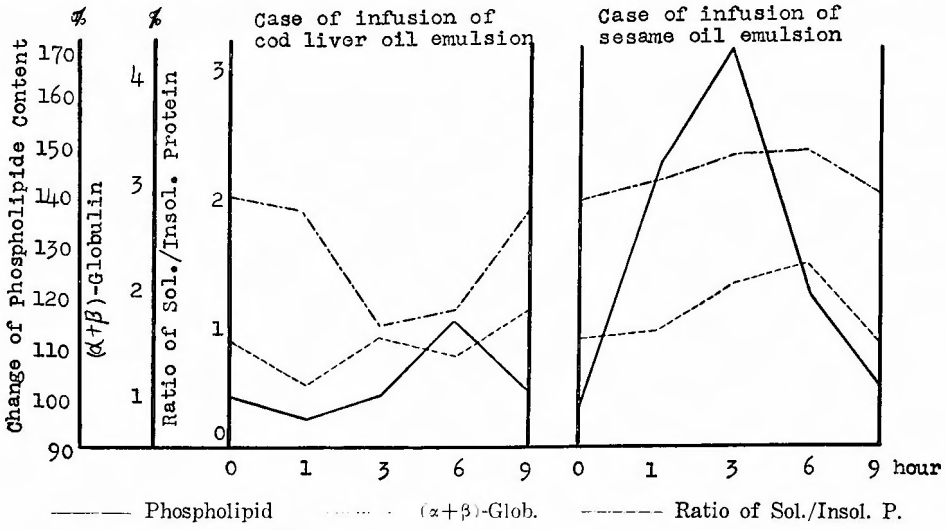


Table 7 Changes of Liver Protein Following Intravenous Administration of Sesame Oil Emulsion (Rats).

Time after Infusion	Tissue Protein (g%)		Ratio of Saline-Soluble to Insoluble Protein	
	Liver	Kidney	Liver	Kidney
0	17.12	8.81	1.41	2.07
1 hr	17.75	9.81	1.71	2.15
3 hrs	18.18	9.98	1.82	2.19
6 hrs	17.93	10.26	1.95	2.26
9 hrs	17.46	8.63	1.88	2.03

Table 8 Changes of Liver Protein Following Intravenous Administration of Sesame Oil Emulsion (Gastric Ulcer Cases).

Time after Infusion	Kunkel-Unit		Ratio of Saline-Soluble to Insoluble Potein	Electrophoretic Fractions of Liver Protein (g%)			
	Serum Lipid	Saline-Soluble Liver Lipid		Alb.	α-Glob.	β-Glob.	γ-Glob.
0	24	9.3	0.84	4.05	1.77	0.94	3.17
3 hrs	3[illegible]	15.0	1.34	5.67	2.14	1.45	2.63
6 hrs	21	8.3	1.04	5.38	1.57	1.27	3.81
12 hrs	26	9.3	0.89	6.01	1.46	0.95	2.59

of the infusion of cod liver oil emulsion than sesame oil emulsion, and the liver is the chief site for the formation of ketone bodies[36] (Table 9). HASHINO[49], in our laboratory, reported that blood ketone body levels and their urinary excretions increase after the infusion of sesame oil emulsion alone or with methionine, but their increase were not shown in the case of the simultaneous infusion of riboflavin, pantothenic acid, nicotinic acid and vitamin C (Table 10).

Table 9

A: Rate of Ketone Body Production in Tissues Following Intravenous Infusion of Cod Liver Oil Emulsion into Rats.
(Values were expressed as μ moles of acetoacetic acid per 100 mg dry weight of tissue per hour.)

Time after infusion		0	1 hr	3 hrs	6 hrs	9 hrs	12 hrs
Liver	Mean	3.86	4.11	5.63	8.91	6.74	5.82
	Change (%)	0	+ 6	+46	+131	+75	+51
Kidney	Mean	0.81	0.78	0.85	0.87	0.76	0.79
	Change (%)	0	− 4	+ 5	+ 7	− 6	− 2
Skeletal Muscle	Mean	0.66	0.68	0.63	0.68	0.64	0.67
	Change (%)	0	+ 3	− 5	+ 3	− 3	+ 2

B: Rate of Ketone Body Production in Tissues Following Simultaneous Infusion of Methionine with Cod Liver Oil Emulsion into Rats.
(Values were expressed as μ moles of acetoacetic acid per 100 mg dry weight of tissue per hour.)

Time after infusion		0	1 hr	3 hrs	6 hrs	9 hrs	12 hrs
Liver	Mean	3.86	4.30	6.65	8.92	7.41	6.04
	Change (%)	0	+11	+72	+131	+92	+56
Kidney	Mean	0.81	0.83	0.89	0.84	0.86	0.87
	Change (%)	0	+ 2	+10	+ 4	+ 6	+ 7
Skeletal Muscle	Mean	0.66	0.67	0.69	0.60	0.69	0.67
	Change (%)	0	+ 2	+ 5	− 9	+ 5	+ 2

C: Rate of Ketone Body Production in Tissues Following Intravenous Infusion of Sesame Oil Emulsion into Rats.
(Values were expressed as μ moles of acetoacetic acid per 100 mg dry weight of tissue per hour.)

Time after infusion		0	1 hr	3 hrs	6 hrs	9 hrs	12 hrs
Liver	Mean	3.86	4.16	5.57	7.43	5.98	5.29
	Change (%)	0	+ 8	+44	+92	+55	+37
Kidney	Mean	0.81	0.86	0.85	0.89	0.83	0.85
	Change (%)	0	+ 6	+ 5	+10	+ 2	+ 5
Skeletal Muscle	Mean	0.66	0.72	0.75	0.73	0.69	0.70
	Change (%)	0	+ 9	+14	+11	+ 5	+ 6

OSA[27], in our laboratory, obserbed that the fat emulsion with such various vitamins spared not only protein but also storage fat (Table 11), and HSÜ[53] and TAKEDA[52], in our laboratory, recognized that the concentration of vitamin C and the ester-form of riboflavin in blood, liver, kidney and cardiac muscle decreased

Table 10

A: Effect of Simultaneous Infusion of Various Drugs with Sesame Oil Emulsion into Rabbits (Single Infusion).

Remarks	Changes of Blood Ketone Body Levels						Urine	
	0	3 hrs	6 hrs	9 hrs	12 hrs	18 hrs	Concentration (mg/dl)	Excretion (mg)
Emulsion	0	+ 48	+111	+160	+324	+233	1.86	2.86
Emulsion+Methionine	0	+247	+322	+344	+393	+354	3.03	2.81
Emulsion+Methionine+ Riboflavin+Vitamin C	0	+ 59	+ 83	+ 76	+ 4	+ 18	1.70	1.52
Emulsion+Methionine+ Riboflavin+Vitamin C +Nicotinic Acid+ Pantothenic Acid	0	+ 20	+ 7	+ 22	+ 14	+ 33	1.31	1.18
Control(7% Glucose Solution)	0	+ 47	+ 71	+ 54	+ 44	+ 53	1.45	1.29

B: Effect of Simultaneous Infusion of Various Drugs with Sesame Oil Emulsion into Rabbits (Repeated Infusion).

Remarks	Ketone Bodies in Blood (mg/dl)	Ketone Bodies in Urine (mg/day)	Change of Body Weight (%)
Emulsion	1.05	5.26	+ 15.1
Emulsion+Methionine+Riboflavin+ Vitamin C	1.03	2.43	+ 17.6
Emulsion+Methionin+Riboflavin+ Vitamin C+Nicotinic Acid+ Pantothenic Acid	1.12	1.78	+ 18.8

Table 11 Effect of Daily Infusion of Sesame Oil Emulsion for 20 Days into Rabbits Fed with Low Protein and Caloric Diet.*

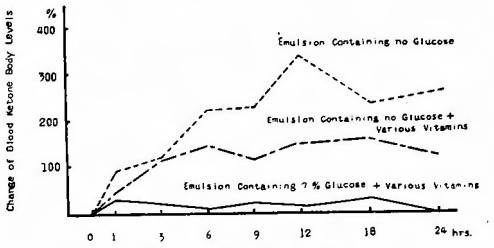
Infused Drugs	Loss of Body Weight (%)	Nitrogen Balance (g)	Loss of Storage Fat (g)
None	39.1	− 12.383	469.0
Emulsion	17.8	− 4.359	236.0
Emulsion+Methionine	17.5	− 4.168	266.0
Emulsion+Methionine+ Riboflavin	13.7	− 3.624	194.0
Emulsion+Methionine+ Riboflavin+5% Glucose (10cc)	8.1	− 1.808	113.0
Emulsion+Methionine+ Riboflavin+Vitamin C+ Nicotinic Acid+5% Glucose (10cc)+Vitamin B_1	6.6	− 1.121	106.0

Remarks

*The reducing diet of 27 g of wheat bran, 75 g of radish leaves, and water in adequate volume was given. The nitrogen content was 540 mg for wheat bran, 600 mg for radish leaves, which is the equivalent of total of 116 Cal. For the first 10 days the fat emulsion was daily infused at the rate of 0.5 g of fat per kg body weight, and for the latter 10 days, at the rate of 1.0 g of fat per kg body weight.

after receiving sesame oil emulsion intravenously. In the above mentioned experiments, the fat emulsions containing 7 per cent glucose were used (Fig. 10); however, blood ketone body levels increased remarkably after infusion of the fat emulsions containing no glucose, even if various vitamins were given simultaneously. It is evident from these results that the additional amount of glucose is necessary to utilize effectively and smoothly the infused fat in the case of lack or deficiency of glycogen.

Fig. 10 Is Simultaneous Infusion of Glucose Necessary or not for the Intravenous Administration of Sesame Oil Emulsion into Normal Rabbits?



KUROKAWA[54], in our laboratory, reported that blood levels of pyruvic acid, lactic acid and ketone bodies in dogs increased after ether anesthesia and remarkably decreased in the case of the injection of vitamin B_1, riboflavin and vitamin C before anesthesia. Same findings were obtained even in the case of starved or fasted anesthetized dogs, when the subcutaneous injection of additional amount of glucose was made before anesthesia. Consequently, these results indicate that the disturbance of the fat metabolism in anesthesia is a secondary change due to defective carbohydrate metabolism.

It is emphasized here that in starvation storage fat are mobilized and the fatty acids are oxidized excessively in the liver. In fact, phospholipids appeared in the hepatic parenchymatous cells in far greater quantities than in the case of the infusion into normal rabbits, when the standard dose of the sesame oil emulsion was infused intravenously into the starved rabbits (Fig. 11). However, when glucose and the above mentioned drugs were administered intravenously with sesame oil emulsion into the starved rabbits, the amounts of the phospholipids appearing in the hepatic parenchymatous cells were only observed to an extremely slight degree (Fig. 12).

From this fact, it is presumed that the role of glucose in fat metabolism is to accelerate the formation of Adenosine-triphosphate which is produced by the oxidation processes of carbohydrate, to "spark" the initial reaction of fatty acid oxidation and to raise the production of oxaloacetic acid which is necessary to lead Acetyl•CoA into the Tricarboxylic Acid Cycle.

The determination of respiratory quotient in the rat tissues receiving fat emulsion gave an evidence that riboflavin and nicotinic acid, which are concerned as a hydrogen carrying system, pantothenic acid, which is the principal component of coenzyme A, and vitamin C, which activates aconitase, played the important role in the process of fatty acid oxidation[55] (Table 12).

These findings on the catabolic process of fat metabolism in vivo were in accordance with the results in vitro by GREEN, OCHOA and LYNEN, who studied it biochemically using mitochondria. Namely, that fatty acid —— also in vivo —— breaks down to Acetyl•CoA by the Fatty Acid Cycle (after LYNEN) and, in the presence of oxaloacetic acid, enters into the Tricarboxylic Acid Cycle, in which it

Fig. 11

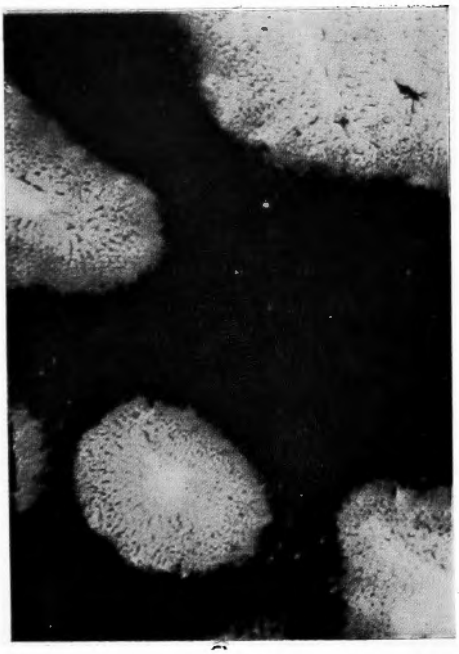


Fig. 12

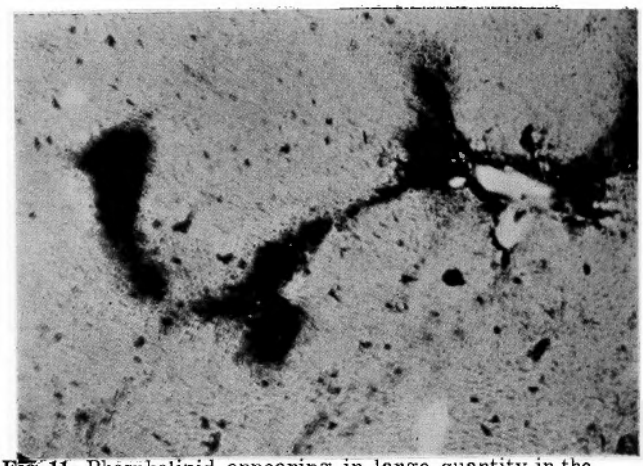


Fig. 11. Phospholipid appearing in large quantity in the hepatic parenchymatous cells 3 hours after intravenous administration of sesame oil emulsion into starved rabbits(*Smith-Dietrich*'s stain).

Fig. 12. Phospholipid appearing in small quantity in the hepatic parenchymatous cell 3 hours after simultaneous infusion of glucose and various vitamins with sesame oil emulsion into starved rabbits (*Smith-Dietrich's* stain).

Table 12

A: Q_{O_2}, Q_{CO_2} and R.Q. of Various Tissues Following Intravenous Infusion of Sesame Oil Emulsion into Rats (Mean).

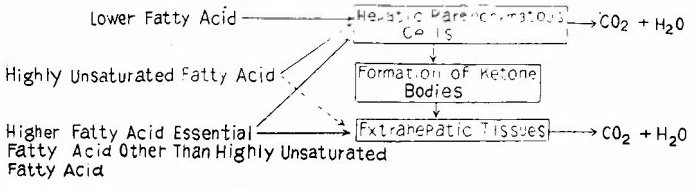
Time after infusion		0	1 hr	3 hrs	6 hrs	9 hrs	12 hrs
Liver	Q_{O_2}	7.30	9.56	13.94	16.94	17.73	18.68
	Q_{CO_2}	6.35	7.55	8.92	8.34	10.64	13.26
	R.Q.	0.87	0.79	0.64	0.51	0.60	0.71
Kidney	Q_{O_2}	21.09	22.89	27.93	32.14	33.05	34.09
	Q_{CO_2}	18.35	19.00	20.95	22.50	24.46	26.59
	R.Q.	0.87	0.83	0.75	0.70	0.74	0.78
Spleen	Q_{O_2}	10.15	11.24	14.06	16.60	17.27	18.25
	Q_{CO_2}	8.83	9.44	10.69	12.28	13.13	14.24
	R.Q.	0.87	0.85	0.76	0.74	0.76	0.78
Lung	Q_{O_2}	10.51	11.84	12.80	13.49	15.28	16.80
	Q_{CO_2}	9.25	9.95	10.11	10.12	11.77	13.27
	R.Q.	0.88	0.84	0.79	0.75	0.77	0.79
Cardiac Muscle	Q_{O_2}	5.42	5.82	6.39	6.72	7.40	7.70
	Q_{CO_2}	4.72	4.83	5.05	5.04	5.62	6.08
	R.Q.	0.87	0.83	0.79	0.75	0.76	0.79
Skeletal Muscle	Q_{O_2}	1.30	1.36	1.43	1.52	1.63	1.66
	Q_{CO_2}	1.14	1.16	1.14	1.16	1.26	1.33
	R.Q.	0.88	0.85	0.80	0.76	0.77	0.80

B: Qo_2, Qco_2 and R.Q. of Various Tissues Following Simultaneous Infusion of Methionine, F. A. D., Vitamin C and Pantothenic Acid with Sesame Oil Emulsion into Rats (Mean).

Time after infusion		0	1 hr	3 hrs	6 hrs	9 hrs	12 hrs
Liver	Qo_2	7.30	10.22	14.67	18.47	20.07	20.73
	Qco_2	6.35	7.46	9.53	11.08	12.44	14.51
	R.Q.	0.87	0.73	0.65	0.60	0.62	0.70
Kidney	Qo_2	21.09	24.96	31.97	35.04	35.23	36.00
	Qco_2	18.35	19.97	23.36	24.87	26.06	27.77
	R.Q.	0.87	0.80	0.73	0.71	0.74	0.77
Spleen	Qo_2	10.15	11.38	16.20	18.36	19.01	19.78
	Qco_2	8.83	8.99	12.06	13.18	14.05	15.00
	R.Q.	0.87	0.80	0.74	0.72	0.74	0.76
Lung	Qo_2	10.51	12.09	13.68	15.07	17.06	18.00
	Qco_2	9.25	9.80	10.16	10.91	12.72	13.82
	R.Q.	0.88	0.81	0.74	0.71	0.75	0.77
Cardiac Muscle	Qo_2	5.42	6.06	6.96	7.20	7.68	8.06
	Qco_2	4.72	4.97	5.26	5.17	5.75	6.21
	R.Q.	0.87	0.82	0.76	0.72	0.75	0.77
Skeletal Muscle	Qo_2	1.30	1.46	1.64	1.69	1.74	1.83
	Qco_2	1.14	1.20	1.26	1.23	1.31	1.41
	R.Q.	0.88	0.82	0.77	0.73	0.75	0.77

is oxidized to water and carbon dioxide through the route as shown in Fig. 13.

Fig. 13



IV. SUMMARY

The processes of the fat metabolism in vivo were studied with the fat emulsion which was produced in our laboratory.

In the case of the oral administration of fat, the fats which were absorbed by the chyle in the form of glyceride are first phagocytized by the alveolar phagocytes, KUPFFER'S stellate cells and reticuloendothelial cells of the spleen, then are changed into phospholipids in these cells and diffuse into the blood stream. Afterwards, the phospholipids, being changed from glycerides of highly unsaturated fatty acids and lower fatty acids, enter chiefly into the hepatic parenchymatous cells in the form of lipoprotein, while the phospholipids, being changed from higher fatty acids other than highly unsaturated fatty acids, enter not only into the hepatic parenchymatous cells but also into the extrahepatic tissues. However, even highly unsaturated fatty

acids can directly enter into the extrahepatic tissues in small quantities.

Even in the case of intravenous administration, the glycerides containing in our artificial fat emulsion are oxidized through the same metabolic processes as those containing in the chyle which is physiologically absorbed by the oral administration. Thus, fatty acids break down finally to water and carbon dioxide in the hepatic parenchymatous cells and the extrahepatic tissues. However, in the liver, the major part of fatty acids are converted to the stage of ketone bodies which diffuse into the blood stream with an increase in ketone body levels. When ketone bodies are carried to the extrahepatic tissues to enter into the final metabolic pathway, an increase in oxygen consumption in these tissues is observed. Accordingly, the process of fatty acid oxidation divides into direct oxidation, by which fatty acids are directly oxidized in tissues, and indirect oxidation, by which they are oxidized finally in the extrahepatic tissues after conversion to ketone bodies.

When the fat emulsion are given simultaneously with methionine, riboflavin, vitamin C, pantothenic acid and nicotinic acid, the infused fat is utilized more smoothly and effectively. The importance of glucose and vitamin B_1 in fat metabolism was also clearly demonstrated.

From the stand-point of the above mentioned catabolic processes of fat metabolism and the mechanism of fat absorption, it is suggested that the emulsive form of the glycerides of the higher fatty acids than lauric acid (myristic, palmitic, stearic, oleic, linoleic, linolenic acid) are extremely effective as parenteral nutritional supplements[48),56),57)].

V CONCLUSION

We have carried on our fundamental studies on the parenteral nutritional supplement of fat for the past several years and have also been able to clarify for the most part the catabolic process of fat metabolism. Nowadays, a safe, effective sesame oil emulsion without side effects for clinical use has been successfully prepared in our laboratory.

In closing, the writers wish to thank Dr. S. Kato and members of his staff of Research Laboratory, Osaka Factory, Dainippon Pharmaceutical Co.. The writers are also grateful to Lecturer Dr. S. Tomizawa of the Pharmacological Division, Keio University, for his valuable suggestions and criticisms throughout the present investigation. Thanks are due to Assist. Prof. M. Noda of the Biochemical Laboratory, Saikyo University, for invaluable advice and guidance in examining the writer's chromatogram.

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