EXPERIMENTAL STUDIES ON THE INTRAVENOUS ADMINISTRATION OF A FAT EMULSION FOR NUTRITIONAL PURPOSES

by

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Surgical operation has two main dangers, one caused by operation technique itself, and the other dependent upon the patient's pathological condition. For the purpose of preventing the latter danger, a surgeon administers parenteral nutrition to the patient. Accordingly, for the purpose of more effectively utilizing amino acids which have been parenterally administered, even though quantitatively insufficient, for tissue protein synthesis, there would be no alternative but to supplement the resultant caloric insufficiency with carbohydrate and fat. This is the very reason why we surgeons expect on the "*Protein Sparing Effect*" of carbohydrate and fat. But, notwithstanding, fat compares far more advantageously with carbohydrate with respect to calories. Thus far a very few studies on parenteral nutrition with fat have been performed. Independent of studies of McKIBBIN and SHAFIROFF, in 1949, we succeeded in our laboratory in producing a fat emulsion which could be safely given intravenously, and since then we have continued this experimental study, and the clinical observation on the same. TSUKADA in our laboratory reported that cod liver oil emulsion, which was infused intravenously, was changed in the body through the stage of lipoprotein for once when the infused fat was effectively utilized in the body. However, quite different from the oral administration of fat, in order to achive the objective of nutrition with fat by infusing a fat emulsion intravenously such as we have done experimentally, a fat emulsion which principally contains triglycerides of long chain fatty acids, such as oleinic acid, etc., should be used, as ascertained by SHIROTANI in our laboratory in his study on the fat content in thoracic duct lymph. On the other hand, from the results obtained in the experiments on hemolytic phenomenon, it was also found that the use of triglycerides of short chain fatty acids should absolutely be avoided. Thus, it became evident that the use of cod liver oil emulsion containing comparatively large quantities of short chain fatty acids as mentioned above, is undesirable. For these reasons, we have prepared and used sesame oil emulsion which does not contain any short chain fatty acids. The author has done electrophoretical determinations of the changes in circulating lipoprotein in the case of intravenous infusion of sesame oil emulsion. These results have been compared with the previous reports by TSUKADA, in which he used a cod liver oil emulsion. The author studied the

Table 1

Electrophoretic Fractions in Normal Rabbit Serum. (Value shows mean of 90 samples.)

T. P. g/dl	Alb. %	α-Glob. %	β-Glob. %	γ-Glob. %
5.94 (5.4～7.4)	60.7 (48.9～63.5)	8.9 (6.3～16.7)	18.1 (13.3～28.3)	12.3 (5.0～20.9)

Table 2

Electrophoretic Fractions of Rabbit Serum before and after Infusion of Fat Emulsion.

Time after infusion	Rabbit No.	T. P. g/dl	Alb. %	α-Glob. %	β-Glob. %	γ-Glob. %
0	18	6.2	59.5	10.4	18.4	11.7
10 min.		6.4	61.4	10.9	16.7	11.0
0	20	5.8	57.8	6.2	20.6	15.4
30 min.		5.8	65.4	7.2	17.3	10.1
0	21	5.6	66.5	8.2	17.0	8.3
1 hrs.		5.4	65.6	6.5	19.4	8.5
0	22	5.4	62.6	10.3	22.4	5.0
3 hrs.		5.0	62.7	11.8	20.4	5.1
0	23	6.4	48.9	9.7	19.1	22.3
6 hrs.		6.0	49.7	8.6	21.2	20.5
0	24	5.6	60.0	8.6	23.1	8.3
12 hrs.		5.0	60.5	9.1	22.1	8.3
0	25	5.6	64.9	8.8	18.5	7.8
24 hrs.		5.6	64.7	9.1	18.5	7.4
0	26	7.0	60.1	7.1	17.3	15.5
36 hrs.		6.4	50.6	10.2	24.9	14.3
0	27	7.2	61.6	7.5	15.8	15.1
48 hrs.		6.4	58.3	8.0	19.3	14.4

Table 3

Electrophoretic Fractions of Rabbit Serum before and after Simultaneous Infusion of Fat Emulsion with Methionine.

Time after infusion	Rabbit No.	T. P. g/dl	Alb. %	α-Glob. %	β-Glob. %	γ-Glob. %
0	36	6.4	61.4	8.9	14.2	15.5
10 min.		6.3	62.7	9.3	13.4	14.6
0	28	6.3	54.3	8.9	19.9	16.9
30 min.		6.0	60.0	9.4	17.5	13.1
0	29	5.6	59.5	8.6	20.6	11.3
1 hrs.		5.4	59.2	7.7	23.7	9.4
0	30	6.0	63.5	8.4	15.9	12.2
3 hrs.		5.4	64.2	6.8	16.6	12.4
0	31	6.1	66.8	7.9	15.8	9.5
6 hrs.		5.7	65.2	8.6	16.4	9.8
0	32	6.6	43.7	5.9	15.9	34.5
9 hrs.		5.7	44.4	5.7	16.4	33.5
0	33	5.6	65.1	8.6	16.1	10.2
12 hrs.		5.4	62.7	9.7	16.5	11.1
0	34	6.4	60.5	7.7	13.8	17.9
24 hrs.		6.4	57.1	7.7	19.1	15.8
0	35	6.0	52.5	12.2	24.1	11.2
36 hrs.		6.0	59.9	10.4	19.5	10.3

problem of whether or not the fat, which is parenterally administered in the form of this emulsion, is effectively utilized in the body with the "*Protein Sparing Effect*," by observing the changes of body weight, serum protein, nitrogen balance, and the effects on the processes of tissue protein synthesis.

MATERIALS AND METHODS

Fat Emulsion: The following fat emulsion was used; 15 per cent sesame oil emulsion with 7 per cent glucose. In the present experiment, this fat emulsion in the amount of 0.5 g of fat per kg body weight, was infused intravenously into rabbits.

Experimental Animals: Adult male rabbits, in cages with urine collecting apparatus, were fed for at least 3 weeks with a standard diet (Wheat bran, 800 g : Protein 11.0 g 256 Cal., Radish leaves, 150 g : Protein 7.6 g. 60 Cal. and water in adequate volume.). In certain cases, adult male dogs were used.

Serum Protein: Blood samples were removed from the auricular vessels 24 hours after the supplement with diet. Total serum protein concentration was estimated refractometrically by means of HITACHI's refractometer. The electrophoretic analysis of the sera was performed by use of HITACHI's HT-B type of electrophoretic apparatus. In the present experiments, veronal buffer solution, containing 0.1 M

Table 4

Controls. Electrophoretic Fractions of Rabbit Serum before and after Simultaneous Infusion of Glucose Solution with Methionine.

Time after infusion	Rabbit No.	T. P. g/dl	Alb. %	α-Glob. %	β-Glob. %	γ-Glob. %
0	40	6.0	55.2	9.0	21.8	14.0
1 hrs.		5.7	63.0	7.0	18.6	15.4
0	41	6.3	63.4	6.9	14.5	15.2
3 hrs.		6.0	65.1	6.5	14.2	14.2
0	42	5.4	60.4	11.7	17.6	10.3
6 hrs.		5.6	60.1	12.2	17.9	9.8
0	43	6.1	64.5	8.0	17.4	10.1
12 hrs.		6.0	62.4	8.3	18.6	10.7
0	45	5.8	68.5	7.7	16.5	7.3
24 hrs.		5.8	63.6	7.2	21.5	7.7

$C_8H_{11}O_3N_2Na$, 0.02 M $Co(NH)_2(Co)_2C(C_2H_5)_2$, with a pH of about 8.6 and an ionic strength of 0.1, was used. Determination of circulating serum volume was made electorophotometrically by use of 0.3 per cent Evans-blue. The estimation of serum water was made by KURODA's modified method.

Nitrogen Determination: Urinary and fecal nitrogen excretion were determined by KJELDAHL analysis.

Experimental hypoproteinemia in rabbits, fed by ordinary low protein, low calorie feeding for 7 days, was induced by plasmapheresis, leading to decrease of about 4.0 g per cent in total serum protein concentration.

The Determination of Liver Tissue Protein: Physiological saline-soluble or insoluble protein was determined by FISHMAN, & VEEN'S method.

RESULTS

1. EXPERIMENTAL RESULTS IN NORMAL HEALTHY RABBITS.

(A) Total serum protein concentration and electrophoretic-fractionated serum protein were as shown in Table 1, in 90 cases of adult healthy rabbits, each weighing about 2.0 kg.

(B) Changes in serum protein components in case of single infusion of fat emulsion : ASADA, NAKATA, SENŌ, NISHINO, and others in our laboratory previously carried out the histological and biochemical studies on the metabolic process of fat when cod liver oil emulsion was infused intravenously. They pointed out the fact that the infused fat is firstly phagocytized by the alveolar phagocytes, KUPFFER's cells, reticuloendothelial cells of the spleen, etc., and also that the neutral fat gradually changes over to phospholipid in these cells, and then enters the hepatic parenchymatous cells where it is oxidized. Furthermore, TSUKADA studied the metabolic process of fat in body from the viewpoint of protein metabolism, and revealed that

Table 5

Changes in Serum Protein and Serum Water Following Intravenous Infusion of Fat Emulsion. (Each values show mean of samples.)

Remarks	Serum	0	10 min.	30 min.	1 hrs.	3 hrs.	6 hrs.	9 hrs.	12 hrs.	24 hrs.
Emulsion + Methionine	T. P. (g/dl)	5.2	5.35	5.2	5.2	4.9	4.8	5.2	5.5	5.2
	S. W. (%)	93.31	93.25	93.32	93.34	93.89	93.80	93.48	92.92	93.01
Control	T. P. (g/dl)	6.2	6.0	5.8	5.8	5.8	6.0	6.2	6.0	6.2
	S. W. (%)	92.34	92.44	92.64	92.68	92.58	92.31	92.18	92.55	92.11

infused fat must pass through the phospholipid stage in order to be utilized in body. It has thus been clarified that plasma lipids combine with protein to form lipoprotein, and exist in plasma in the form of α- and β-globulin fractions, and that β-lipoprotein consists of 25 per cent protein and 75 per cent lipids. Furthermore, it has recently become evident that 30 per cent of the above lipids are phospholipids, 45 per cent of the latter being cholesterol ester and free cholesterol. On the other hand, it has been found that α-lipoprotein contains 65 per cent protein and 25 per cent lipids. Thus α- and β-globulin are comprised of lipids in excess of 50 per cent. The increase in α- and β-globulin fractions affects the refractive index of serum protein fraction as an increase in lipids rather than increase in protein. This becomes recognizable as though an increment of α-and β-globulin fraction in the case of the infusion of the cod liver oil emulsion for the infused fat formed lipoprotein, and for its ability to change step by step into the blood lipids inherent in the body. The author studied the fluctuation in α- and β-globulin in the infusion of sesame oil emulsion, and compared the results with those obtained in TSUKADA's experiments.

Table 6

Electrophoretic Fractions of Dog Serum before and after Infusion of Fat Emulsion.
(Each values show mean of 2 samples.)

Remarks	Time after infusion	T. P. g/dl	Alb. %	α-Glob. %	β-Glob. %	γ-Glob. %
Emulsion + Methionine	0	7.1	43.65	22.35	22.15	11.85
	30 min.	6.2	43.30	26.05	18.55	12.10
	1 hrs.	6.45	42.70	23.80	22.05	11.45
	2 hrs.	6.4	43.55	23.60	20.85	12.00
	4 hrs.	6.1	44.30	22.90	21.45	11.35
	6 hrs.	6.1	44.15	22.45	21.75	11.65

Table 7A

Controls. Nitrogen Balance. (Each values show mean of 2 samples.)

Remarks		Days of infusion 0	10	20	Total
Body weight	kg	2.10	1.52	1.26	
Weight loss	g		580	260	840
Decrease	%		29.3	9.8	39.1
Urinary nitrogen output	g		18.015	8.785	26.800
Fecal nitrogen output	g		1.803	0.809	2.612
Nitrogen balance	g		-9.169	-3.211	-12.383

Table 7B

Controls. Electrophoretic Fractions of Serum.
(Each values show mean of 2 samples.)

Serum protein		Days of infusion 0	10	20
T. P.	g/dl	5.8	5.1	
Alb.	%	59.45	59.2	
α-Glob.	%	8.15	8.15	
β-Glob.	%	16.4	18.8	
α-Glob.	%	16.0	13.85	

(i) EXPERIMENTAL RESULTS IN RABBITS.

Single Administration of the Fat Emulsion: It is obvious that the total serum protein and the electrophoretic components must undergo a change when repeated blood samples of 7 cc were removed from the same test rabbit for electrophoretic analysis. Therefore, to avoid this, collection of blood samples from the same rabbit was not done successively. When the first blood speciments

Table 8

Effect of Daily Infusion of Fat Emulsion on Nitrogen Balance. (Each values show mean of 3 samples.)

Remarks		Days of infusion 0	10	20	Total
Body weight	kg	2.130	1.880	1.760	
Weight loss	g		250	120	370
Decrease	%		11.9	5.9	17.8
Urinary nitrogen output	g		11.655	10.651	22.306
Fecal nitrogen output	g		1.788	1.564	3.352
Nitrogen balance	g		-2.793	-1.566	-4.359

Table 9

Effect of Daily Infusion of Fat Emulsion on Electrophoretic Fractions of Serum. (Each values show mean of 3 samples.)

Serum protein		Days of infusion 0	10	20
T. P.	g/dl	5.3	5.5	5.6
Alb.	%	62.1	60.2	56.6
α-Glob.	%	10.4	9.9	10.7
β-Glob.	%	16.6	18.7	19.4
γ-Glob.	%	10.9	11.2	13.3

Table 10

Effect of Daily Infusion of Fat Emulsion with Methionine on Nitrogen Balance. (Each values show mean of 3 samples.)

Remarks		Days of infusion 0	10	20	Total
Body weight	kg	2.213	1.947	1.827	
Weight loss	g		266	120	386
Decrease	%		12.4	5.1	17.5
Urinary nitrogen output	g		10.967	11.097	22.064
Fecal nitrogen output	g		1.768	1.632	3.400
Nitrogen balance	g		-2.088	-2.080	-4.168

were collected, total serum protein concentration and electrophoretic fraction were measured. Then 7 days later the fat emulsion was intravenously infused at the rate of 0.5 g of fat per kg body weight into a rabbit which was in the postabsorptive state. The blood samples were drawn from each different rabbit 10 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, 12 hours, 24 hours, 36 hours and 48 hours, respectively, after the above infusion of fat emulsion, and, the total serum protein concentration and the electrophoretic fraction were estimated, so as to make comparative study of the levels before and after the infusion.

As indicated in Table 2, the total serum protein concentration slightly increased 10 minutes after infusion. Thirty minutes after infusion and thereafter it decreased progressively. No abnormal changes were observed in the electrophoretic patterns. The albumin showed a slight tendency to increase 10～30 minutes after infusion, but no pronounced change was observed until 24 hours after. The α- and β-globulin showed a slight tendency to increase 30 minutes～one hour after infusion, and continued only slight fluctuations up to 6 hours. The increase in the γ-globulin was not clearly observed. As compared with the findings in case of the cod liver oil emulsion reported by TSUKADA, it was observed that the sesame oil emulsion could be utilized far more smoothly than the cod liver oil emulsion. According to the experimental results by IZUKURA, when sesame oil emulsion, which contains no short chain fatty acids, was infused intravenously into rabbits, the infused fat, which was changed into phospholipid from neutral fat by the reticulo-

endothelial cells was processed not only in the liver but in every tissue of the body where it could be oxidized. On the contrary, in case of infusion with cod liver oil emulsion, triglycerides of short chain fatty acids which are contained in a comparatively large quantity, was always oxidized only in the hepatic parenchymatous cells after it had been changed into phospholipid, and could not be oxidized in the extrahepatic tissues, with the result that there occurred a remarkable accumulation of phospholipid in the hepatic parenchymatous cells. A similar conclusion can be reached from the results obtained in the experimental observation by HASHINO that cod liver oil emulsion is obligatory ketogenic, whereas sesame oil emulsion is facultative ketogenic. Hence, it seems logical that the elevation of blood lipid levels, which diffused into the circulating blood after having been phagocytized by the reticuloendothelial cells, is liable to occur in case of infusion with cod liver oil emulsion for the reason that the cod liver oil emulsion is apt to be slow in the oxidative process of phospholipid in tissues as compared with the sesame oil emulsion. We believe that the elevation of blood lipid levels influenced the electrophoretic patterns, especially the changes of α- and β-globulin, and caused a significant difference between the cod liver oil emulsion and the sesame oil emulsion.

Simultaneous Infusion of the Fat Emulsion with Methionine: ASADA, and others previously clarified the facts that methionine enhances the activity of the reticuloendothelial cells to utilize fat,

Table 11

Effect of Daily Infusion of Fat Emulsion with Methionine on Electrophoretic Fractions of Serum.
(Each values show mean of 3 samples).

Serum protein		Days of infusion		
		0	10	20
T. P.	g/dl	5.96	5.7	5.5
Alb.	%	62.6	63.7	56.5
α-Glob.	%	9.8	8.6	11.8
β-Glob.	%	16.4	18.3	20.3
γ-Glob.	%	11.2	9.4	11.4

Table 12A

Controls. Effect of Daily Infusion of 7 per cent Glucose with Methionine on Nitrogen Balance.
(Each values show mean of 2 samples.)

Remarks		Days of infusion			Total
		0	10	20	
Body weight	kg	2.26	1.66	1.30	
Weight loss	g		600	360	960
Decrease	%		22.1	20.3	42.4
Urinary nitrogen output	g		21.063	15.275	36.338
Fecal nitrogen output	g		1.745	0.832	2.577
Nitrogen balance	g		-12.158	-9.777	-21.935

Table 12B

Controls. Effect of Daily Infusion of 7 per cent Glucose with Methionine on Electrophoretic Fractions of Serum.
(Each values show mean of 2 samples.)

Serum protein		Days of infusion		
		0	10	20
T. P.	g/dl	6.4	5.0	
Alb.	%	61.7	61.4	
α-Glob.	%	9.2	6.9	
β-Glob.	%	17.1	19.0	
γ-Glob.	%	12.0	12.7	

and that neutral fat is smoothly and rapidly changed into phospholipid in these cells, leading to the secondary acceleration of the process of oxidation of fatty acids in tissues. In order to determine what alteration the lipoprotein would undergo when the present fat emulsion was intravenously infused simultaneously with methionine, the author added 10 mg of *l*-methionine per kg body weight. As controls, 7 per cent glucose solution, 3.3 cc and *l*-methionine, 10 mg per kg body weight were simultaneously infused. As indicated in Table 3, a slight decrease in the total serum protein concentration was observed after infusion in all cases. However, no abnormal peak was shown in the electrophoretic patterns. A slight increase in the albumin was observed 10～30 minutes after infusion. Thirty minutes～one hour after, the α- and β-globulin showed a slight increase. Three hours after, no change in the α- and β-globulin was observed. Twenty-four hours after, the β-globulin again increased. No abnormal increase in the γ-globulin was shown. On the contrary, the controls (Table 4) indicated a slight decrease in the β-globulin one hour after and showed a increase after 24 hours. Therefore, the increase in the β-globulin after 24 hours which occurred in case of the simultaneous infusion with the fat emulsion and methionine is presumed to be due to the fact that the depot fat in body been mobilized by methionine. As above-mentioned, in case of single infusion of cod liver oil emulsion reported by TSUKADA, a remarkable increase was observed in the α- and β-globulin 24 hours after, and in case of the combination with methionine, the same remarkable increase in the α- and β-globulin occurred very rapidly. However, from the results obtained in the present experiments using sesame oil emulsion, there was noticed no significant difference in the changes of the α- and β-globulin between the infusion of the fat emulsion alone and the infusion of the fat emulsion in combination with methionine. This fact indicates that even in the case of the infusion of fat emulsion alone, the infused fat is smoothly and rapidly utilized, and there occurs no elevation of lipoprotein in blood.

Changes of the serum protein concentration : When this fat emulsion is intravenously infused, it is often noticed that the serum protein concentration is usually lowered in respective of whether or not methionine is simultaneously used. However, it does not seem reasonable that this result is attributable to the decrease in total circulating albumin. The increase in the serum water was observed at the very same time that the total serum protein concentration began to decrease as indicated in Table 5. As compared with the controls, the total serum protein concentration was lowered for a longer time, and the degree of the increment in the volume of water was more remarkable. The cause of these phenomena can hardly be considered due to the water in the emulsion. The mechanism of this phenomenon is thought to be due to the transitory elevation of blood lipid levels caused by the infusion of the fat emulsion, which elevates the plasma osmotic pressure, inducing the transport of tissue water into the circulating blood.

(ii) EXPERIMENTAL RESULTS IN DOGS.

As previously mentioned concerning rabbits, repeated collection of blood samples seriously effects the test animals. Therefore, dogs were used since they are cosidered to be comparatively unaffected by the repeated collection of blood samples. Studies on the serum protein before and after the infusion of the fat emulsion were carried out, and the results were compared with those obtained in rabbits. As shown in Table 6, similar to rabbits, the total serum protein concentration showed a tendency to decrease gradually, and failed to return to previous levels even 6 hours after infusion. Regarding the pattern, 2 or 3-irregular minor peaks appeared over the β-globulin boundary between 30 minutes and one hour. After that, the β-globulin boundary showed a single peak. No change was observed in the albumin fraction. As time passed, slight fluctuations in the α- and β-globulin fractions were noted. The γ-globulin fraction showed no remarkable fluctuation. In short, it seems that, similar to rabbits, the infused fat was smoothly utilized, and there was no elevation of lipid levels.

2. EXPERIMENTAL RESULTS DURING LOW DIET.

According to the recent studies on the oxidative process of fatty acid "*in vitro*", it has been shown that various enzyme systems participate in this metabolic process similar to other nutriments. HASHINO studied ketone body formation and its relations with enzyme systems "*in vivo*". The present author further studied the relations between fat and enzyme systems as related to protein metabolism, and determined whether or not the present fat emulsion would demonstrate the "*Protein Sparing Effect*". Rabbits were fed with a standard dosage diet for more than 3 weeks. 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 a 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

Table 13

Effect of Daily Infusion of Fat Emulsion with Methionine, Riboflavin on Nitrogen Balance.
(Each values show mean of 3 samples.)

Remarks		Days of infusion			Total
		0	10	20	
Body weight	kg	2.200	1.997	1.900	
Weight loss	g		203	97	300
Decrease	%		9.2	4.5	13.7
Urinary nitrogen output	g		10.384	11.234	21.618
Fecal nitrogen output	g		1.784	1.517	3.301
Nitrogen balance	g		-1.519	-2.105	-3.624

Table 14

Effect of Daily Infusion of Fat Emulsion with Methionine, Riboflavin on Electrophoretic Fractions of Serum.
(Each values show mean of 3 samples.)

Serum protein		Days of infusion		
		0	10	20
T. P.	g/dl	5.9	5.8	5.7
Alb.	%	59.8	59.7	59.7
α-Glob.	%	9.7	8.6	10.2
β-Glob.	%	19.1	19.1	19.2
γ-Glob.	%	11.4	12.6	10.9

per kg body weight.

A. *Control.*

As shown in Table 7 A and 7 B, when the rabbits were fed by the above low calorie diet without any treatment, the body tissue protein was decomposed considerably, resulting in death before the end of the experiment.

Table 15A

Controls. Effect of Daily Infusion of 7 per cent Glucose with Methionine, Riboflavin on Nitrogen Balance.
(Each values show mean of 2 samples).

Remarks		Days of infusion			Total
		0	10	20	
Body weight	kg	2.130	1.600	1.270	
Weight loss	g		530	330	860
Decrease	%		25.1	13.4	38.5
Urinary nitrogen output	g		14.390	14.043	28.433
Fecal nitrogen output	g		1.689	1.142	2.831
Nitrogen balance	g		-5.429	-6.660	-12.089

Table 15B

Controls. Effect of Daily Infusion of 7 per cent Glucose with Methionine, Riboflavin on Electrophoretic Fractions of Serum.
(Each values show mean of 2 samples.)

Serum protein		Days of infusion		
		0	10	20
T. P.	g/dl	6.1	5.5	
Alb.	%	59.9	56.3	
α-Glob.	%	8.3	6.7	
β-Glob.	%	16.2	17.5	
γ-Glob.	%	15.6	19.5	

Table 16

Effect of Daily Infusion of Fat Emulsion with Methionine, Riboflavin, Addition of Glucose on Nitrogen Balance.
(Each values show mean of 3 samples.)

Remarks		Days of infusion			Total
		0	10	20	
Body weight	kg	2.140	2.060	1.967	
Weight loss	g		80	93	173
Decrease	%		3.5	4.6	8.1
Urinary nitrogen output	g		9.269	10.609	19.878
Fecal nitrogen output	g		1.685	1.545	3.230
Nitrogen balance	g		-0.304	-1 504	-1.808

B. *Group Infused with Fat Emulsion Alone.*

In the case of daily infusion of the fat emulsion alone for 20 days, protein sparing effect was observed to a remarkable degree (Table 8), but the β- and γ-globulin increased (Table 9). This fact shows that the infused fat could not be completely utilized. Histologically, however, traces of neutral fat could be observed in the reticuloendothelial cells, without abnormal findings such as fatty liver.

C. *Group Infused with Fat Emulsion and Methionine Simultaneously.*

As stated in the preceding chapter, it has been demonstrated by researchers of our laboratory that methionine accelerates phagocytosis and lipoidization of fat in the reticuloendothelial cells, and secondarily influences favorably the fatty acid oxidation in tissues. Based upon those experimental results, the fat emulsion was daily infused in combination with methionine, 5 mg per 0.5 g of fat for 20 days. As presented in Table 10 and 11, remarkable protein sparing effect was shown as compared with the controls, with 7 per cent glucose solution, 3.3 cc, and *l*-methionine, 5 mg per kg body weight (Table 12A, 12B).

However, compared with the group in which the fat emulsion alone was infused, no significant difference was found, besides the α- and β-globulin increased. Those results indicate that the infused fat could not be smoothly utilized by the simultaneous infusion of methionine alone.

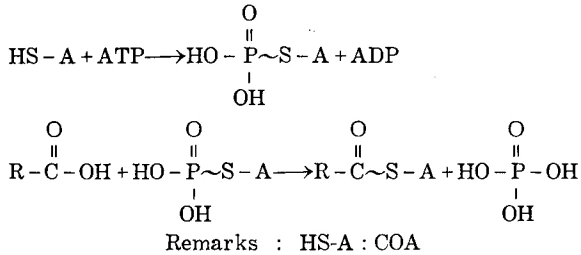
D. *Group Infused with Fat Emulsion, Methionine and Riboflavin Simultaneously.*

TSUKADA previously reported that it was indispensable to add methionine and riboflavin to the infusion of cod liver oil emulsion. Furthermore, HASHINO pointed out the importance of riboflavin being infused simultaneously, basing this on his experimental studies. NATH recently demonstrated that continuous injection of sodium acetoacetate and β-hydroxybutyrate into rabbits for 3 months produced a state of riboflavin and nicotinic acid deficiency. From these experimental results, *l*-methionine, 5 mg, riboflavin-5'-phosphate sodium solution, 2 mg per 0.5 g of fat, were infused simultaneously with the fat emulsion. As shown in Table 13 and 14, remarkable protein sparing effect was shown as compared with the above-mentioned two groups (B, C). On the contrary, the control (Table 15A, 15B), with 7 per cent glucose solution 3.3 cc, *l*-methionine, 5 mg and riboflavin, 2 mg per kg body weight, showed a marked decomposition of body tissue protein. In brief, from the fact that the decrease in the albumin and the increase in the α- and β-globulin could not be observed for the first time when riboflavin is used simultaneously, it is presumed that the infused fat could be utilized smoothly.

E. *Group Infused with Fat Emulsion, Methionine, Riboflavin and Glucose Solution Simultaneously.*

According to the recent studies of the process of fatty acid oxidation "*in vitro*", it has been clearly demonstrated that adenosine triphosphate(A. T. P.) and coenzyme A (CoA) is essential to "*spark*" the initial reaction of fatty acid oxidation. A. T. P. is produced from inorganic phosphate by progressing the process in which pyruvic acid, carbohydrate intermediate, is oxidized to carbon dioxide and water, namely the five-oxidation process in the tricarboxylic acid cycle (T. C. A. cycle). Thus CoA accepts phosphate from A. T. P., and the sulphydryl radical (SH radical) is phosphorylated. Phosphorylated CoA combines with fatty acid to activate the carboxyl radical of the fatty acid. (See Fig. 1) Fatty acid, which is activated by combination with CoA, enters into the fatty acid cycle to be broken down to acetyl-CoA. In other words, fatty acid oxidation is "*sparked*" by oxidation of a "*sparker*" (intermediates in T. C. A. cycle) which is produced by carbohydrate metabolism. Acetyl-CoA reacts with oxaloacetic acid, which is produced by carbohydrate metabolism, to enter into the T. C. A. cycle. The combined use of glucose should be considered in the intravenous infusion of fat emulsion for the purpose of the parenteral nutrition. Therefore, fat emulsion, to

Fig. 1



Remarks : HS-A : COA

which is added 7 per cent glucose solution, has been practically used. However, in the case of the present animals, it was obvious that the glycogen depots were liable to be deficient, and that an addition of 7 per cent glucose solution was insufficient to smooth the oxidation process of the contained fatty acids. To correct this, 5 per cent glucose solution, 5 cc per 0.5 g of fat was further added to the fat emulsion and simultaneously infused into the rabbits with *l*-methionine, 5 mg and riboflavin, 2 mg per 0.5 g of fat. The results are given in Table 16 and 17. As shown in Table 18A and 18B, no significant difference was recognized between the case of this control and the case of controls as mentioned in Table 12A, 12B and 15A, 15B. This fact signifies that the additional amount of glucose improved the process of fat metabolism, thus allowing the infused fat to be utilized more effectively and smoothly, producing such a remarkable protein sparing effect.

Table 17

Effect of Daily Infusion of Fat Emulsion with Methionine, Riboflavin, Addition of Glucose on Electrophoretic Fractions of Serum. (Each values show mean of 3 samples.)

Serum protein		Days of infusion		
		0	10	20
T. P.	g/dl	5.8	5.66	5.6
Alb.	%	61.1	61.2	61.4
α-Glob.	%	8.4	9.4	8.9
β-Glob.	%	17.2	17.9	18.2
γ-Glob.	%	13.3	11.5	11.5

Table 18A

Controls. Effect of Daily Infusion of Addition of Glucose with Methionine, Riboflavin on Nitrogen Balance. (Each values show mean of 2 samples.)

Remarks		Days of infusion			Total
		0	10	20	
Body weight	kg	2.150	1.750	1.440	
Weight loss	g		400	310	710
Decrease	%		18.8	14.7	33.5
Urinary nitrogen output	g		13.129	14.689	27.818
Fecal nitrogen output	g		1.796	1.605	3.401
Nitrogen balance	g		-1.375	-5.644	-10.019

Table 18B

Controls. Effect of Daily Infusion of Addition of Glucose with Methionine. Riboflavin on Electrophoretic Fractions of Serum. (Each values show mean of 2 samples.)

Serum protein		Days of infusion		
		0	10	20
T. P.	g/dl	6.2	5.8	5.4
Alb.	%	66.4	61.1	52.4
α-Glob.	%	7.4	8.3	10.3
β-Glob.	%	15.8	16.8	14.1
γ-Glob.	%	10.4	13.8	23.2

F. *Group Infused with Fat Emulsion, Methionine, Riboflavin, Vitamin B_1, Vitamin C and Nicotinic Acid Amide Simultaneously.*

As above mentioned, sufficient glucose is necessary for the fat metabolism to progress smoothly. However, in order to smooth the carbohydrate metabolism, Vitamin B_1 is necessary. Deficiency not only in Vitamin B_1 but also nicotinic acid amide and Vitamin C is liable to occur during these continued reducing diets. HIKASA and ISHIGAMI demonstrated that the combined use of ascorbic acid with the fat emulsion intensified the activity of lipase in the liver and serum, and that effectively influenced the metabolic process of the infused fat. Hence, the fat emulsion plus *l*-methionine, 5 mg,

Table 19

Effect of Daily Infusion of Fat Emulsion with Methionine, Riboflavin, Nicotinic Acid Amide, Thiamin Hydrochloride, Ascorbic Acid, and Addition of Glucose on Nitrogen Balance. (Each values show mean of 3 samples.)

Remarks		Days of infusion			Total
		0	10	20	
Body weight	kg	2.153	2.106	2.013	
Weight loss	g		47	93	140
Decrease	%		2.1	4.4	6.6
Urinary nitrogen output	g		9.072	9.941	19.013
Fecal nitrogen output	g		1.780	1.627	3.407
Nitrogen balance	g		-0.203	-0.918	-1.121

Table 20

Effect of Daily Infusion of Fat Emulsion with Methionine, Riboflavin, Nicotinic Acid Amide, Thiamin Hydrochloride, Ascorbic Acid, Addition of Glucose on Electrophoretic Fractions of Serum. (Each values show mean of 3 samples.)

Serum protein		Days of infusion		
		0	10	20
T. P.	g/dl	6.2	6.2	6.1
Alb.	%	62.5	63.0	62.5
α-Glob.	%	11.0	9.4	9.8
β-Glob.	%	15.6	15.7	16.3
γ-Glob.	%	10.9	11.9	11.4

Table 21

Changes of Dept Fat for 20 Days. (Each values show mean of samples.)

	Remarks	Weight loss (g)	Depot Fat loss (g)
Control	Without Infusion	840	469
	Glucose + Methionine	960	330
	Glucose + Methionine + Riboflavin	860	434
	G. + M. + R. + Addition of Glucose	710	412
Fat Emulsion	Fat Emulsion	370	236
	F. E. + Methionine	386	266
	F. E. + M. + Riboflavin	300	194
	F. E. + M. + R. + Glucose	173	113
	F. E. + M. + R. + G. + Thiamin Hydrochloride + Ascorbic Acid + Nicotinic Acid Amide.	140	106

riboflavin, 2.5 mg, 5 per cent glucose solution, 5 cc, thiamin hydrochloride, 5 mg, ascorbic acid, 10 mg and nicotinic acid amide, 5 mg per 0.5 g of fat were infused simultaneously into rabbits. The results of the experiments are presented in Table 19 and 20. Protein sparing effect in this group was more remarkable than that in above mentioned groups (B, C and D). According to the method of MOOR, the fat depots consumption of the respective groups during the present experiment was calculated, as indicated in Table 21. We could thus observe that the present fat emulsion spared not only protein but also fat depots.

3. RESULTS IN HYPOPROTEINEMIC RABBITS.

As described above, it has been demonstrated that the present fat emulsion shows a sparing effect of protein. Furthermore, the author examined the fluctuations in the serum protein and the liver tissue protein when fat emulsion was infused into hypoproteinemic rabbits, produced by plasmapheresis. Prior to the present experiment, the liver tissue protein in normal healthy rabbits was determined (Table 22). Rabbits were fed a daily low calorie diet with 50 g of Okara (Protein 2.3 g, 35 Cal), 200 g of Hakusai leaves (Protein 2.7 g, 24 Cal) and 12 g of Starch (40 Cal), while plasmapheresis was performed for 7 days. As indicated in Table

Table 22

Serum Protein and Liver Protein of Normal Rabbit.

Rabbit No.	Body weight	T. P.	Circulating serum volume	Circulating serum protein	Saline-soluble liver protein (A)	Saline-insoluble liver protein (B)	Ratio A/B
	kg	g/dl	cc	g	g/%	g/%	
101	2.00	6.2	96	5.95	11.9	6.7	1.77
102	2.50	6.6	101	6.66	11.6	6.9	1.68
103	2.54	5.8	108	6.26	11.3	6.8	1.66
Mean	2.34	6.2	101.6	6.29	11.6	6.8	1.70

Table 23

Serum Protein and Liver Protein in Experimental Hypoproteinemic Rabbit.

(Before plasmapheresis.)

Rabbit No.	Body weight kg	T. P. g/dl	Circulating serum volume cc	Circulating serum protein g	Alb. %	α-Glob. %	β-Glob. %	γ-Glob. %	Ratio A/G
106	2.10	6.4	98	6.27	62.1	9.1	11.2	17.6	1.63
107	2.10	6.2	85	5.27	72.2	9.7	13.2	4.9	2.59
108	2.36	6.4	85	5.44	61.2	9.2	14.2	15.3	1.57
mean		6.3	89.3	5.66	63.3	9.3	12.8	12.6	1.93

(After plasmapheresis)

										Liver Protein		
										(A)	(B)	A/B
106	1.90	4.2	89	3.73	49.2	11.1	25.5	11.2	0.96	7.6	6.8	1.11
107	1.80	3.8	82	3.11	60.4	9.8	24.9	4.9	1.52	7.5	6.6	1.13
108	2.10	4.3	80	3.44	51.5	13.7	29.8	5.0	1.06	7.8	6.9	1.13
mean		4.1	83.6	3.42	53.7	12.5	26.7	7.1	1.18	7.6	6.7	1.12

Table 24

Effect of Daily Infusion of Fat Emulsion on Recovery of Serum Protein and Body Weight.

(Each values show mean of 2 samples.)

Remarks	Days of infusion after plasmapheresis	Body weight kg	T. P. g/dl	Alb. %	α-glob. %	β-glob. %	γ-glob. %
Fat Emulsion + Various Vitamins	Before plasmapheresis	2.13	5.9	60.8	11.3	14.9	13.0
	0	2.05	4.0	53.1	14.3	24.2	8.4
	5	2.02	5.3	54.7	13.1	21.2	11.0
	10	2.08	5.9	55.3	13.0	19.6	12.1
	15	2.13	6.0	59.6	11.7	15.4	13.3
Control	Before plasmapheresis	2.00	6.0	59.0	10.9	15.5	14.6
	0	1.90	3.9	50.3	11.1	29.2	9.4
	5	1.64	5.0	51.5	11.6	24.8	12.1
	10	1.76	5.4	51.2	13.8	21.0	14.0
	15	1.77	5.8	52.3	11.5	22.1	14.1

23, the serum protein and the saline-soluble liver protein markedly decreased. The experimental hypoproteinemic rabbits thus prepared were fed with a ration supplying a daily intake of 300 g of Okara (Protein 13.8 g, 210 Cal), while fat emulsion was daily infused in combination with the above-mentioned vitamins. As shown in Table 24, the group infused with the fat emulsion returned to the previous levels in serum protein and body weight far more rapidly than the controls. The above facts signify that protein orally supplied, although insufficient in amount, was utilized more effectively for the tissue protein synthesis when compared with the controls. Furthermore, the author continued to feed the hypoproteinemic rabbits with the same low calorie diet and infused the fat emulsion and vitamins. The liver protein on the 5th day of the experiment was determined. As shown in Table 25, the saline-soluble liver protein of the controls decreased markedly as compared with those cases infused with the fat emulsion. Moreover, no abnormal findings, such as fatty liver, were observed histologically. From the above results, it was ascertained that the intravenous infusion of fat emulsion into the rabbits in a state of hypoproteinemia did not impair their condition, but caused more effective utilization of the oral protein in the synthesis of plasma and tissue protein.

Table 25

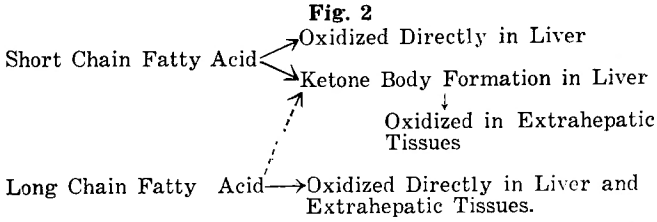
Effect of Daily Infusion of Fat Emulsion on Recovery of Liver Protein in Experimental Hypoproteinemic Rabbit.

Remarks	Rabbit No.	Saline-soluble protein g/%(A)	Saline-insoluble protein g/%(B)	Ratio A/B
Fat Emulsion + Various Vitamins	122	7.5	6.8	1.10
	123	7.5	6.7	1.11
	124	7.6	6.7	1.12
	mean	7.55	6.72	1.11
Control	120	6.6	6.6	1.00
	121	6.8	6.7	1.01
	mean	6.7	6.65	1.00

DISCUSSION

It has been pointed out by DERMAN and LEITES that neutral fat is changed into fatty acid or phospholipid in the reticuloendothelial cells. This fact has been more clearly demonstrated, since the fat emulsion, produced in our laboratory, was safely infused intravenously. The infused fat globules are phagocytized by the reticuloendothelial cells in the lung, liver and spleen. The fatty acids, contained in the infused fat emulsion, are oxidized after neutral fat is changed into phospholipid in these cells. In case triglycerides of short chain fatty acids are used as components of the fat emulsion, phospholipid, which is changed from these fatty acids, enters into the hepatic parenchymatous cells. However, in the fat emulsion which is principally composed of triglycerides of long chain fatty acids, phospholipid enters into not only the hepatic parenchymatous cells but all the cells of body to be oxidized. Therefore, the increase in blood ketone body levels is far more remarkable in the former fatty acids, as compared with the latter. This indicates that despite the fact that short chain fatty acids are principally oxidized to the stage of ketone body in the liver, long chain fatty acids are partially oxidized to the stage of ketone body in the liver and then diffuse into the blood stream to be

Fig. 2



carried to the extrahepatic tissues, and the major part of these are metabolized finally to water and carbon dioxide in the extraphepatic tissues (See Fig. 2). As shown in Fig. 1, it has been established "*in vitro*" that fatty acids, which enter into the tissue cells in the form of phospholipid, produce fatty acid-CoA compound at the first stage in process of fatty acid oxidation. Fatty acid-CoA compound enters into the fatty acid cycle, yields 2-carbon fragments viz. acetyl coenzyme A (acetyl-CoA) and fatty acid containing two carbon atoms less than the parent fatty acid by way of the stage of β-hydroxy acid and β-keto acid. By repeating such a reaction formula, fatty acid breaks down completely to acetyl-CoA, and, in the presence of oxaloacetic acid, enters into the tricarboxylic acid cycle (T. C. A. cycle) in which it is oxidized completely to carbon dioxide and water. Various enzyme systems play an important role in the process of fatty acid oxidation. The simultaneous use of various vitamins should be considered in the infusion of the present fat emulsion. As demonstrated in this experiment, riboflavin is one of the vitamins belonging to this category, and is physiologically effective only in the form of flavin adenine dinucleotide (F. A. D.) or flavin mononucleotide (F. M. N.). When the fatty acid-CoA compound enters into the fatty acid cycle of LYNEN to be oxidized, F. A. D. and diphospho-pyridine-nucleotide (D. P. N.) are essential to smooth this oxidation process. Thus the importance of pyridine enzyme and flavin enzyme as the hydrogen carriers has been recognized. After acetyl-CoA enters into the T. C. A. cycle, all hydrogens which are liberated by turn of the cycle are transported by pyridin nucleotide, flavoprotein and cytochrom system to finally combine with oxygen. And thus fatty acids are completely decomposed to carbon dioxide and water. Pyridine nucleotide and flavoprotein each contain nicotinic acid amide and riboflavin. It is obvious that riboflavin and nicotinic acid amide are indispensable as elements of the hydrogen carrring system in fatty acid oxidation. It has been recently demonstrated that Vitamin C not only enhances the activity of lipase in the liver and serum, as stated previously, but also activates aconitase and succinic dehydrogenase. Therefore, by means of the simultaneous infusion of vitamins with the fat emulsion, the fatty acid contained in the infused fat emulsion was utilized smoothly and effectively, inducing a marked protein sparing effect. With such a reducing diet as used in the present experiment, the addition of 7 per cent glucose solution is insufficint, and the addition of a larger amount of glucose and combination with Vitamin B_1 should be considered. The present results further explain what an important role the glucose does play in fat metabolism. The above facts demonstrate the important roles of these drugs "*in vivo*", which have already been demonstrated "*in vitro*". If the present fat emulsion had been infused simultaneously with pantothenic acid, which is the principal component of CoA, more marked protein sparing effect could possibly have been obtained. The fact that the increase in the α- and β-

globulin components is far less in the case of the infusion of sesame oil emulsion than in the case of the infusion of cod liver oil emulsion, reveals that the former emulsion is more rapidly and smoothly utilized than the latter. In the modern conception of lipoprotein, abnormal elevation of α- and β-globulin peak indicates at least the catabolic phase of fat and at the same time makes it presumable that the catabolism of fat does not follow a smooth. The results obtained from the present experiments illustrate that infused fat emulsion for the purpose of parenteral nutrition is utilized more effectively when the appropriate methods, as described above, are employed.

SUMMARY AND CONCLUSIONS

The present experiments were carried out to determine whether or not the sesame oil emulsion produced in our laboratory show any marked "*Protein Sparing Effect*" by intravenous infusion into normal rabbits fed a reducing diet, as well as rabbits in a hypoproteinemic state, and to observe from the viewpoint of protein metabolism whether or not various vitamins, which have been found recently to show biochemical activity "*in vitro*", show any significance "*in vivo*".

The author reached the following conclusions.

(1) This fat emulsion shows a remarkable protein sparing effect and enables the protein orally taken in, although insufficient in amount, to be utilized more effectively for plasma protein and tissue protein synthesis.

(2) Because of the simultaneous infusion of methionine, riboflavin, nicotinic acid amide, ascorbic acid and thiamin hydrochloride with the fat emulsion, the infused fat is utilized more smoothly and effectively.

(3) The sesame oil emulsion can be utilized more smoothly and effectively than the cod liver oil emulsion.

(4) Abnormal elevation of α- and β-globulin peak in serum indicates that its metabolism is not proceeding smoothly.

(5) From these experimental results, the intravenous infusion of fat emulsion into lower animals (i. e. rabbits and dogs) has been shown to be a valid method of parenteral nutrition.

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References

1) Asada, S. : Histochemical studies on the intravenously infused fat emulsion. Arch. Jap. Chir., **22**; 77, 217, 1953, Acta Med. Univ. Kioto, **31**; 171, 1954. 2) Armstrong, S. H. Jr., Budka, M. J. E. and Morrison, K. C. : Preparation and properties of serum and plasma proteins. XI. Quantitative interpretation of electrophoretic schlieren diagrams of normal human plasma proteins. J. Amer. Chem. Soc., **69**; 416, 1947. 3) Addis, T., Poo, L. J. and Lew, W. : The quantities of protein lost by the various organs and tissue of the body during a fast. J. B. C., **115**; 111, 1936. 4) Blix, G., Tiselius, A. and Svensson, H. : Lipids and polysaccharides in electrophoretically separated blood

serum proteins. J. B. C., **137**; 485, 1941. 5) Blix, G. : Electrophoresis of lipid-free blood serum. J. B. C. **137**; 495, 1941. 6) Benditt, E. P., Humphreys, E. M., Wissler, R. W., Steffee, C. H., Frazier, L. E. and Cannon, P. R. : The dynamics of protein metabolism. I. The interrelationship between protein and caloric intake and their influence upon the utilization of ingested protein for tissue synthesis by the adult protein-depleted rat. J. Lab. & Clin. Med., **33**; 257, 1948. 7) Cannon, P. R., Frazier, L. E. and Hughes, R. H. : Fat emulsion as caloric supplements in parenteral nutrition, with particular reference to amino acid utilization. J.Lab. & Clin. Med., **44**; 250, 1954. 8) Canttarow, A. and Trumper, D. : Lipid metabolism. Clin.Biochem., 1949. 9) Dunham, L. J. and Brunschwig, A. : Intravenous administration of fat for nutritional purposes. Experimental study. Arch. Surg., **48**; 395, 1944. 10) Fishman, W. and Veen, H. : A method of partitioning liver proteins and its application to the study of the effects of acute hemorrage in the dogs. Ann. Surg., **127**; 256, 1948., Blood and liver proteins in surgical patients as related to protein depletion. Ann. Surg., **127**; 264, 1948. 11) Fujiwara, M. : Some studies of riboflavin metabolism in the human bodies. Vitamin, **6**; 787, 1953. 12) Gorens, S. W., Geyer, R. P., Matthews, L. W. and Stare, F. J. : Parenteral nutrition. X. Observation on the use of a fat emulsion for intravenous nutrition in man. J. Lab. & Clin. Med., **34**; 1627, 1949. 13) Geyer, R. P., Chipman, J. and Stare, F. J. : In vivo oxidation of emulsified radioactive trilaurin administered intravenously. J. B. C., **176**; 1469, 1948. 14) Geyer, R. P., Matthews, L. W. and Stare, F. J. : Metabolism of emulsified trilaurin (-C^{14} 00-) and octanoic acid (-C·400-) by rat tissue slices. J. B. C., **180**; 1037, 1949. 15) Goldman, D. S., Chaikoff, I. L., Reinhardt, W. O., Entenman, C. and Dauben, W. G. : The oxidation of palmitic acid-1-C^{14} by extrahepatic tissues of the dog. J. B. C., **184**; 719, 1950. 16) Dermann, G. L. und Leites. S. : Die Rolle der Lunge, Leber und Milz in Fettstoffwechsel. Virch. Arch., **268**; 440, 1928. 17) Hikasa, Y., Asada, S., Zaitsu, A., Tsukada, A. and Nakata, K. : Studies on the intravenous administration of fat emulsion. Arch. Jap. Chir., **21**; 1, 1952. 18) Hikasa, Y., Ishigami, K., Asada, S., Zaitsu. A, and Nakata, K.: Studies on the intravenous administration of fat emulsion. J. Jap. Surg. Soc, **52**; 298, 1951. 19) Hirai, H. and Shimao, K. : Electrophoretic studies of plasma protein. J. Biochemistry, **21**; 54, 109, 1949. 20) Hara, M. and Suda, M. : Aceton body formation and mitochondrial enzyme system activity. Symp. Enzyme. Chem., **9**; 66, 1954. 21) Hashino, H. : Experimental studies on fat metabolism from the view-point of ketone body formation. Arch. Jap. Chir., **24**; 488, 1955. 22) Izukura, T. : unpublished. 23) Ikeda, H. : unpublished. 24) Kuroda, K. : Microdetermination of blood water component. Jap. J. Med. Prog., **35**; 197, 1948. 25) Longsworth, L. G. and MacInnes, D. A. : An electrophoretic study of nephrotic sera and urine. J. Exp. Med., **71**; 77, 1940. 26) Longsworth, L. G., Shedlorsky, T. and MacInnes, D, A. : Electrophoretic patterns of normal and pathological human blood serum and plasma. J. Exp. Med., **70**; 399, 1939. 27) Lehninger, A. L. : Fatty acid oxidation and the Krebs tricarboxylic acid cycle. J. B. C., **161**; 413, 1945, On the activation of fatty acid oxidation. J. B. C., **161**; 437, 1945. 28) Mc Kibbin, J. M., Ferry, R. M., Jr. and Stare, F. J. : Parenteral nutrition. Ⅱ. The utilization of emulsified fat given intravenously. J. Clin. Invest., **25**; 679, 1946. 29) Mann, G. V., Geyer, R. P., Watkin, D. M. and Stare, F. J. : Parenteral nutrition. IX. Fat emulsion for intravenous nutrition in man. J. Lab. & Clin. Med., **34**; 699, 1949. 30) Maddan, S. C., George, W. E., Waraich, G. S. and Whipple, G. H. : Blood plasma protein regeneration as influenced by fasting, infection and diet factores. Variable reserve stores of plasma protein building material in the dog. J. Exp. Med., **67**; 675, 1938. 31) Maddan, S. C. and Whipple, G. H. : Plasmaproteins : their source, production and utilization. Phys. Rev., **20**; 194, 1940. 32) MacKay, E. M., Wick, A. N. and Barnum, C. P. : Ketogenic action of short chain, even numbered carbon fatty acids in carbohydrate-fed animals. J. B. C., **135**; 183, 1940. 33) Moor, F. D. : Bodily changes in surgical convalescence. I. The normal sequence-observat ons and interpretations. Ann. Surg., **137**; 302, 1953. 34) Maddan, S. C. and Whipple, G. H. : Amino acids in the production of plasma protein and nitrogen balance. Am. J. Med. Sci. **211**; 149, 1946. 35) Nath, M. C. and Chakrabarti, C. H. : Effect of acetoacetate and β-hydroxybuty-

rate on riboflavin and nicotinic acid. Proc. Soc. Exp. Biol. Med., **82**; 5, 1953. 36) Nakata, K. : Experimental studies on fat metabolism in the lung. Arch. Jap. Chir., **23**; 455, 1954. 37) Nishino, T. : Laboratory studies on the intravenous administration of the fat emulsion in the light of tissue metabolism. Arch. Jap. Chir., **23**; 607, 1954. 38) Ōtani, S. : unpublished. 39) Rittenberg, D., Schoenheimer, R. and Evans, E. A. Deutrium as an indicator in the study of intermediary metabolism. X. The metabolism of butyric and capric acids. J. B. C., **120**; 503, 1937. 40) Schoenheimer, R. and Rittenberg, D. : Deutrium as an indicator in the study of intermediary metabolism Ⅲ. The role of the fat tissues. J. B. C., **111**; 175, 1935. Ⅸ. The conversion of stearic acid into palmitic acid in the organism. J. B. C., **120**, 155, 1937. 41) Schoenheimer, R., Ratner, S., Rittenberg, D. and Heidelberger, M. : Indicator of bloodproteins of rat with dietary nitrogen. J B. C., **144**; 541, 1942. 42) Senō, A. : A study of the fat metabolism in the isolated perfused liver. Arch. Jap. Chir., **24**; 1 79, 1955. 43) Shirotani, K. : unpublished. 44) Shafiroff, B. G. P., Baron, H. and Roth, E. : Intravenous infusion of a combined fat emulsion into dog. Proc. Soc. Exp. Biol. Med., **69**; 387, 1948. 45) Stern, J. R. and Ochoa, S. : Enzymatic synthesis of citric acid. V. Reaction of acetyl coenzyme A. J. B. C., **198**; 313, 1952. 46) Strang, J. M., McCluggage, H. B. and Evans, F. M. : The nitrogen balance during dietary correction of obesity. Am. J. M. Sc., **181**; 336, 1931. 47) Saitō, M. : Electrophotometrical clinical method. Nanzando. 1953. 48) Tiselius, A. : New apparatus for electrophoretic analysis of colloidal mixtures. Trans. Farad. Soc., **33**; 524, 1937. 49) Tsukada, A. : Studies on the intravenous administration of the fat emulsion in the light of the protein metabolism. Arch. Jap. Chir., **23**; 215, 1954. 50) Weinhouse, S., Medes, C. and Floyd, N. F. : The mechanism of fatty acid oxidation. J. B. C., **153**; 689, 1944. 51) Weinhouse, S., Millington, R. H. and Friedman, B. : The effect of carbohydrate on the oxidation of fatty acids by liver slices. J. B. C., **181**; 489, 1949. 52) Whipple, G. H. : Protein production and exchange in the body including hemoglobin, plama protein and cell protein. Am. J. Med. Sci., **196**; 609, 1938. 53) Whipple, G. H. : Hemoglobin and plasma proteins: Their production, utilization and interrelation. Am. J. Med. Sci., **203**; 477, 1942. 54) Yoshikawa, H. : Protein metabolism. Clinic all-Round, **3**; 115, 280, 1954. 55) Zeldis, L. J. and Alling, E. L. : Plasma protein metabolism electrophoretic studies. Restoration of circulating proteins following acute depletion by plasmapheresis. J. Exp. Med., **81**; 515, 1945. 56) Zeldis, L. J., Alling, E. L., Mc Cood, A. B. and Kulka, J. P. : Plasma protein metabolism - electrophoretic studies. Chronic depletion of circulating protein during low protein feeding. J. Exp. Med., **82**; 157, 1945. 57) Zeldis, L. J., Alling, E. L., McCood, A. B. and Kulka, J. P. : Plasma protein metabolism electrophoretic studies. The influence of plasma lipids on electrophoretic patterns of human and dog plasma. J. Exp. Med., **82**; 411, 1945.

経静脈性脂肪輸入の蛋白節約作用に関する実験的研究

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教室創製の経静脈性輸入可能な脂肪乳剤（胡麻油乳剤）を減食飼育家兎，或は実験的低蛋白血症家兎群の静脈内へ注入し，果たして蛋白節約作用を示すや否やを検討した．又脂質代謝に関与する各種ビタミン類の重要性を，蛋白代謝の面よりin vivoに於て観察し次の所見を得た．

(1) 本脂肪乳剤は著明な蛋白節約作用を有し，経口的に摂取された蛋白質をして，より一層効果的に血漿蛋白，組織蛋白の再建に利用せしめる．

(2) 本脂肪乳剤の蛋白節約作用は，リボフラビン，ニコチン酸アミド，ビタミンC等の脂肪酸酸化酵素系の併用により，更に一層増強せしめられる．

(3) 減食試獣を対象とした際には，7%の糖添加では不充分であり，更に多量の糖添加，及びビタミンB1の併用により本脂肪乳剤の栄養的効果は著明になる．

(4) 胡麻油乳剤は肝油乳剤に比して利用され易い．

(5) 血清中のα-,β-Globulinの異常増加は脂質の異化的方向への過程を示すもので，而もその処理の不円滑なることを示すものである．

(6) 従つて本脂肪乳剤の適切なる静脈内注入法を行えば，脂質の非経口的栄養補給の役割を充分果たし得るものと思われる．