EXPERIMENTAL STUDIES ON THE FAT METABOLISM BY THE USE OF RADIOACTIVE PHOSPHORUS

by

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I. INTRODUCTION

As is well known, fat metabolism has not been as definitively determined as that of other foodstuffs. Hikasa and his colleagues in our laboratory have succeeded in preparing a sesame oil emulsion which can be given intravenously, and using every possible histochemical and biochemical procedure in patients and experimental animals, they have explored how glycerides given intravenously are utilized in the body.

The experiments reported here were carried out in an attempt to clarify by the use of radioactive isotope i) whether the experimental results obtained by conventional biochemical and histochemical procedures are correct, and ii) how much some anesthetics influence fat metabolism.

II. MATERIALS AND METHODS

1) MATERIALS

A) Experimental Animals: Healthy adult dogs weighing about 10 kg were used. They were in the so-called postabsorptive state at the onset of the experiments, after fasting for 18 hours.

B) Radioactive Phosphorus: Radioactive phosphorus (P^{32}) in the form of $Na_2H\overset{*}{P}O_4$ was injected in the gluteal muscles of experimental animals in a dose of 0.5 mc per kg of body weight.

C) Fat Emulsion: A 20% sesame oil emulsion, produced in our laboratory, was given intravenously at a dosage level of 5 cc per kg 3 hours after the injection of P^{32}. Then serum was collected on successive times.

D) Anesthetics: As general anesthetics, Ravonal (Thiopentalsodium), Morphine, Ether and Cyclopropane were used.

Ravonal: A 2.5% solution was given intravenously in a dose of 1 cc per kg at first, then one third of the above dose was given every 30 minutes to maintain the depth of anesthesia.

Morphine: A 4% Narcopon-scopolamine solution was given subcutaneously in a dose of 0.1 cc per kg only once.

Ether and Cyclopropane: Closed circuit intubation anesthesia inhaling ether or cyclopropane was performed using a Dräger's apparatus. Intubation was done

without using any other narcotics than succinylcholine (S. C. C., 2 cc injected intravenously), in order to investigate the influence of each anesthetic on fat metabolism.

The depth of anesthesia was maintained at the second phase of the third stage as constantly as possible.

2) MATERIALS

A) Determination of Electrophoretic Fractions of Serum Protein and Lipoprotein: Electrophoresis of serum was continued for 10 hours, using a KOBAYASHI's paper electrophoretic apparatus (horizontal type, Toyo filter paper No. 51, current 0.4 mA per cm), and MICHAELIS' Buffer of pH 8.6 and ionic strength 0.059 μ.

Protein was marked on a paper strip by staining with B. P. B. and lipoprotein with Sudan Black B. Equal samples of serum collected successively from the same animal was placed on the same paper strip to compare the protein and lipoprotein fractions.

B) Autoradiogram: A FUJI's X-ray film was placed on the above mentioned paper strip for 3 weeks to prepare an autoradiogram.

C) Preparation of Acid-soluble and Lipid Phosphorus in Serum and Tissues: This was performed by the method of SCHMIDT and THANHAUSER.

D) Measurement of the Radioactivity: A GEIGER-MÜLLER's counter (METRO ELECTRIC & Co.) was used for this measurement at 1350 volts.

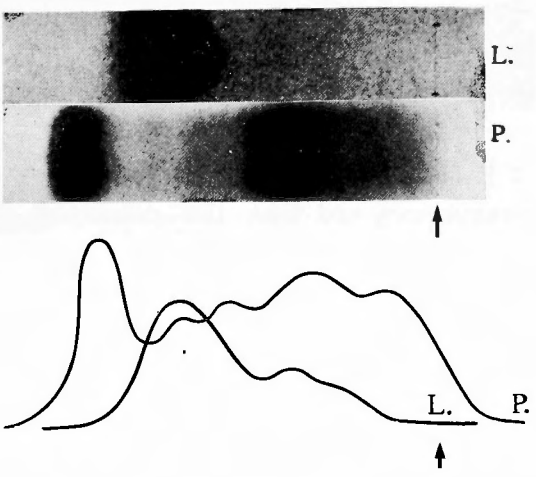
E) Determination of Phosphorus: The method of FISKE and SUBBAROW was used, using an electrophotometer (BECKMANN's type) at a wave-length of 620 mμ.

III. RESULTS AND DISCUSSION

1) Paper Electrophoretic Fractions of Serum Protein and Lipoprotein in Normal Dogs

In 1950, ONCLEY, GOFMAN and others asserted that most of the serum lipids were present in the α- or β-globulin fractions as lipoproteins.

Fig. 1 Paper electrophoretic patterns of a normal dog serum in the fasting state. (The paper strip marked P is stained for proteins, that marked L is stained for lipids)



Further, in 1954, ELAINE, BOSSACK and others demonstrated that α-lipoprotein was contained in albumin and α_1-globulin, β-lipoprotein in α_2-, β_1- and β_2-globulin and that the other lipids were in the γ-globulin fraction in the form of O-Lipoprotein.

Paper electrophoretic patterns of serum protein and lipoprotein in normal dogs are shown in Figs. 1, 2 and Tables 1, 2.

Fig. 2 Paper electrophoretic patterns stained for lipids of sera collected successively during the fasting state (Fat emulsion not infused).

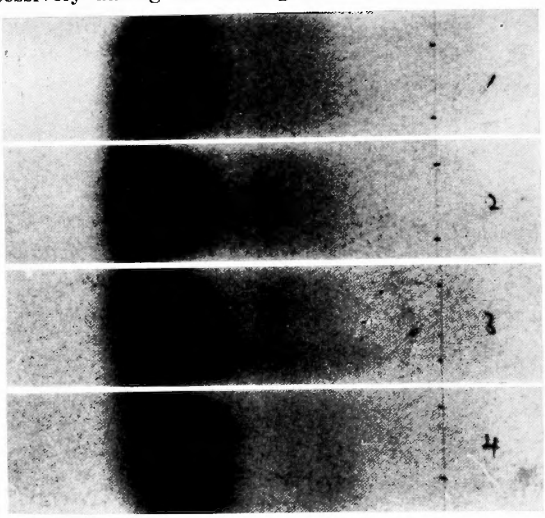


Table 1 Paper electrophoretic fractions of normal serum protein (%)

	Albumin	α-Globulin	β-Globulin	γ-Globulin
Dog	37.9	20.7	23.9	17.5
Man	60.7	8.6	12.8	17.9

(Mean value)

Table 2 Paper electrophoretic fractions of normal serum lipoprotein (%)

	α-lipoprotein	β-lipoprotein	o-lipoprotein
Dog	79.6	20.4	0
Man	37.4	45.7	16.9

(Mean value)

From these results, it is apparent that serum lipoproteins of normal dogs consist of α-lipoprotein and β-lipoprotein, and that the amount of the former is four to five times that of the latter. In the so-called postabsorptive state the O-lipoprotein in the γ-globulin fraction is hardly perceptible.

2) Fate of the Fat Given Intravenously

A) Changes in Serum Protein and Lipoprotein Fractions Following a Single Infusion of Fat Emulsion

After a single intravenous infusion of 20% sesame oil emulsion in a dose of 5

cc per kg of body weight in animals in the postabsorptive state, serum was collected on successive times. Their protein and lipoprotein fractions are shown in Figs. 3, 4 and Tables 3, 4.

Fig. 3 Paper electrophoretic patterns stained for proteins of sera collected successively following the infusion of fat emulsion.

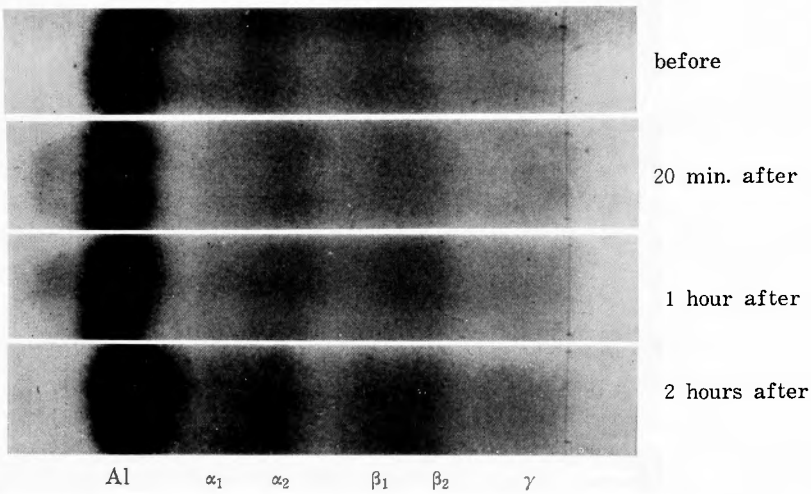


Fig. 4 Paper electrophretic patterns stained for lipids of sera collected successively following the infusion of fat emulsion.

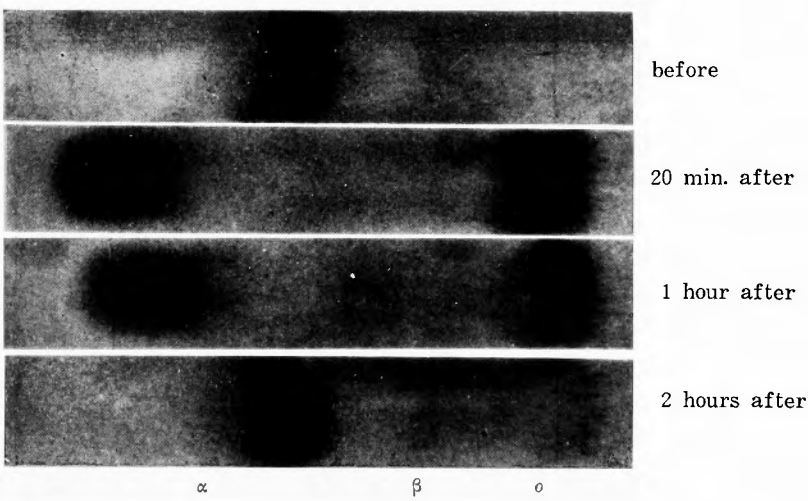


Table 3 Changes in electrophretic fractions of serum protein following the infusion of fat emulsion (%)

Intervals after infusion	Albumin	α-Globulin	β-Globulin	γ-Globulin
Before	37.9	20.7	23.9	17.5
20 min. after	34.4	22.4	21.5	18.7
1 hour after	32.8	24.0	25.6	17.6
2 hours after	34.8	23.8	25.0	16.4

(Each values show means of 5 samples)

Table 4 Changes in electrophoretic fractions of serum lipoprotein following the infusion of fat emulsion (%)

Inervals after infusion	α-lipoproten	β-lipoprotein	o-lipoprotein
Before	79.6	20.4	0
20 min. after	27.1	15.6	57.3
1 hour after	38.4	17.1	44.5
2 hours after	60.6	27.1	12.3

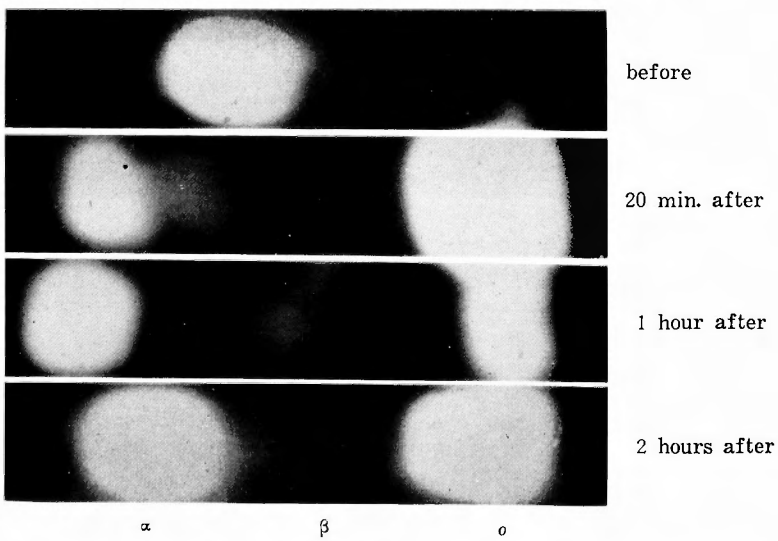
(Each values show means of 5 samples)

When glycerides in an emulsified form were infused intravenously, *O*-lipoprotein appeared first in the position of the γ-globulin fraction accompanying an increase in γ-globulin. But about 20 minutes later, *O*-lipoprotein began to decrease again, and it was hardly perceptible 2 hours after the infusion. γ-globulin changed in parallel with *O*-lipoprotein. However, α-and β-lipoprotein increased after showing a transient but marked decrease. α-and β-globulin showed the same change.

B) Autoradiogram of Electrophoretic Patterns of Serum Collected on Successive Times Following a Single Infusion of Fat Emulsion

Radioactive phosphorus (P^{32}) was injected intramuscularly in the form of $Na_2H\overset{*}{P}O_4$ in a dose of 0.5 mc per kg of body weight, 3 hours before the intravenous infusion of fat emulsion (Fig. 5).

Fig. 5 Autoradiogram of paper electrophoretic patterns of sera collected successively following the infusion of fat emulsion employing P^{32}.



Radioactive P^{32} was introduced markedly into the α-lipoprotein fraction 3 hours after the injection of P^{32}, as shown in the first line of Fig. 5.

When emulsified glycerides were administered intravenously to these animals, serum α-lipoprotein of a low lipid content and of a high density, being labeled with P^{32}, gathered together around the infused glycerides, and formed *O*-lipoprotein with a high lipid content and a low density.

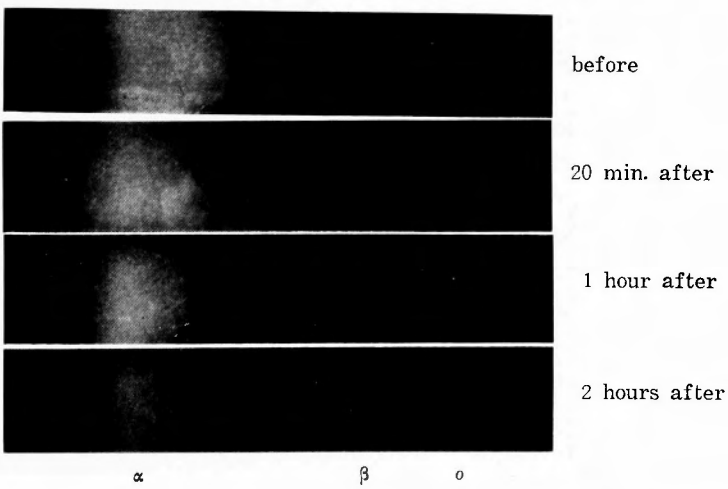
It has been believed from these results, presented in the second line of Fig. 5, that the infused glycerides are stabilized by the above-mentioned process. P^{32} which had appeared in the α-lipoprotein fraction was introduced into the *O*-lipoprotein fraction 20～30 minutes after infusion of the glycerides, so that P^{32} in the α-lipoprotein fraction decreased markedly while that in the *O*-lipoprotein fraction increased (Second stage). This stage, however, continued for only 30 minutes, thereafter the infused glycerides diminished, being phagocytized in the form of *O*-lipoprotein by alveolar phagocytes, KUPFFER's stellate cells of the liver and reticuloendothelial cells of the spleen.

These findings coincide with the histochemical findings obtained by our colleagues IZUKURA, ASADA and SHIROTANI, and the biochemical findings of NAKATA and SENO.

When the glycerides were converted to phospholipids in these cells, P^{32} in the α-lipoprotein fraction increased markedly while that in the *O*-lipoprotein fraction decreased (Third stage). Moreover, 2 hours after the infusion of the glycerides, P^{32} in the α-lipoprotein fraction increased still more (Fourth stage). These findings suggest that α-lipoprotein contains phospholipids in a larger amount than β-lipoprotein, as stated by ELAINE and others, and that the infused glycerides are converted to phospholipids in the above mentioned cells.

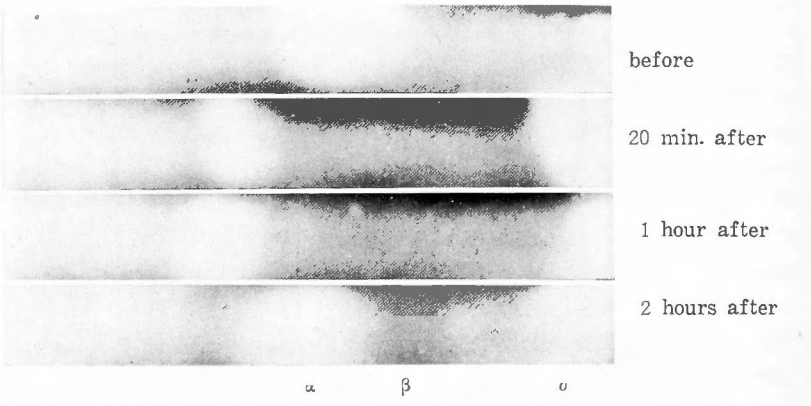
In control animals receiving no emulsion P^{32} in the α-lipoprotein fraction decreased gradually after its injection, not showing the changes of P^{32} observed in the emulsion infused dogs (Fig. 6).

Fig. 6 Autoradiogram of paper electrophoretic patterns of sera collected successively during the fasting state (Fat emulsion not infused).



In addition, autoradiography was performed after adding the same volume of P^{32} in the form of $Na_2H\overset{*}{P}O_4$ in vitro to the serum used in preparing the autoradiogram shown in Fig. 5. P^{32} added to serum in vitro did not combine with lipids, so that it did not appear between the *O*-lipoprotein and the α-lipoprotein fractions, but shifted much more than the α-lipoprotein fraction (Fig. 7). It was evident, therefore, that P^{32} which appeared in the α- or *O*-lipoprotein fraction on the autoradiogram

Fig. 7 Autoradiogram of electrophoretic patterns of mixtures of a solution of inorganic phosphorus ($Na_2H\overset{*}{P}O_4$) and sera collected successively following the infusion of fat emulsion.



The spots of inorganic P are shifted to the left side of the paper strips beyond the original place of lipid P.

shown in Fig. 5 was that combined with lipids.

Although these results obtained by autoradiography coincide for the most part with those obtained by photoelectric measurements of lipoproteins stained by Sudan Black B, there is a significant difference between them.

Serum *O*-lipoprotein which increased markedly immediately after infusion of fat emulsion decreased again as the glycerides were phagocytized by alveolar phagocytes, stellate cells of the liver and reticuloendothelial cells of the spleen. In the autoradiography using P^{32}, however, *O*-lipoprotein increased following infusion, decreased about one hour later, but increased again thereafter. These results seem to be contradictory.

When glycerides are introduced into the blood stream, serum α-lipoprotein, which is low in P^{32} content, accumulates around the infused glycerides, but in the course of time, these glycerides are phagocytized by the above-mentioned cells and actively form phospholipids with the introduced P^{32}. These processes lead to a formation of α-lipoprotein which contains a large amount of P^{32}. This α-lipoprotein accumulates again around the remaining glycerides in the blood stream. Therefore, it seemed as if *O*-lipoprotein had increased in the autoradiogram shown in the 4th line of Fig. 5.

C) Changes in Serum Phospholipids Labeled with P^{32} after Infusion of Fat Emulsion

Changes in radioactivity of acid-soluble phosphorus and phospholipid phosphorus in serum following an infusion of fat emulsion are shown in Table 5. No significant difference in serum acid-soluble P^{32} was observed between the group given the fat emulsion and the control group, and both groups showed a decrease of about 50% in the course of the experiments.

On the other hand, P^{32} in phospholipids increased in both groups, much more in the fat emulsion infused group. Therefore, it was considered that the increase in lipid P^{32} in the emulsion infused group was due to the formation of phospholipids

Table 5 Changes of radioactive P in sera collected successively following the infusion of fat emulsion.

Counts × 10^{-5}/min./ ml of serum

Intervals after infusion	Fat emulsion infused group		Control group (Fat emulsion not infused)	
	acid-soluble P	lipid P	acid-soluble P	lipid P
Before	4.5 (± 0)	0.73 (± 0)	4.6 (± 0)	0.74 (± 0)
20 min. after	3.3 (−27)	0.81 (+ 8)	3.6 (−22)	0.75 (+ 1)
1 hour after	3.0 (−38)	0.99 (+35)	3.0 (−35)	0.79 (+ 7)
2 hours after	2.5 (−45)	1.36 (+86)	2.3 (−50)	0.84 (+13)

(Each values show means of 5 samples)

from the infused glycerides. Moreover, these facts became more evident when the specific radioactivity was considered. The specific radioactivity of lipid P^{32} showed only a 15% increase in the control group, but a 50% increase in the emulsion infused group. Therefore, the ratio of lipid P^{32} to acid-soluble P^{32}, i. e. the relative specific activity, showed an increase of 95% in the control group and a 266% increase in the emulsion infused group (Table 6).

Table 6 Turn over rate of lipid P in the serum following the infusion of fat emulsion. (Each values show means of 5 samples)

Intervals after infusion	Fat emulsion infused group			Control group (Fat emulsion not infused)		
	Sp. ac. of acid-sol. P	Sp. ac. of lipid P	Relative Sp. ac. (%)	Sp. ac. of acid-sol. P	Sp. ac. of lipip P	Relative Sp. ac. (%)
Before	69 (± 0)	8.5 (± 0)	12.3 (± 0)	66 (± 0)	8.6 (± 0)	13.0(± 0)
20 min. after	57 (−12)	9.0 (+ 6)	15.8 (+ 28)	53 (−20)	8.7 (+ 1)	16.4(+26)
1 hour after	44 (−36)	10.9 (+28)	22.5 (+ 83)	44 (−23)	9.3 (+ 8)	21.1(+62)
2 hours after	34 (−51)	15.3 (+80)	45.0 (+266)	39 (−41)	9.9 (+15)	25.4(+95)

Specific activity : counts × 10^{-3}/min. /mg

Relative specific activity : specific activity of lipid P/specific activity of acid-soluble P

D) Introduction of Phospholipids Labeled with P^{32} into Tissues Following Infusion of Fat Emulsion

From the above findings, it was concluded that serum α-lipoprotein accumulated around the intravenously infused glycerides to form low density *O*-lipoprotein, which was phagocytized by alveolar phagocytes, KUPFFER's stellate cells of the liver and reticuloendothelial cells of the spleen to be converted into phospholipids. And it was postulated that these phospholipids entered the blood stream again in the form of α- and β-lipoprotein (especially in the former) and at last were carried to all tissues. In order to clarify this point more accurately, the following experiments were performed.

According to our colleagues ASADA, IZUKURA, SHIROTANI, KUYAMA, SHIGENAGA and TOBE the amount of phospholipid in tissues reaches its maximum about 3 hours after infusion of the fat emulsion. In the following experiments, therefore, the radioactivity of acid-soluble phosphorus and lipid phosphorus in tissues was measured

3 hours after infusion.

Liver, kidney and heart muscle were the organs selected for study, since these organs oxidize fat more actively than the others.

The radioactivity of lipid P^{32} increased much more in the emulsion infused group than in the control (Table 7, 8), and these results became much more evident when the specific activity and the relative specific activity were considered (Table 9). The amount of phospholipid introduced into the tissues in the form of α- and

Table 7 Introduction of radioactive P in tissues 3 hours after the infusion of fat emulsion. (Counts × 10^{-5}/min. /100gr of wet tissue)

	Fat emulsion infused group		Control group (Fat emulsion not infused)	
	acid-soluble P	lipid P	acid-soluble P	lipid P
Liver	93	52	103	27
Kidney	98	33	83	13
Heart muscle	93	4.4	106	1.7

(Each values show means of 5 samples)

Table 8 Distribution of phosphorus to tissues 3 hours after the infusion of fat emulsion. (mg/100gr of wet tissue)

	Fat emulsion infused group		Control group (Fat emulsion not infused)	
	acid-soluble P	lipid P	acid-soluble P	lipid P
Liver	123	109	113	87
Kidney	94	86	91	64
Heart muscle	119	55	106	51

(Each values show means of 5 samples)

Table 9 Turn over rate of lipid P in tissue 3 hours after the infusion of fat emulsion.

	Fat emulsion infused group			Control group (Fat emulsion not infused)		
	Sp. ac. of acid-sol. P	Sp. ac. of lipid P	Relative sp. ac.	Sp. ac. of acid-sol. P	Sp. ac. of lipid P	Relative Sp. ac.
Liver	75	47	62.6	93	31	33.3
Kidney	104	38	36.5	92	21	22.8
Heart muscle	78	7.9	10.1	88	3.4	3.9

(Each values show means of 5 samples)

β-lipoprotein was largest in the liver. These findings agree with the results obtained by KUYAMA and SHIGENAGA in our laboratory.

Histochemical and microautoradiographical examinations of tissue slices of the liver were performed in the following manner.

Radioactive P^{32} in the form of $Na_2H\overset{*}{P}O_4$ was injected intramuscularly into a normal healthy dog three hours prior to an infusion of the fat emulsion. Three hours after infusion of the emulsion the animal was sacrificed by bleeding and tissue slices were taken from the liver to examine histochemically using Smith-Dietrich's staining, and microautoradiographically. But no distinct preparations were obtained

by using the above-mentioned doses of radioactive phosphorus and fat emulsion, therefore, the dosages were doubled in this experiment.

In the emulsion infused group, radioctive phosphorus was observed diffusely in the hepatic cells in the periphery of the hepatic lobules, where phospholipids stained by Smith-Dietrich's method were also clearly found (Fig. 8, 9), while in the controls these findings were not present. It was apparent that these phospholipids were made from the infused glycerides.

Fig. 8 Microautoradiogram of liver 3 hours after the infusion of fat emulsion employing P^{32} (by SHIROTANI)

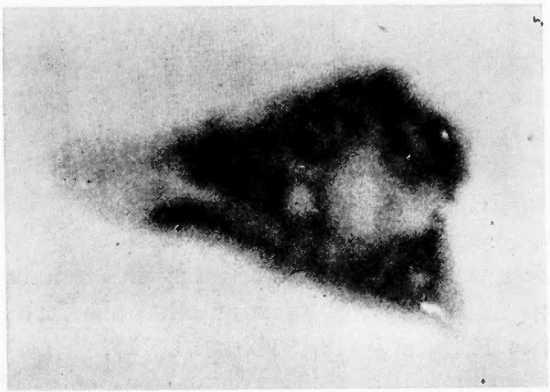
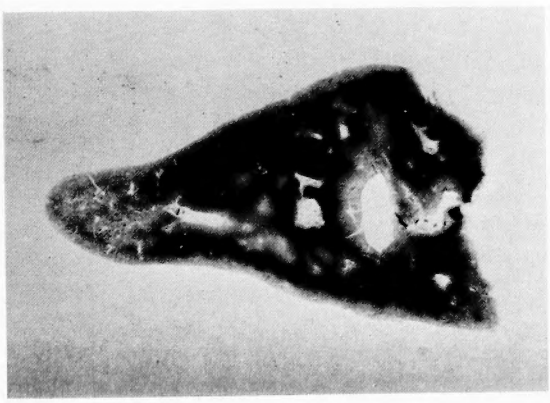


Fig. 9 Liver 3 hours after the infusion of fat emulsion (SMITH-DIETRICH's stain of identical preparate with Fig. 8) (by SHIROTANI)



From the above results, it is evident that the methods of histochemical and biochemical examination which have been employed in our laboratory and our explanations for the metabolic process of fat in vivo derived from these experimental methods are quite reasonable.

3) Influence of Anesthetics on Fat Metabolism

According to SELYE's general adaptation theory, destruction of body protein, increased urinary nitrogen excretion, retention of sodium and chloride and loss of potassium due to an operative insult are a series of phenomena of stress response.

It has been noted that dépot fat is mobilized and decreases rapidly as a result

of an operative insult. Total fatty acids in the blood increase, phospholipids in the blood and liver decrease; dépot fat is mobilized and introduced into the metabolic process in the body.

There is, however, some disagreement as to whether all of these changes in fat metabolism could be regarded as stress responses. Some investigators regard them as only a secondary phenomenon due to a loss of carbohydrate and protein. Our colleagues performed some experiments in regard to these problems. They injected androgen and cortison into animals and observed that the mobilization of dépot fat following an operative insult was one of the defense reactions to stress.

Since anesthesia almost always accompanies surgery, it is necessary to evaluate accurately the influence of different anesthetics on fat metabolism in order to clarify the changes in the metabolic process of fat following an operation.

After administrations of ravonal or morphine, or after ether or cyclopropane general anesthesia continued for about 3 hours a 20% sesame oil emulsion was infused, and the metabolic process of the infused fat was compared with that of the untreated controls, using radioactive phosphorus.

A) Changes in the Serum Lipoprotein

When the emulsion was infused intravenously in animals under anesthesia with ravonal, narcopon-scopolamine, cyclopropane or ether the formation of serum lipoprotein changed as follows (Figs. 10～13, Tables 10, 11).

Both the rate of formation of *O*-lipoprotein from α-lipoprotein and the infused glycerides, and the facility of the phagocytes to phagocytize *O*-lipoprotein were reduced. Consequently *O*-lipoprotein remained in blood much longer, and the changes of α-lipoprotein were less than in the unanesthetized animals.

B) Influence of Anesthetics on the Serum Phospholipids Labeled with P^{32} Following an Intravenous Infusion of Fat Emulsion

When the emulsion was infused intravenously in anesthetized dogs, the specific

Fig. 10 Paper electrophoretic patterns stained for proteins of sera collected successively following the infusion of fat emulsion under thiopental-sodium anesthesia.

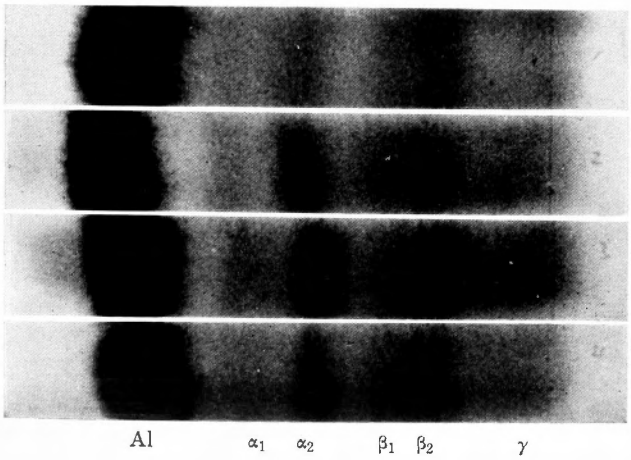


Fig. 11 Paper electrophoretic patterns stained for lipids of sera collected successively following the infusion of fat emulsion under thiopental-sodium anesthesia.

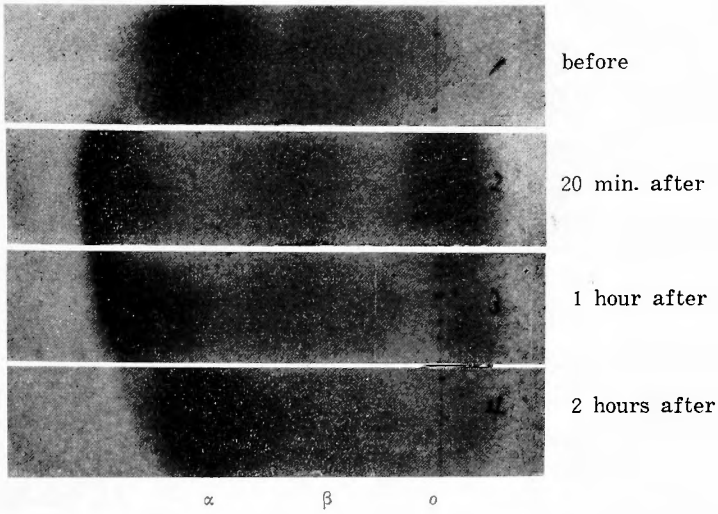
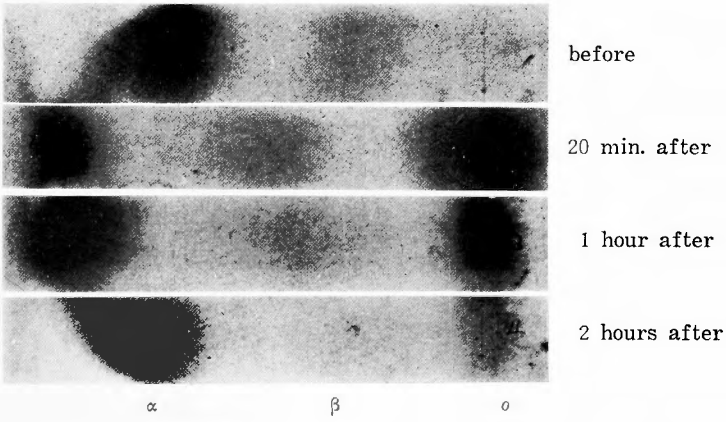


Fig. 12 Paper electrophoretic patterns stained for lipids of sera collected successively following the infusion of fat emulsion under narcopon-scopolamin anesthesia.



activities of acid-soluble P^{32} in serum decreased in the course of time. The degree of this change increased in the following order: ether, narcopon-scopolamine, ravonal, cyclopropane and unanesthetized group. The specific activities of serum lipid P^{32}, on the other hand, increased following the infusion and the degree of this change increased in the following order: ether, narcopon-scopolamine, cyclopropane, ravonal and unanesthetized group (Table 12). These results became more distinct when the relative specific activity was considered (Fig. 14).

All the anesthetics disturb markedly the formation of phospholipids from the infused glycerides, ether and narcopon-scopolamin more seriously than ravonal and cyclopropane. Therefore, from the standpoint of fat metabolism the latter two can be said to be good anesthetics.

Fig. 13 Paper electrophoretic patterns stained for lipids of sera collected successively following the infusion of fat emulsion under closed circuit anesthesia with ether.

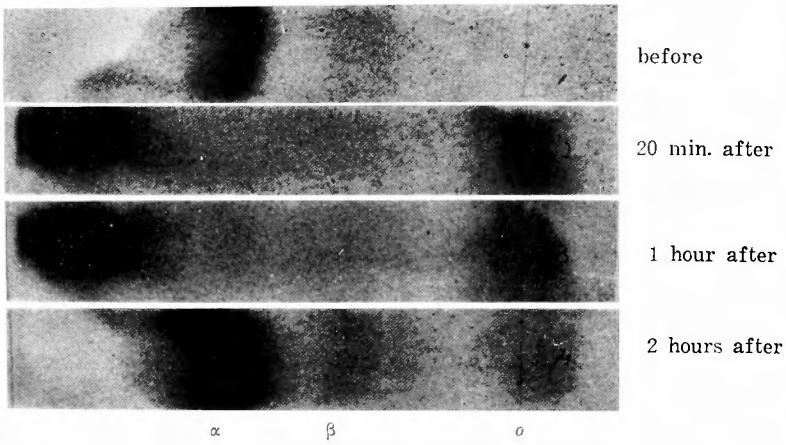


Table 10 Influences of ravonal on electrophoretic fractions of serum lipoprotein (%)

Intervals after infusion	α-lipoprotein	β-lipoprotein	o-lipoprotein
Before	77.5	22.5	0
20 min. after	43.0	20.8	36.2
1 hour after	55.3	26.3	28.4
2 hours after	58.1	24.9	17.0

(Each values show means of 3 samples)

Table 11 Influences of ether on electrophoretic fractions of serum lipoprotein (%)

Intervals after infusion	α-lipoprotein	β-lipoprotein	o-lipoprotein
Before	75.7	24.3	0
20 min. after	49.4	21.8	28.8
1 hour after	50.3	26.3	23.4
2 hours after	51.2	28.0	20.8

(Each values show means of 3 samples)

It was also clarified that disturbances in fat metabolism caused by anesthesia were reduced by daily administration of some vitamins (10mg of vitamin B_1, 10mg of vitamin B_2 and 100mg of vitamin C per day) for five days prior to anesthesia. Therefore, vitamin deficiencies should be corrected before the performance of an operation.

These results were also proved by the influence of anesthetics on the turn over rate of the phospholipids labeled with P^{32} into the tissues (Table 13).

IV. CONCLUSION

The fat absorbed from the intestine enters the blood stream by way of the

Table 12 Influences of some anesthetics on turn over rate of lipid P in the serum following the infusion (Each values show means of 3 samples).

	Intervals after infusion	Ravonal	Narcopon-scopolamin	Cyclopropane	Ether	Ether (+Vitamin)*
Sp. ac. of acid-solub. P×10⁻³	Before	72 (± 0)	66 (± 0)	64 (± 0)	69 (± 0)	7 (± 0)
	20 min. after	64 (− 12)	55 (− 17)	57 (− 11)	58 (− 16)	64 (− 10)
	1 hour after	51 (− 30)	44 (− 33)	46 (− 28)	55 (− 20)	54 (− 24)
	2 hours after	37 (− 49)	37 (− 44)	32 (− 50)	38 (− 45)	39 (− 46)
Sp ac. of lipid P×10⁻³	Before	7.9(± 0)	7.7(± 0)	9.9 (± 0)	7.5(± 0)	7.9(± 0)
	20 min. after	8.5(+ 7)	7.9(+ 3)	9.6 (+ 8)	8.1(+ 8)	8.6(+ 9)
	1 hour after	9.9(+ 25)	8.5(+ 10)	10.8 (+ 21)	9.4(+ 21)	9.7(+ 23)
	2 hours after	13.8(+ 74)	12.8(+ 66)	15.2 (+ 71)	11.0(+ 46)	12.7(+ 61)
Relative Sp. ac. (%)	Before	10.9(± 0)	11.6(± 0)	13.9 (± 0)	10.8(± 0)	11.1(± 0)
	20 min. after	13.2(+ 21)	14.3(+ 22)	16.8 (+ 22)	13.9(+ 30)	13.4(+ 20)
	1 hour after	19.4(+ 76)	19.3(+ 65)	23.4 (+ 70)	16.5(+ 53)	18.1(+ 63)
	2 hours after	37.2(+239)	34.5(+196)	47.5 (+245)	28.9(+163)	32.5(+193)

Vitamin*: Vitamines (10mg of Vit. B_1, 10mg of Vit. B_2, and 100mg of Vit. C per day) were injected daily for 5 days prior to anesthesia.

Fig. 14 Changes in the relative specific activity of lipid P. (Influences of some anesthetics)

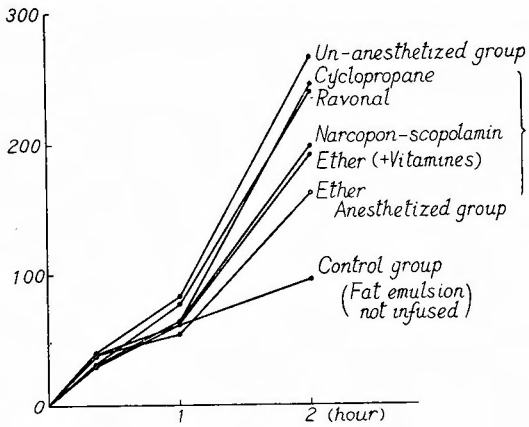


Table 13 Influences of some anesthetics on turn over rate of lipid P to the tissues 3 hours after the infusion.

		Ravonal	Narcopon-scopolamin	Cyclopropane	Ether	Ether (+Vitamin)*
Sp. ac. of acid-sol. P×10⁻³	Liver	92	86	94	102	98
	Kidney	101	87	108	86	88
	Heart muscle	79	103	102	94	97
Sp. ac. of lipid P×10⁻³	Liver	45	38	43	38	43
	Kidney	37	33	38	35	32
	Heart muscle	5.4	9.9	6.2	8.6	8.0
Relative sp. ac. (%)	Liver	48	44	45	37	43
	Kidney	36	37	35	40	36
	Heart muscle	6	9	6	9	8

(Each values show means of 3 samples)

thoracic duct for the most part in the form of microscopic globules less than 0.5 μ in diameter. These globules of glycerides were named chylomicra by FISH.

It has been demonstrated by LUDLUM, TAFT and NUGENT that chylomicra are stabilized not by lecithine but by a thin layer of protein, because chylomicra precipitate at near the isoelectric point of serum protein (pH 4.7～5.3), and lose their stability to form large droplets by the action of acids or alkalies which salt the protein.

ELAINE, BOSSACK and others have demonstrated that α-lipoprotein is contained in the albumin and α_1-globulin fractions, and β-lipoprotein in the α_2-, β_1- and β_2-globulin fractions, and that α-lipoprotein contains 5 % cholesterol and 45～50% phospholipids and β-lipoprotein contains 40% cholesterol and 35% phospholipids. The other lipids are present in the γ-globulin fraction in the form of *O*-lipoprotein. Nowadays, it is possible to separate lipoproteins by ultracentrifugation, since the specific gravity of lipoproteins is less than that of protein because of their content of lipids. The flotation constant is ordinarily represented in SVEDBERG units (Sf); the larger the Sf is, the smaller the protein content and the larger the fat content are. Most of the phospholipids and cholesterol in serum are contained in lipoproteins of Sf less than 10.

It has been considered that chylomicra, entering the blood stream from the thoracic duct, diverge to form finer particles and are introduced into the tissues through capillary walls. Factors participating in these processes are called clearing factors. ANFINSEN et al. have reported that heparin promotes this clearing action. Moreover, it has been postulated that the clearing of serum lipids following lipid absorption from the intestine is based upon such a specific action of heparin.

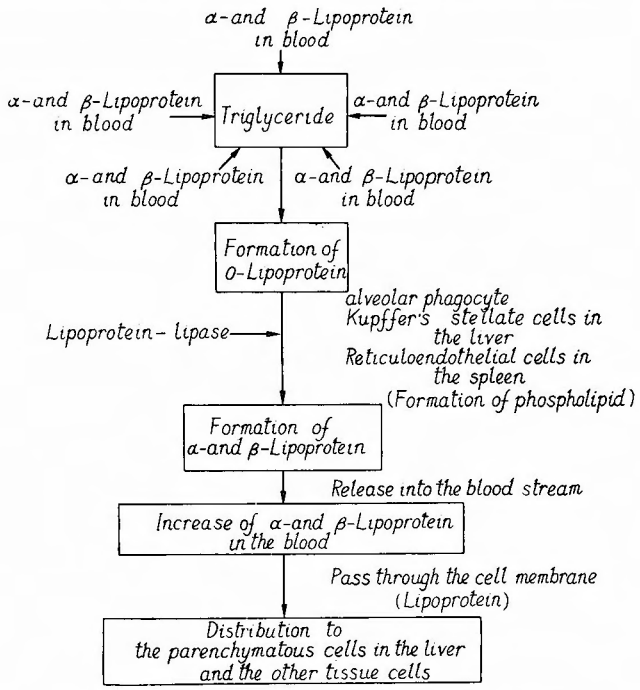
According to our colleagues ASADA, IZUKURA and SHIROTANI, the intravenously infused fat globules, circulating in the blood vessels of the whole body, accumulate in the capillaries of the lung, the sinuses of the liver and the follicles of the spleen where the blood stream is somewhat delayed, and they are phagocytized by alveolar phagocytes, KUPFFER's cells of the liver, reticuloendothelial cells and splenocytes of the spleen to form phospholipids, which enter the blood stream again.

When the above mentioned primary disposal of the infused fat advances more or less, phospholipids begin to appear diffusely in the parenchymatous cells of the peripheral zone of liver lobules, and their amount reaches its maximum three hours after the infusion of the fat.

Consequently it is considered that the infused glycerides are phagocytized by alveolar phagocytes, liver KUPFFER's cells and reticuloendothelial cells of the spleen to form phospholipids, which are transported to all the tissue cells by the blood stream. These results agree with the biochemical results obtained by KUYAMA in our laboratory.

Moreover, it has been demonstrated that higher saturated fatty acids, oleic acid and essential fatty acids are metabolized not only in the liver tissues but also in the extrahepatic tissues, while lower fatty acids, highly unsaturated fatty acids, eicosenoic and docosenoic acid are metabolized only in the liver tissues.

Fig. 15 Fate of glycerides flowed into the blood vessels.



On the whole, the results of the above histochemical and biochemical studies suggest that the clearing of chylomicra is achieved by the phagocytes of the lung, liver and spleen.

In the present report the metabolic process of intravenously infused sesame oil emulsion was studied by autoradiography of the serum with the use of radioactive phosphorus, and by measuring the relative specific activities of the serum and tissues. It has been clarified by these experiments that α-lipoprotein of a high density accumulates around the intravenously infused glycerides to form *O*-lipoprotein (Chylomicra). Glycerides, stabilized in these processes, are phagocytized by alveolar phagocytes, KUPFFER'S stellate cells of the liver and reticuloendothelial cells of the spleen, where the glycerides are converted to phospholipids. These phospholipids enter again the blood stream in the combined forms of α- and β-lipoprotein, and are carried to all tissues, especially to the liver.

The results obtained using radioactive phosphorus as a tracer agree completely with the results and explanations for the metabolic process of fat obtained by histochemical and biochemical studies in our laboratory (Fig. 15). Furthermore, it has been clarified that postoperative disturbances in fat metabolism are improved markedly by the careful management of preoperative nutritional states and by the improvements in anesthesia, and that not all of the postoperative changes in fat metabolism can be regarded as a stress response.

V. SUMMARY

A 20% sesame oil emulsion, produced in our laboratory, was administered

intravenously without causing any deleterious effects. Using radioactive phosphorus as a tracer, the metabolic processes of the infused glycerides were studied and the following results obtained.

1) Glycerides infused intravenously are stabilized in the blood stream by the accumulation of α-lipoprotein around them to form *O*-lipoprotein which circulates in the blood vessels.

2) *O*-lipoprotein thus formed is phagocytized by alveolar phagocytes, KUPFFER's cells of the liver and the reticuloendothelial cells of the spleen and is converted into phospholipids, which are delivered again into the blood stream in the combined forms of α- or β-lipoprotein.

3) These α- and β-lipoproteins are introduced into the tissues of the whole body, especially into the liver tissues.

4) The results obtained by using radioactive phosphorus agree completely with those obtained by histochemical and biochemical methods which have been employed in our laboratory, and the reasonableness of our explanations for fat metabolism is established.

5) Some anesthetics exert a serious influence on fat metabolism: narcopon-scopolamine and ether do so more seriously than cyclopropane and ravonal.

6) Not all of the postoperative changes in fat metabolism can be regarded as a stress response. Some of them are the results of deficient preoperative nourishment and of the mismanagement of anesthesia.

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和 文 抄 録

放射性燐 P^{32} を以てする脂質代謝の研究

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われわれの教室に於て創製された静脈内注入可能な脂質乳剤と放射性燐 P^{32} を応用し，さきに教室の先人が行つて来た脈管内注入グリセライドの生体内代謝過程を更に詳細に追究吟味し，次のような結論に到達した．

1) 脈管内注入グリセライドはまづその周囲に血中既存のα-脂蛋白が集積することによつて血液中で安定化され，*O*-脂蛋白として，全身を循環する．

2) この間，脈管内流入グリセライドは *O*-脂蛋白として肺胞喰細胞，肝星細胞，脾臓の網内系細胞により摂取され，それら細胞内で1次的処理を受け，再び流血中に放出されるが，その際には既にグリセライドは燐脂質に変じており，而も遊離型の燐脂質としてではなく，常にα-あるいはβ-脂蛋白という結合形態をとつている．

3) 以上の過程で新しく形成せられた燐脂質は脂蛋白の型で血流を介して全身臓器組織に移行する．而してその際肝臓に於てその単位体積当りの燐脂質移行量は最も大であつた．

4) 放射性燐 P^{32} を用いて行つた本実験成績は従来教室で採用されて来た組織顕微化学的並に生化学的検索方法による結果とよく一致し，生体内脂質代謝過程についてのわれわれの従来いだいて来た見解の妥当性が一層確然とされるに至つた．

5) 麻酔剤は生体内脂質代謝に対しても著るしい障碍作用を及ぼしている．而してその障碍の程度はサイクロプロペィン，ラボナールでは比較的僅微であるが，ナルコポン・スコポラミン，エーテルでは相当顕著である．

6) 従来唱えられて来た様に，手術後の脂質代謝の様相をすべてStress responseとしての現象と解釈することは出来ず，それらのあるものは術前の栄養状態，麻酔管理法の不備にもとづく．