

PARENTERAL ADMINISTRATION OF FATS

III. Qualitital Investigation on Nutritional Effects of Fats and Recent Studies on Fat Metabolism in Vivo.

by

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Received for publication Feb. 5, 1959

I. INTRODUCTION

The nutritional effects of fat have not been taken as seriously up to this time as those of other nutriments, and the oral administration of fat in pathological conditions has met with opprobrium. This has been due to the imperfect studies on the metabolic processes of fat in vivo and to inadequate methods for deciding their nutritional effects.

Hence, the nutritional effects of fat should be rescrutinized from a new standpoint and by new experimental methods, as the metabolic processes of fat in vivo have now been clarified for the most part.

This report concerns problems of the nutritional effects varied by the qualitital difference of fat.

II. EXPERIMENTAL MATERIALS AND METHODS

In this investigation, a fat emulsion having the same components as that reported in the previous paper^{1,2)} was employed, unless otherwise stated.

As experimental animals, dogs and cats representing carnivorous animals, rats representing omnivorous animals and rabbits representing herbivorous animals were used. Adult rabbits, weighing 2.0~2.5kg and rats of the Wistar strain, weighing 50~250g were bred in a constant temperature room at 20°C.

Paper chromatography was done by NODA-HIRAYAMA's method^{3)~6)}, i. e. the development was made as the *p*-bromophenacyl ester 2, 4-dinitrophenylhydrazones of fatty acids.

(i) Filter paper: "Toyo" filter paper No. 2 was used. A starting line was penciled 3 cm above the upper border of the surface of the solvent.

(ii) Solvent systems: As the moving solvent, methanolglacial acetic acid-petroleum hydrocarbon (10 : 2 : 1.2 by volume) was used, and petroleum hydrocarbon

was used as the stationary solvent.

(iii) Procedure: The paper was spotted with samples and was uniformly sprayed with the stationary solvent, petroleum hydrocarbon. Then the chromatogram was developed with the moving solvent by the ascending technique at 30°C. The development time required for a satisfactory separation was 5~6 hours by this method.

(iv) Spot identifications: The spot identifications are as follows: The number represents the carbon atoms,

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 (Ctoleic acid),		EI; Eicosenoic acid,
HU; Highly unsaturated acids,		

S; Standard, i. e. a mixture of the derivatives of all the even-numbered saturated acids from C_4 to C_{20} .

Furthermore, 0.5 m C per kg body weight of radioactive P^{32} in the form of Na_2HPO_4 was injected intramuscularly into adult dogs. Fat emulsions were infused intravenously 3 hours after the injection of radioactive P^{32} , as the concentration of serum organic phosphate reached its maximum at that time. And then sera of dogs was successively collected. The paper electrophoresis of serum was continued for 10 hours at 0.4 mA/cm. MICHAELIS' buffer solution of pH 8.6 and ionic strength 0.059μ was used. Protein marked on a strip with B. P. B. and lipid on another with Sudan Black B, and the protein and lipid on these strips were estimated directly with a densitometer of electrophotocells. The autoradiography of the electrophoregram of the serum lipoprotein was done for 25 days using "Fujii" X-ray film. In addition, acid soluble phosphorus and phosphorus in phospholipid of various tissues were determined by SCHMIDT-THANHAUSER's method⁸⁾.

Male albino rats from the Wistar strain weighing about 100 g were given a diet containing 30 % fat for a period of 30 days. Natural fats such as sesame oil, olive oil (containing higher saturated fatty acids, oleic acid and essential fatty acids), butter fat, coconut oil (containing lower fatty acids), and cod liver oil (containing highly unsaturated fatty acids, docosenoic acid and eicosenoic acid), and synthetic triglycerides such as simple glyceride of C_3 , C_5 , C_{10} , C_{18} , C_{13} and triolein were used. After fasting for 12 hours, all the rats were injected with 1% alloxan solution (160 mg per kg body weight) intraperitoneally. After 10 days, their blood sugar levels were measured by SOMOGYI's method⁹⁾. Any rat whose blood sugar level was over 150 mg/dl was judged to be diabetic. We also used as an aid in judging diabetes, urinary sugar values measured by BENEDICT's method¹⁰⁾, applying it on the 3rd, 6th, and 9th day after alloxan injection.

III. EXPERIMENTAL RESULTS AND DISCUSSION

1) THE LATEST STUDIES ON FAT METABOLISM

We have studied the intravenous administration of fat as a parenteral nutrient and fat metabolism in vivo, and recently these studies have been promoted by the use of radioactive P^{32} . After radioactive P^{32} in the form of $Na_2HPO_4^*$ was injected intramuscularly into dogs, the serum was successively examined by paper electrophoresis and autoradiography. Then, radioactive P^{32} was introduced into α -lipoprotein in serum. When the fat emulsion (sesame oil) was administered intravenously in such a state (3 hours after the intramuscular injection of radioactive P^{32}), α -lipoprotein in serum, which had high density and contained lipids in small quantities, being labeled radioactive P^{32} , gathered together around the infused fat corpuscles (triglyceride), and formed O -lipoprotein, which had low density and contained lipids in large quantities. These findings seem to show that the infused fat corpuscles (triglyceride) were stabilized in such a form (Chylomicrons), because P^{32} in α -lipoprotein appeared before the infusion of fat emulsion introduced into O -lipoprotein up to 20-30 minutes after the infusion. However, this state continued for only 30 minutes, in which the infused fat globules finished being phagocytized by the alveolar phagocytes, KUPFFER's stellate cells of the liver and reticuloendothelial cells of the spleen, as shown in a previous paper¹¹. And radioactive P^{32} in α - and β -lipoprotein, especially in the former, increased markedly, when glycerides changed into phospholipids in these cells. These findings suggest that α -lipoprotein contains phospholipids in larger quantities than β -lipoprotein, as stated by ELAINE and others¹¹, and that the infused fat changed into phospholipids from glycerides (Figs. 1 and 2).

On the other hand, in control animals, radioactive P^{32} in α -lipoprotein gradually decreased and could scarcely be found on a strip analysed by paper electrophoresis and autoradiography 4 hours after the intramuscular injection of P^{32} .

Summarizing the above findings, it is postulated that α -lipoprotein in serum gathers together around the triglyceride corpuscles to form O -lipoprotein (chylomicrons) when these corpuscles enter into the blood stream, and then glycerides are stabilized in blood. Furthermore, it is evident that chylomicrons are phagocytized by alveolar phagocytes, KUPFFER's stellate cells of the liver and reticuloendothelial cells of the spleen, in which glycerides change into phospholipids and then phospholipids changed from glycerides enter again into the blood stream in the form of α - and β -lipoprotein, not in the free form, and are carried to all tissues through this stream (Table 1). Since the infused fats changed into phospholipids and entered into the blood stream in the form of α - and β -lipoprotein, radioactive P^{32} in phospholipid in the hepatic parenchymatous cells started to increase and reached the maximum approximately 3 hours after the infusion of fat emulsion (Table 2). According to the results of histochemical and micro-autoradiographical examinations of tissue slices of liver, at such a time, radioactive P^{32} was observed diffusely in the periphery of the hepatic lobules (Fig. 3), in which phospholipids stained by SMITH-DIETRICH' method were also clearly found (Fig. 4), while in controls these findings could not be demonstrated. From the above facts, it is clear that the methods of the histochemical and biochemical examinations employed by

Table 1 PHOSPHOLIPID TURNOVER IN SERUM
(following the administration of fat emulsion)

intervals after injection	FAT EMULSION GROUP			CONTROL GROUP		
	Sp. ac. of acid-soluble $P \times 10^{-3}$	Sp. ac. of phospholipid $P \times 10^{-3}$	relative Sp. ac. (%)	Sp. ac. of acid-soluble $P \times 10^{-3}$	Sp. ac. of phospholipid $P \times 10^{-3}$	relative Sp. ac. (%)
before	69	8.5	12.3	66	8.6	13.0
20 min. after	57	9.0	15.8	53	8.7	16.4
1 hour after	44	10.9	24.7	44	9.3	21.1
2 hours after	34	15.3	45.0	39	9.9	25.4

Table 2 PHOSPHOLIPIDE TURNOVER IN TISSUES
(3 hours after the administration of fat emulsion)

TISSUE	FAT EMULSION GROUP			CONTROL GROUP		
	Sp. ac. of acid-soluble $P \times 10^{-3}$	Sp. ac. of phospholipid $P \times 10^{-3}$	relative Sp. ac. (%)	Sp. ac. of acid-soluble $P \times 10^{-3}$	Sp. ac. of phospholipid $P \times 10^{-3}$	relative Sp. ac. (%)
LIVER	75	47	62.6	93	31	33.3
KIDNEY	104	38	36.5	92	21	22.8
HEART	78	7.9	10.1	88	3.4	3.8
MUSCLE						

us, those results and our explanation for the process of fat metabolism in vivo are quite reasonable.

We have used sesame oil emulsion, cod liver oil emulsion, and synthetic triolein emulsion for oral and intravenous administration in experimental animals, and have carried out histochemical studies of various organs. When cod liver oil, containing a large amount of highly unsaturated fatty acids, docosenoic acid, and eicosenoic acid, or butter, containing lower fatty acids, were given orally or intravenously as an emulsion, much larger amounts of phospholipids were demonstrated in the parenchymatous cells of the liver, in comparison with the cases in which sesame oil emulsion was used, containing nothing but higher saturated fatty acids, oleic acid and the essential fatty acids. And we found the same results as when we studied the phospholipid content of various organs biochemically. According to the results mentioned above, highly unsaturated fatty acids, lower fatty acids, eicosenoic acid, docosenoic acid etc. are shifted only to the parenchymatous cells of the liver; and higher saturated fatty acids, oleic acid, and essential fatty acids are shifted not only to the parenchymatous cells of the liver but also to extrahepatic tissues where they will be disposed of further¹⁾.

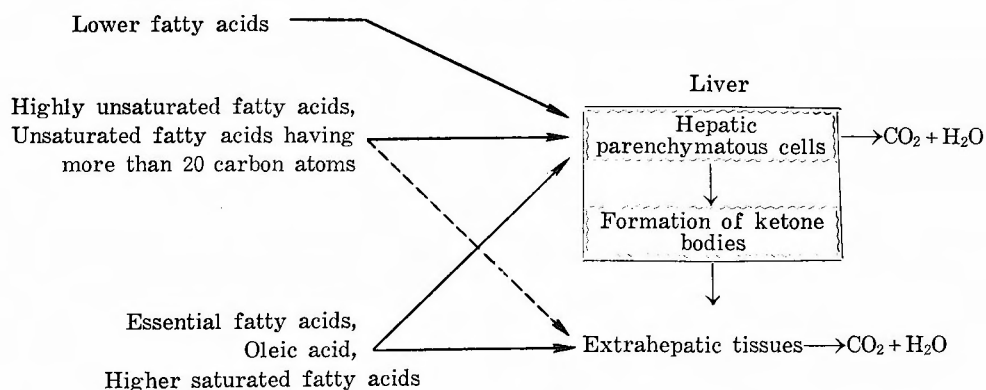
Using paper chromatography of fatty acids, we reaffirmed the fact that the amount of fatty acids in various organs differs according to the kind of fatty acids, which we have indirectly proved using histochemical and biochemical methods.

Cats, representing carnivorous animals having a high capacity for the disposition of fat, were used. The synthesized simple glycerides of lower fatty acids, tricaproin, tricaprylin and tricaprin were mixed in the same proportion and

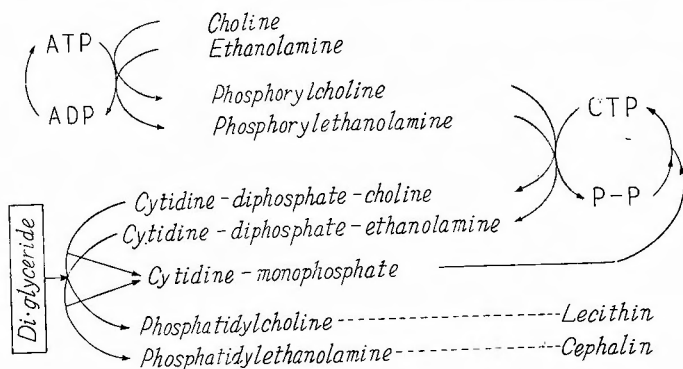
emulsified, and were administered intravenously (Fig. 5). Histochemically it was proved that the amount of phospholipids shifted to the parenchymatous cells of the liver reached the maximum 3 hours after injection. At this stage the experimental animals were sacrificed by bleeding, and the liver, kidney and heart, which are important parenchymatous organs for the disposition of fat, were removed, and the fatty acids were separated and analysed by paper chromatography. Caproic acid, caprylic acid, and capric acid in the group of lower fatty acids were shifted only to the liver and nowhere else (Fig. 6). When cod liver oil emulsion was administered, highly unsaturated fatty acids, docosenoic acid, eicosenoic acid etc. contained in the cod liver oil in large amounts, also seemed to be concentrated in the liver. These fatty acids, however, were sometimes demonstrated in the extrahepatic tissues such as heart muscle in the postabsorptive state, therefore one had to assume that a portion of these fatty acids were shifted to the extrahepatic tissues, though the major portion were shifted to the liver (Figs. 5 and 7). After the administration of sesame oil emulsion, the amount of fatty acids contained in various organs showed a marked increase, with almost no change in the composition of the fatty acids (Figs. 5 and 8).

In other words, lower fatty acids, highly unsaturated fatty acids, docosenoic acid, eicosenoic acid etc. are shifted only to the parenchymatous cells of the liver in the form of phospholipids and undergo further metabolism, and they are the ones which undergo, for the most part, so-called indirect oxidation. Whereas higher saturated fatty acids, oleic acid, the essential fatty acids etc. are shifted in the form of phospholipids not only to the parenchymatous cells of the liver but also directly to the extrahepatic tissues and undergo further metabolism, and they are the ones which, for the most part, undergo so-called direct oxidation. The metabolism of fat *in vivo* can be summarized schematically as shown in Fig. 10 according to the various types of fatty acids.

Fig. 10 METABOLIC PROCESS OF FAT IN VIVO



Up until now studies of phospholipid synthesis have not been conclusive. Recently, KENNEDY^{12)~20)} stated that cytidine triphosphate (CTP) took part in the synthetic process of phospholipid and that the lipid acceptor of phosphorylcholine was diglyceride and was not phosphatidic acid. As shown in Fig. 11, phosphoryl-

Fig. 11 PATHWAY OF THE PHOSPHOLIPID SYNTHESIS (KENNEDY)

choline and phosphorylethanolamine, which are produced from choline and ethanolamine, change into cytidine diphosphate choline and cytidine diphosphate ethanolamine. And then, phosphatidylcholine, which is lecithin, and phosphatidylethanolamine, which is cephalin, are synthesized from cytidine diphosphate choline and cytidine diphosphate ethanolamine with the presence of diglycerides, and cytidine monophosphate is utilized to synthesize cytidine triphosphate. Therefore, diglyceride plays an important role in phospholipid synthesis.

NAKATA²¹⁾, in our laboratory, made a perfusion experiment of the isolated lung of rabbits and cats with fat emulsion and obtained the results that triglycerides in the circulating fluid decreased, while phospholipids and free fatty acids in the circulating fluid increased as did the levels of lipase in both circulating fluid and organs. SENO²²⁾, in our laboratory, reported the same results obtained by perfusion experiments with isolated liver. We believe that diglycerides are produced in the alveolar phagocytes, KUPFFER's stellate cells of the liver and reticuloendothelial cells of the spleen.

Recently, it is believed that lipoprotein lipase and free fatty acid in the plasma increase for clearing the chylomicrons in the serum as stated by ROBINSON^{23)~29)} and others. These facts are in accord with the results obtained by NAKATA²¹⁾ and SENO²²⁾. Thus produced diglyceride changes into phospholipids, such as lecithin and cephalin, in the presence of cytidine diphosphate choline and cytidine diphosphate ethanolamine. The infused fat can enter into cells of various parenchymatous tissues after being changed into phospholipid in the form of α - and β -lipoprotein. So, we can presume that the above mentioned primary disposal of the infused fat is necessary in order to pass the cell membranes (lipoprotein) in the parenchymatous tissues.

It is generally believed that choline, inositol and essential fatty acids have a lipotropic action. In our investigation, essential fatty acids alone could always be found in all organs of fasting animals, even if they were somewhat decreased, while higher saturated fatty acids and oleic acid were markedly decreased in those cases. From these results, it is well understood that the former fatty acids are a constant element and the latter a variable element. Therefore, essential fatty acids are fundamental substances in the construction of cell membrane, which is made

by a kind of lipoprotein. According to the experimental results of NAGASE²⁹⁾ in our laboratory, the deficiency of essential fatty acids brought about an increase in permeability of the capillary wall. In such a condition, the increased glyceride in the plasma can infiltrate into the hepatic parenchymatous cells and fatty infiltration in the liver results. Furthermore, the deficiency of choline and inositol which are important substances in phospholipid synthesis, promotes the occurrence of fatty liver, through disturbing the disposal of glyceride in the plasma. Therefore, we can agree with the experimental results of ENGEL³⁰⁾ that the lipotropic action of choline becomes insufficient when there is a great lack of essential fatty acids and vitamin B₁₂ in the body.

2) DIFFERENCES BETWEEN THE NUTRITIONAL EFFECTS OF VARIOUS KINDS OF FATS.

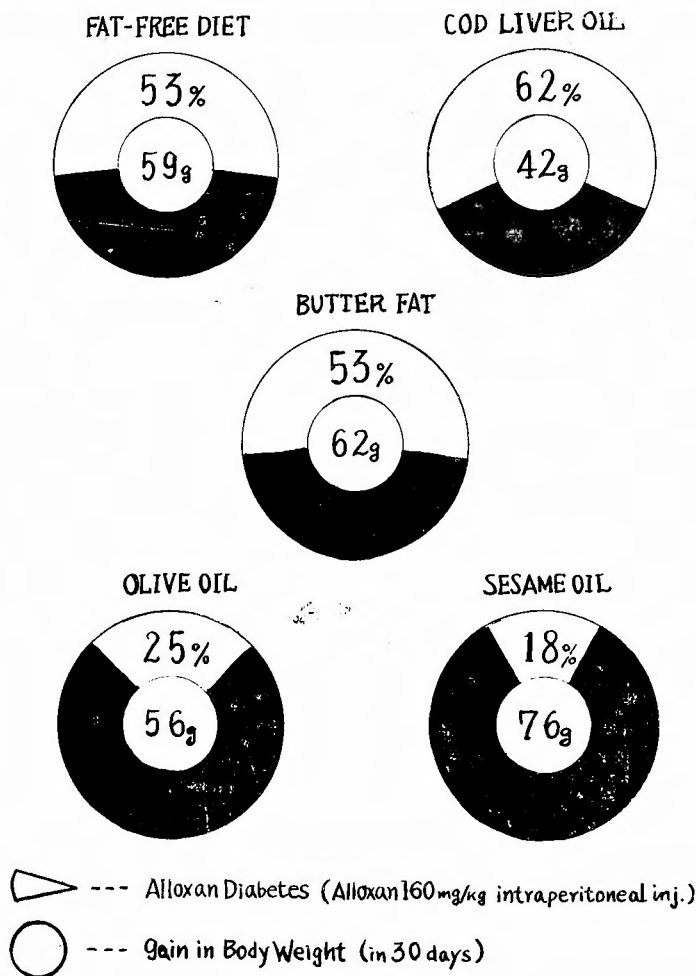
It is evident from the above studies that the process of fat metabolism *in vivo* is divided into two as follows. All or almost all of the lower fatty acids, highly unsaturated fatty acids having more than 4 unsaturated bonds and unsaturated fatty acids having more than 20 carbon atoms (even if they have only one unsaturated bond) enter into the hepatic parenchymatous cells in the form of phospholipid—lipoprotein, and only a part of them (except lower fatty acids) enter slowly into the extrahepatic tissues in slight degree. On the other hand, other higher saturated fatty acids, oleic acid and essential fatty acids, such as linoleic and linolenic acids, enter not only into the hepatic parenchymatous cells, but also into the extrahepatic tissues to be oxidized. Accordingly, the nutritional effects of fats should be decided considering these two metabolic processes. That is, fatty acids of the former group are a burden to the liver, markedly increase ketone body production and may cause a ketosis, while fatty acids of the latter group lighten the burden of the liver and produce ketone bodies in smaller quantities than those of the former group (Fig. 10).

It has been recognized generally up to this time that the increase and decrease of fat in the liver is inversely proportional to that of glycogen and an increase of fat in this organ is injurious to the liver. In our laboratory, sesame oil emulsions containing higher saturated fatty acids, oleic acid, essential fatty acids, etc. which are mostly directly oxidized in the body, were injected intravenously into rabbits in large quantities and for approximately 20 weeks without the simultaneous injection of various vitamins. The blood sugar levels of these animals were maintained during the experiment and the glucose tolerance curve was also normal in the 20 th week after repeated infusions. Furthermore, in these cases, liver glycogen was found in larger quantities than in the controls. These findings show that the administration of glycerides which consist of higher saturated fatty acids, oleic acid, essential fatty acids etc., which in general, undergo direct oxidation in the body, is rather favorable to liver function. However, with repeated infusions of cod liver oil emulsion containing highly unsaturated fatty acids, eicosenoic acid, docosenoic acid etc. which undergo, for the most part, indirect oxidation, the blood sugar levels were slightly higher than in the control and lipids in the liver

increased, while liver glycogen greatly decreased. If further repeated infusions of sesame oil emulsion into these rabbits were continued for two weeks, lipids in the liver decreased again, liver glycogen increased and the glucose tolerance curve recovered to normal²⁾. Thus, the administration of mixed glycerides which consist of higher saturated fatty acids, oleic acid and essential fatty acids, were not only harmless to the liver, but also very effective nutritiously and increased liver glycogen, while the administration of mixed glycerides containing highly unsaturated fatty acids, eicosenoic acid and docosenoic acid, especially in the deficiency of vitamin C and riboflavin in the body, should be avoided from the view-point of liver function.

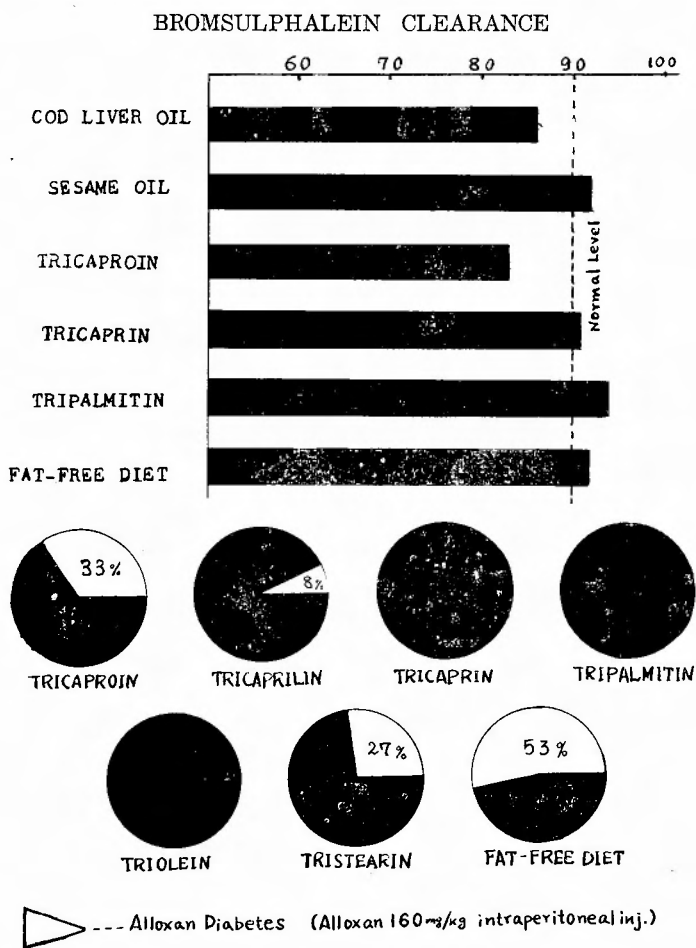
Furthermore, the administration of butter fat and cod liver oil containing lower fatty acids, highly unsaturated fatty acids, docosenoic acid, eicosenoic acid etc., which undergo, for the most part, indirect oxidation, greatly increased the

Fig. 12 INFLUENCE OF DIETARY FAT (30% IN DIET) ON INCIDENCE OF ALLOXAN DIABETES. AND EFFECT OF THOSE FATS ON GROWTH OF RAT.



sensitivity to alloxan, while olive oil and sesame oil, which consist of higher saturated fatty acids, oleic acid and essential fatty acids, lowered it markedly (Figs. 12 and 13)³¹⁾.

Fig. 13 EFFECT OF DIETARY FAT (30% IN DIET) ON LIVER-FUNCTION AND INFLUENCES OF VARIOUS SIMPLE GLYCERIDES ON ALLOXAN DIABETES (albino-rats from Wistar-strain were used, and they were kept in a room regulated about 20°C)



From these findings we can conclude that triglycerides such as sesame oil seem to be best suited to oral or parenteral administration of fat, since they contain no lower fatty acids, no highly unsaturated fatty acids, docosenoic acid, or eicosenoic acid, etc. which for the most part are those undergoing so-called indirect oxidation; but do contain higher saturated fatty acids and oleic acid which are used in the body as variable elements, imposing little burden on the liver, having little possibility of forming ketosis, and undergoing for the most part direct oxidation; and also containing linoleic acid and linolenic acid which are indispensable in the composition of tissue cells as constant elements.

In the experimental administration of fat, the presence of peroxide in the auto-oxidized products, which are produced in rancid fat, should be strictly avoided. The poisonous action of fish oils may be caused by highly unsaturated fatty acids, which undergo, for the most part, indirect oxidation, and peroxide, which is produced by the auto-oxidation of these fats. Recently, KANEDA³²⁾ and others reported the fact that peroxide accumulates in tissue cells, destroying the mitochondria, when the auto-oxidized products enter the body. Therefore, the intake of fat with a very high peroxide value, should be avoided even in health, and especially in riboflavin and vitamin E deficiency. And the administration of fat containing fatty acids, which undergo, for the most part, indirect oxidation, and also containing peroxide easily causes fatty liver. After all, the effects of fat and the occurrence of fatty liver are decided by the composition of fat in the diet.

KISHIMOTO in our laboratory has affirmed that sesame oil produces an auto-oxidized substance of rancidity when the sesame oil is heated (under 90°C) or treated by a solar light for a long time, showing a marked increase in peroxide value and a marked decrease in the iodine value. When this auto-oxidized substance is produced, although there is no change in the amount of saturated fatty acids, essential fatty acids having unsaturated double bonds show a definite decrease. At the time of this reaction the amount of 2,4-dinitrophenylhydrazones of *p*-bromophenacyl ester produced is very little, and a black viscous substance which is ether insoluble is precipitated (Fig. 14 [A]). When sesame oil is heated over 200°C polymerization takes place at the position of the double bond, and although there is no change in the amount of saturated fatty acids in the sesame oil, the amount of essential fatty acids decreases (Fig. 14 [B]). When the auto-oxidized product enters the body, it is accumulated in the tissue cells, destroying the mitochondria, and the proceeding polymerization lowers the absorption and digestion of the fat. Moreover, as this experiment shows, the rancidity or polymerization induces a decrease in the essential fatty acids, which have a much more important nutritional value than as a mere source of calories, and thus seems to deprive the fat of its nutritional value. Therefore, apart from the method of administration of the fat, it is necessary to select a fat which contains no product due to rancidity or polymerization, in other words non-spoiled fat.

The same care is to be taken in the preservation of fat, avoiding light, keeping it at a low temperature, and exposing as little surface as possible to the air. As the reaction is due to self contact, spoiled fat facilitates the reaction; therefore old oil must not be mixed with new. Furthermore, since the heating of fat facilitates polymerization, inhibits digestion and absorption, and causes a decrease in the amount of the essential fatty acids, care must be taken even at the time of cooking.

This has led us to believe that sesame oil is the ideal substance, and one must select refined sesame oil with the lowest peroxide value.

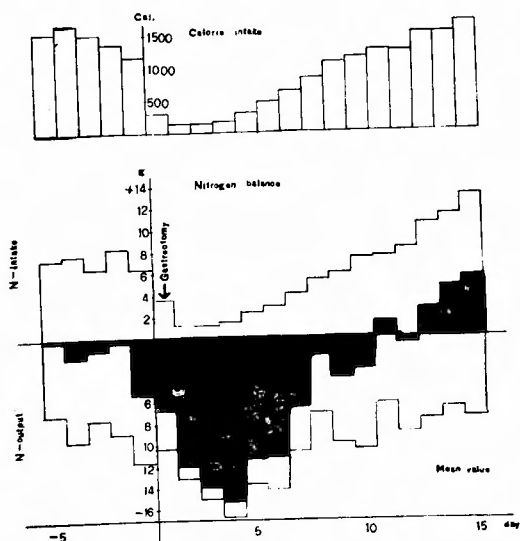
Therefore, we prepared a 20% refined sesame oil emulsion with the lowest peroxide value ("Fatgen".....peroxide value: 0). In a previous paper, we demon-

strated clinically that this sesame oil emulsion can certainly meet our expectations when it is applied with proper amounts of glucose and various vitamins, i. e. the administration of sesame oil emulsion is remarkably effective in economizing protein and maintaining the body weight (Fig. 15)²⁾.

Fig. 15 CHANGE IN NITROGEN BALANCE FOLLOWING GASTRECTOMY

[A]

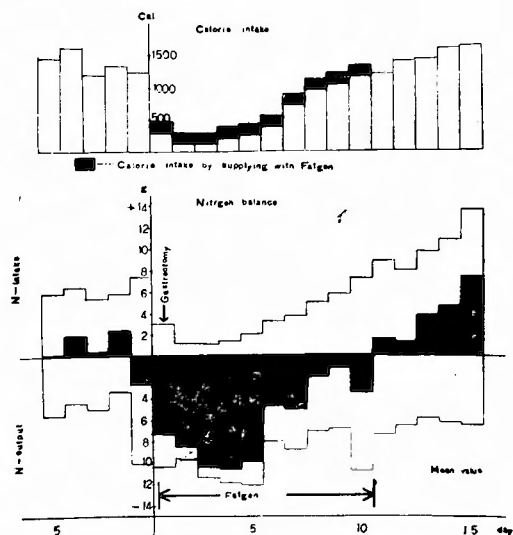
Control Group



[B]

Fat Grout (I)

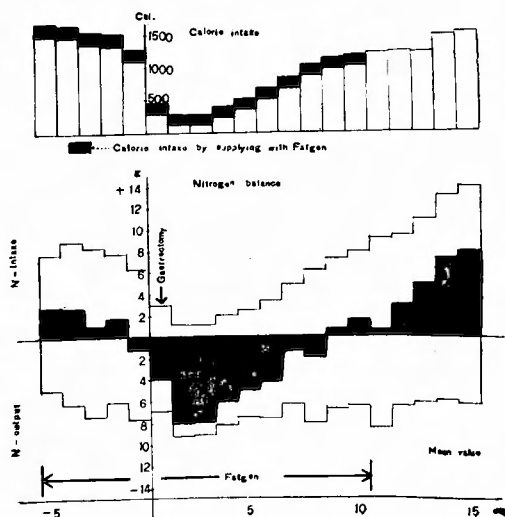
(The sesame oil emulsion was given every day for 10 days after the gastrectomy)



[C]

Fat Group (II)

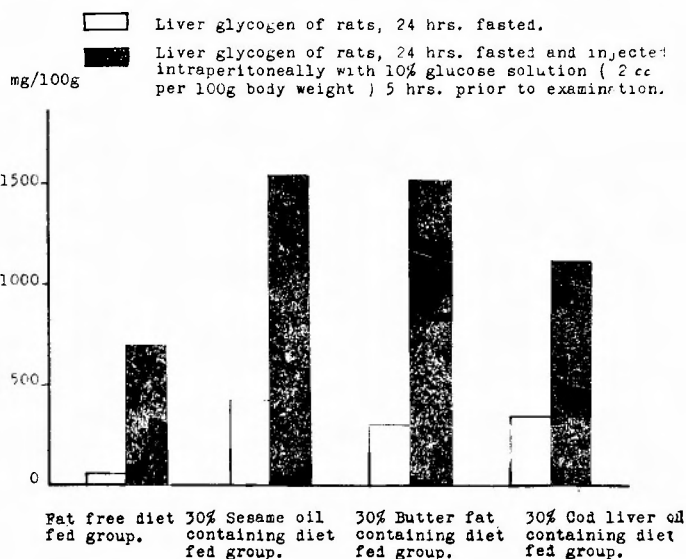
(The sesame oil emulsion was given everyday for 5 days prior to the gastrectomy and for 10 days after it)



Furthermore, we proved that the protein sparing effect of the sesame oil emulsion in postoperative gastrectomized patients was gained much better when the emulsions were administered before and after the operation (Fig. 15 [C]), than when it was supplied only after the operation (Fig. 15 [B]). It is impossible to account for this postoperative effect of a previous supply of fat from its calorogenic action alone. The following results of our investigations are very suggestive in this regard.

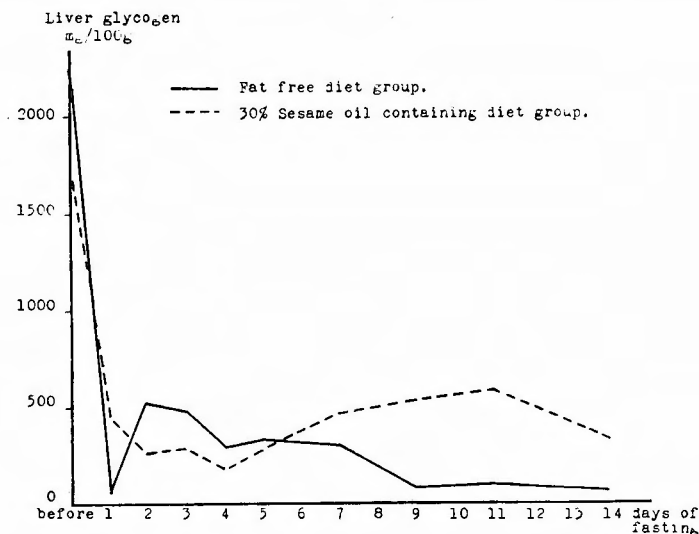
Rats were fed a fat free diet or a diet containing 30% sesame oil for a period of about 1 month, then were subjected to starvation and their liver glycogen measured successively. It was observed that liver glycogen depletion after 24 hours of fast was much less in the fat fed group than in the fat free diet group, and when glucose was given intraperitoneally, glycogenesis occurred to a higher degree in the livers of the fat fed group (Fig. 16). These findings show that previous

Fig. 16 EFFECT OF DIETARY FAT ON GLYCOGENESIS IN LIVER



feeding with fat prior to fasting enabled the animals to preserve more economically the stored and the exogenic carbohydrate during fasting. When starvation continued further in the fat free diet group, an increase of liver glycogen due to glycogenesis associated with protein catabolism was observed to occur as early as the 48th hour of fasting but to decrease again after the 4th day. On the other hand, in the fat fed group, this glycogenesis started later (after the 4th day of the fast) and lasted longer (till the 11th day at least). It is indisputable that the caloric demand of starving animals must be fulfilled mainly by utilization of their own storage fat (Fig. 17). Accordingly animals fed fat free diets which have subsisted mainly on dietary carbohydrate and protein, are forced by starvation to shift acutely the source of energy from carbohydrate to fat, because of rapid depletion of their carbohydrate reserve as was observed. It is easy to surmise that

Fig. 17 CHANGE IN LIVER GLYCOGEN CONTENT DURING FASTING
(RAT)



such a condition may act as a stress and force the organisms to mobilize their adrenal glucocorticoid, which accelerates the glycogenesis associated with protein catabolism. From this point of view, the decrease of liver glycogen in the later phase of fasting can well be interpreted as a manifestation of the exhaustion with such an adaptive function combined with depletion of body protein and slow down of the glycogenetic function in the liver. On the other hand, animals well adapted to fat feeding can utilize most effectively and smoothly their storage fat during a fast so that they prevent the rapid depletion of their carbohydrate reserve, make milder the stress effect of the fast, lessen the protein catabolism and consequently maintain well their liver and adrenal function.

It is assumed that such a fat-adapting effect, in addition to its caloric value, can be expected from the clinical use of fat emulsion, and in fact, we have confirmed it in the cases of gastrectomy as mentioned above. From this standpoint, we consider that not only postoperative but also preoperative administration of fat emulsion is a most reasonable procedure for the purpose of minimizing metabolic disorders and breakdown of body protein which would affect the patients after the operation, especially in such cases as gastrectomy after which patient are forced to withstand a certain period of fasting or poor nutrition.

IV. SUMMARY

We studied again the process of fat metabolism in vivo and the nutritional effects of fat by the use of a fat emulsion produced in our laboratory, and obtained the following new results.

(1) When the triglyceride corpuscles enter the blood stream, α -lipoprotein in the serum gathers together around these corpuscles and forms O-lipoprotein

and then, these stabilized triglyceride corpuscles (chylomicrons) are phagocytized by the alveolar phagocytes, KUPFFER's stellate cells of the liver and reticuloendothelial cells of the spleen. After that, triglycerides, which were phagocytized by these cells in the form of *O*-lipoprotein, change into diglycerides under the action of lipoprotein lipase. The diglycerides thus produced change into phospholipids under the supply of cytidine diphosphate choline and cytidine diphosphate ethanolamine, etc., and then phospholipids enter again into the blood stream in the form of α - and β -lipoprotein, not in the free form. Then, phospholipids are carried to all tissues and undergo further metabolism.

(2) The lower fatty acids are distributed only to the liver. Highly unsaturated fatty acids, docosenoic acid, eicosenoic acid etc. also seem to be distributed to the liver for the most part, but partially to other organs also. However, higher saturated fatty acids, oleic acid, the essential fatty acids etc. seem to go not only to the liver but also to the extrahepatic tissues, and undergo further metabolism. Therefore, the lower fatty acids, highly unsaturated fatty acids, docosenoic acid, eicosenoic acid etc. are the ones which undergo, for the most part, so-called indirect oxidation; and administration of these fatty acids in large quantities seems to impose a heavy burden on the liver and marked ketone body production. Accordingly, material for emulsions must be chosen from among the fats which do not contain lower fatty acids, highly unsaturated fatty acids, docosenoic acid, eicosenoic acid etc., which undergo indirect oxidation; but which contain higher saturated fatty acids and oleic acid which undergo direct oxidation and also essential fatty acids which are indispensable for the composition and function of tissue cells.

(3) When sesame oil is heated (under 90°C) for a long time, it becomes rancid and produces auto-oxidized substances, and the peroxide value rises. In this process, even though saturated fatty acids show no change in amount, linoleic acid having double bonds show a definite decrease in amount, lowering the nutritional value of sesame oil as fat. In the process of polymerization due to heating (over 200°C) there is also a decrease in the amount of essential fatty acids. Therefore, care has to be taken in the preservation and cooking of the oil. Fat for oral and parenteral administration must be chosen from among the fats with the lowest peroxide value. The administration of fat containing large amounts of fatty acids, which in general, undergo indirect oxidation, and also having high peroxide value easily caused fatty liver in animals.

(4) The administration of fats with the lowest peroxide value which do not contain lower fatty acids, highly unsaturated fatty acids, docosenoic acid, eicosenoic acid etc., but which contain higher saturated fatty acids and oleic acid and also essential fatty acids is not only harmless to the liver, but also rather effective nutritiously in normal or even in pathological conditions. We can not expect perfect nutrition with high protein and carbohydrate diet therapy alone.

(5) From the results of our experiments in which the change in the liver glycogen content in starving animals was investigated, we consider that the protein sparing effect gained clinically by preoperative use of fat emulsion is induced

partly by its fat-adapting action. For this reason too, a proper supply with fat should be recommended in surgery.

From the above mentioned view-points, especially in surgery, the intravenous administration of our sesame oil emulsion is extremely effective as a parenteral nutritional supplement of fat.

The authors wish to express sincere gratitude both to the workers in the Laboratory of Dainippon Pharmaceutical Limited Company, and to Dr. KIYOSHI TSURUSU, the Director of the Research Division of this Company (Director : TOKUJIRO MIYATAKE) for their kind assistance in the course of the work.

REFERENCES

- 1) Hikasa, Y. et al. : Parenteral administration of fats. (I) Fat metabolism in vivo, studied with fat emulsion. Arch. jap. Chir., **27**; 396, 1958.
- 2) Hikasa, Y. et al. : Parenteral administration of fats. (II) Clinical application of fat emulsion. Arch. jap. Chir., **27**; 736, 1958.
- 3) Hirayama, O. et al. : Metallic addition compounds of unsaturated fatty acids. Scientific Reports of the Saikyo University, Agriculture, **6**; 86, 1954.
- 4) Hirayama, O. et al. : Paperchromatography of fatty acids as their acetol ester derivatives. Scientific Reports of the Saikyo University, Agriculture, **7**; 43, 1955.
- 5) Inouye, Y. et al. : Separation and identification of fatty acids. Bull. Agr. Chem. Soc., Jap., **20**; 197, 1956.
- 6) Inouye, Y. et al. : Separation and identification of fatty acids. Bull. Agr. Chem. Soc., Jap., **20**; 200, 1956.
- 7) Kunkel, H. G. et al. : Phospholipid studies of different serum lipoproteins employing P^{32} . Proc. Soc. Exper. Biol. Med., **86**; 887, 1954.
- 8) Schmidt, G. et al. : A method for the determination of desoxyribonucleic acid, ribonucleic acid and phosphoproteins in animal tissues. J. Biol. Chem., **161**; 83, 1945.
- 9) Somogyi, M. : Notes on sugar determination. J. Biol. Chem., **195**; 19, 1952.
- 10) Benedict, S. R. : The detection and estimation of glucose in urine. J. A. M. A., **57**; 1193, 1911.
- 11) Elaine, T. et al. : Comparative studies of lipoprotein in various species by paper electrophoresis. Proc. Soc. Exper. Biol. Med., **87**; 637, 1954.
- 12) Kennedy, E. P. et al. : Oxidation of fatty acids and tricarboxylic acid cycle intermediates by isolated rat liver mitochondria. J. Biol. Chem., **179**; 957, 1949.
- 13) Kennedy, E. P. et al. : The products of oxidation of fatty acids by isolated rat liver mitochondria. J. Biol. Chem., **185**; 275, 1950.
- 14) Kennedy, E. P. : Synthesis of phosphatides in isolated mitochondria. J. Biol. Chem., **201**; 399, 1953.
- 15) Kennedy, E. P. : Synthesis of phosphatides by mitochondrial enzymes. Fed. Proc., **13**; 241, 1954.
- 16) Kennedy, E. P. : Synthesis of phosphatides in isolated mitochondria. J. Biol. Chem., **209**; 525, 1954.
- 17) Kennedy, E. P. : Cytidine diphosphate choline: A new intermediate in lecithin biosynthesis. J. Am. Chem. Soc., **77**; 250, 1955.
- 18) Kennedy, E. P. et al. : The function of cytidine coenzymes in the biosynthesis of phospholipids. J. Biol. Chem., **222**; 193, 1956.
- 19) Kennedy, E. P. : The synthesis of cytidine diphosphate choline, cytidine diphosphate ethanolamine, and related compounds. J. Biol. Chem., **222**; 185, 1956.
- 20) Borkenhagen, L. F. et al. : The enzymatic synthesis of cytidine diphosphate choline. J. Biol. Chem., **227**; 951, 1957.
- 21) Nakata, K. : Experimental studies on fat metabolism in the lung. Arch. jap. Chir., **23**; 445, 1954.
- 22) Seno, A. : A study of the fat metabolism in the isolated perfused liver. Arch. jap. Chir., **24**; 179, 1955.

- 23) Robinson, D. S. et al. : The role of albumin in the interaction of chyle and plasma in the rat. *Quart. J. exp. Physiol.*, **38**; 233, 1953.
- 24) Levy, S. W. et al. : The esteratic and chylolytic properties of post-heparin plasma and their role in lipemia clearing. *J. Physiol.*, **127**; 297, 1955.
- 25) Korn, E. D. : Clearing factor, a heparin-activated lipoprotein lipase. *J. Biol. Chem.*, **215**; 15, 1955.
- 26) Grossman, M. I. et al. : Relation of lipolysis to deturbidification and returbidification in heparin-induced lipemia-clearing phenomenon. *Proc. Soc. Exper. Biol. Med.*, **88**; 132, 1955.
- 27) Shore, B. : Heparin-activated enzyme system of human plasma. *Proc. Soc. Exper. Biol. Med.*, **88**; 73, 1955.
- 28) Brown, R. K. et al. : The enzymatic transformation of lipoproteins. *J. Biol. Chem.*, **204**; 423, 1953.
- 29) Nagase, M. : unpublished.
- 30) Engel, R. W. : *J. Nutr.*, **24**; 175, 1942.
- 31) Satomura, K. : Effect of dietary fat on the incidence of experimental diabetes. *Arch. jap. Chir.*, **28**; 1959.
- 32) Kaneda, T. et al. : Nutritive value or toxicity of highly unsaturated fatty acids, (1), (2), (3). *Bull. Jap. Soc. Sci. Fisheries*, **19**; 171, 1948, **20**; 51, 1949, **20**; 658, 1949.
- 33) Kishimoto, A. : unpublished.
- 34) Tobe, T. : Experimental investigation of various fats as to their nutritional value. *Arch. jap. Chir.*, **28**; 184, 1959.
- 35) Hanabusa, S. : unpublished.
- 36) Holman, R. T. et al. : *Progress in the Chemistry of Fats and Other Lipids (I-IV)*, Pergamon Press, London, 1952~1957.
- 37) Sinclair, H. M. : *Essential Fatty Acids*, Fourth International Conference on Biochemical Problems of Lipids, Oxford, 1957.
- 38) Najjar, A. : *Fat Metabolism*, The John Hopkins Press, Baltimore, 1954.
- 39) Flaschenträger, B. et al. : *Physiologische Chemie, Ein Lehr-und Handbuch*, Springer-Verlag, 1954~1956.

Fig. 1 PAPER ELECTROPHOREGRAM OF SERUM LIPOPROTEIN
(following the administration of fat emulsion)

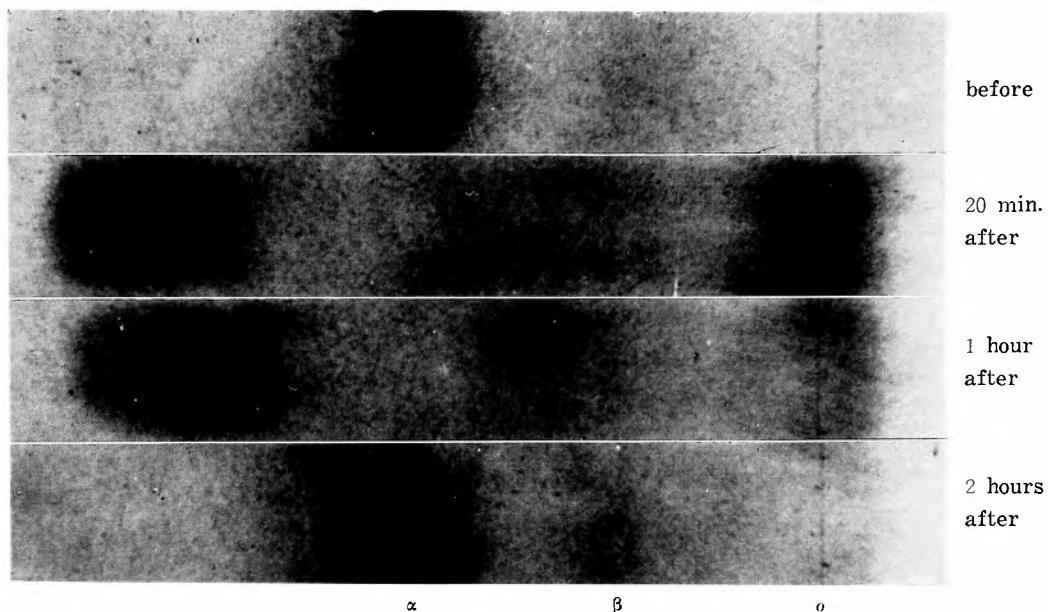


Fig. 2 AUTORADIOGRAM OF PAPER ELECTROPHORETICAL SERUM LIPOPROTEIN
EMPLOYING RADIOACTIVE PHOSPHORUS
(following the administration of fat emulsion)

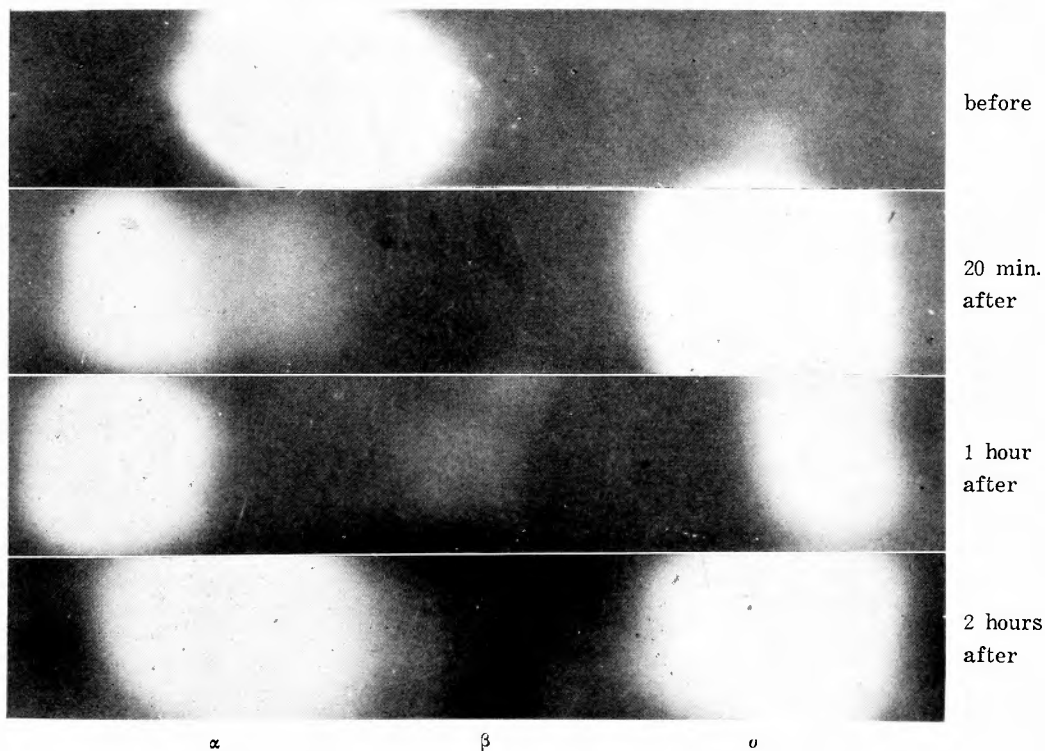




Fig. 3 (Micro-autoradiogram)



Fig. 4 (Smith-Dietrich' stain)

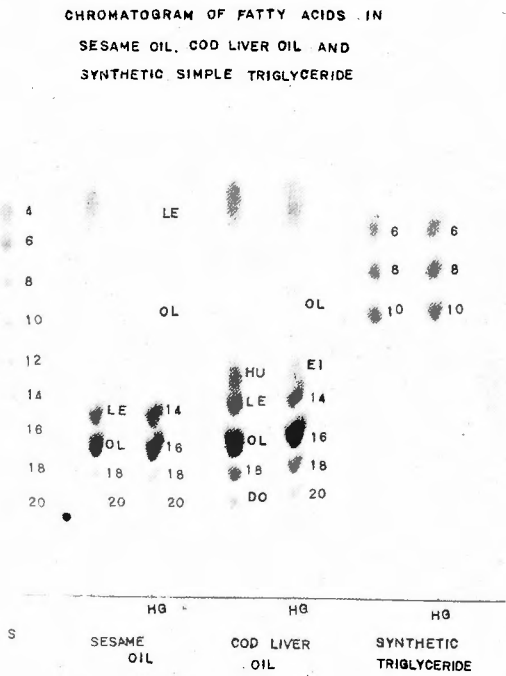


Fig. 5 Sesame oil and cod liver oil were analysed by paper chromatography. Sesame oil was found to contain essential fatty acids which have important nutritional value for the body, such as linoleic acid and linolenic acid, and also higher fatty acids such as myristic acid, palmitic acid, stearic acid, oleic acid etc. Cod liver oil was found to have in addition highly unsaturated fatty acids (having more than 4 double bonds), docosenoic acid, eicosenoic acid, in large amounts.

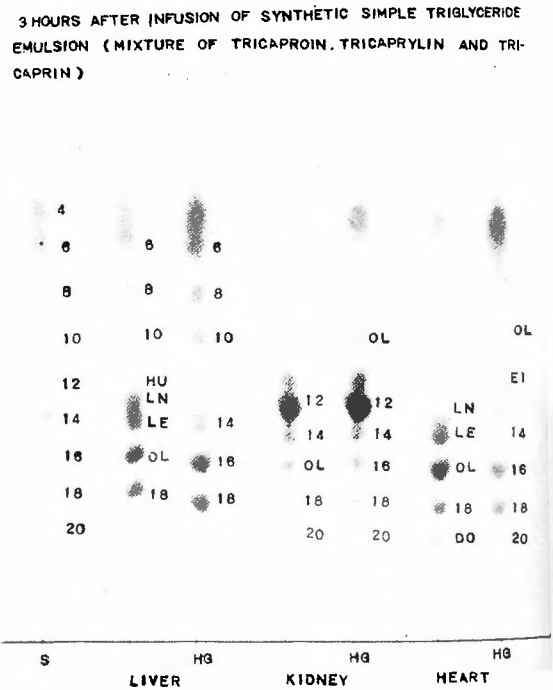


Fig. 6 Tricaproin, tricaprilyn, and tricaprln which are synthetic simple triglycerides of lower fatty acids were mixed in the same proportion, made into an emulsion, and infused into cats intravenously. Three hours later the cats were sacrificed by bleeding and the fatty acid content of their various organs was investigated. We have found that these lower fatty acids had shifted only to the liver, and not to any other organs at all.

HOURS AFTER INFUSION OF COD LIVER OIL EMULSION

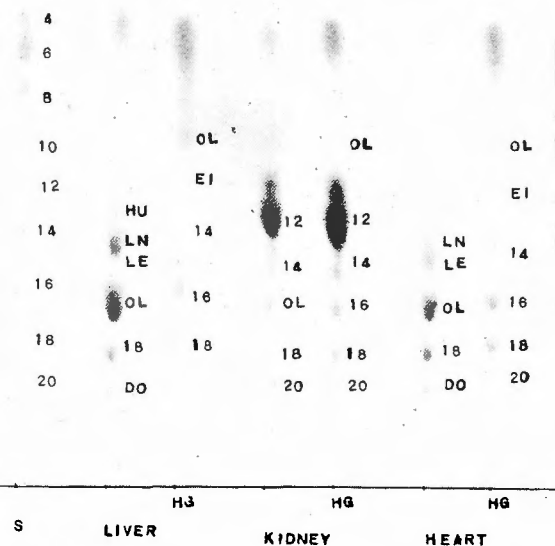


Fig. 7 Cod liver oil emulsion was infused into cats intravenously and three hours later they were sacrificed by bleeding. Their various organs were investigated for the fatty acid content. We have found that highly unsaturated fatty acids, docosenoic acid, and eicosenoic acid had largely shifted to the liver.

3. HOURS AFTER INFUSION OF SESAME OIL EMULSION

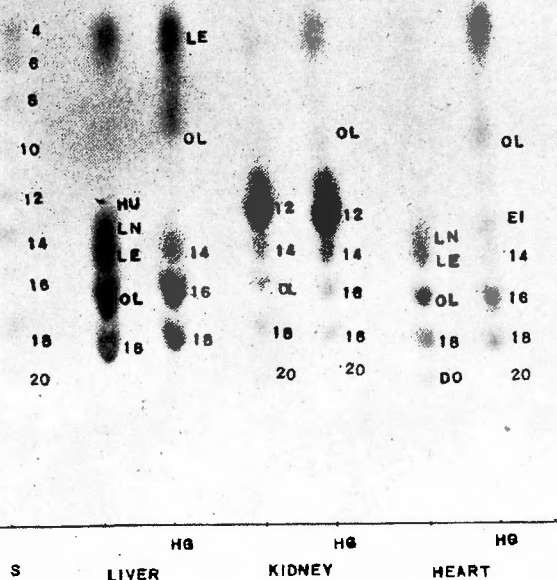


Fig. 8 Sesame oil emulsion was infused into cats intravenously and three hours later they were sacrificed by bleeding. Their various organs were found to contain an increased amount of fatty acids without any change in their composition.

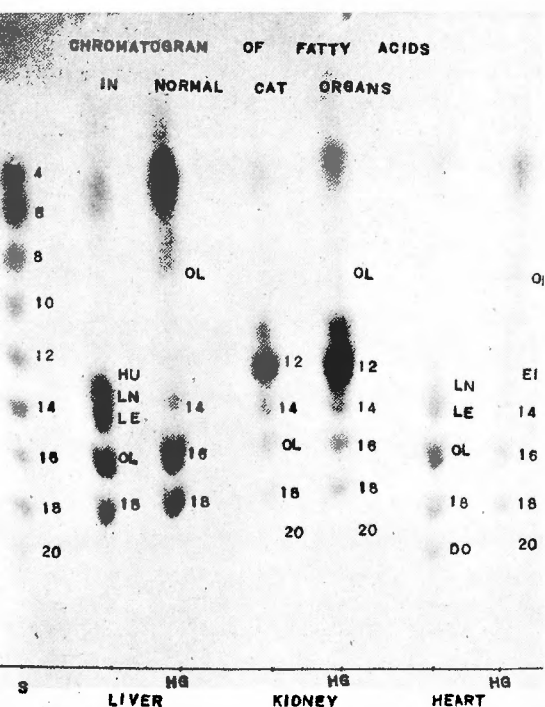
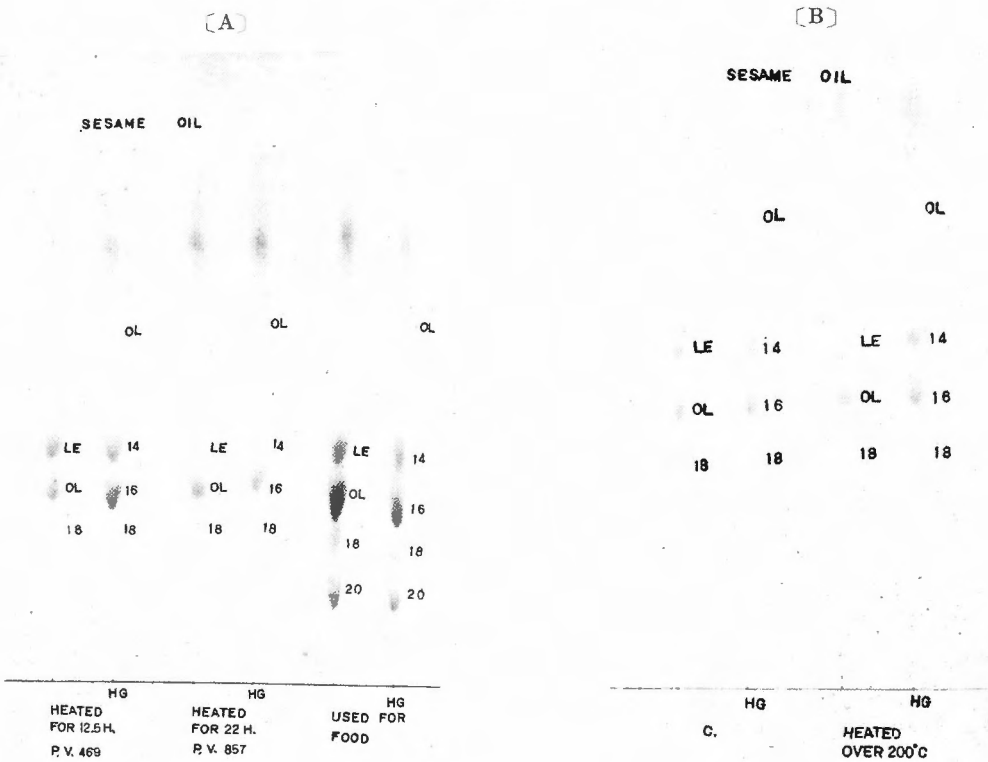


Fig. 9 Liver, kidney, and heart muscle of cats in the postabsorptive state contained only essential fatty acids and higher fatty acids, and no lower fatty acids. A characteristic findings in cats which was not seen in any other experimental animals was the distinct demonstration of lauric acid in the kidney.

Fig. 14



When sesame oil was heated with hot air for a long time, the peroxide value increased and the iodine value decreased. When the sesame oil was analysed by paper chromatography the saturated fatty acids showed no change, and essential fatty acids having double bonds, showed a definite gradual decrease. At the time of this reaction the amount of 2,4-dinitro-phenylhydrazones produced was small and a black viscous precipitate was seen which was insoluble in ether. When eatable sesame oil was heated (over 200°C); it became gradually brown tinged, increased in viscosity and polymerization of fatty oils definitely took place. In this case the essential fatty acids in the sesame oil showed a definite decrease.