ANALYTIC CHEMICAL STUDIES ON FAT METABOLISM BY APPLICATION OF PAPER CHROMATOGRAPHY OF FATTY ACIDS

by

Nobutoshi Tan

From the 2nd Surgical Division, Kyoto University Medical School
(Director: Prof. Dr. Yasumasa Aoyagi)
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I. INTRODUCTION

Recently there has been remarkable progress in the entire field of clinical medicine. One of the contributing factors has been the improvement in nutritional methods. Especially in surgery, consideration should be given to positive nutritional supplements, because in most cases, patients cannot take orally enough nutriments prior to and after surgical operations; besides surgical treatments inevitably accelerate the catabolic process of metabolism. In this case, most convenient is an intravenous supplement of nutriments such as glucose, amino acids, salts, vitamins, etc. This method is currently in a practicable stage. However, intravenous administration of fats still remains a very difficult problem. At our laboratory, the necessity for parenteral administration of fats for supplementary nutrition was early recognized, and efforts have been made to solve this problem. If fatty substances are to be administered intravenously at all, it is necessary that ample thought should be accorded to the qualitative composition of fats —above all their acid components— which are infused intravenously. The reason is this: in the digestive tract, some of the fats which are orally taken, are in the non-hydrolyzed form (triglycerides), some in the partially hydrolyzed form (diglycerides & monoglycerides), and some in the form of isolated free fatty acids, and they are reduced to corpuscles less than 0.5 μ in diameter by bile salts and can be absorbed through the intestinal mucous membrane. This absorption process is regulated by the species of fatty acids as to whether they are absorbed directly into the portal blood or into the lymph of the thoracic duct. The results of indirect examinations by isotopes by Bloom et al. indicate that higher fatty acids can be absorbed mostly into the lymph of the thoracic duct, while lower ones are absorbed into the portal blood. Accordingly, if we are to infuse fatty substances directly into cutaneous veins, glyceride emulsions consisting of higher fatty acids which are apt to be absorbed into thoracic duct lymph should be infused into cutaneous veins. This is because direct infusion of fat emulsion into the portal vein cannot be considered.

In the above-mentioned experiments by Bloom et al. various C^{14}-labeled fatty acids were used, and the use of their radioactivity enabled them to observe the transport pathway of absorbed fatty acids. This is, so to speak, an indirect observation method. It is not by direct experimental verification that lower fatty acids

could actually be absorbed into the portal blood. Even if it is an admitted fact that lower fatty acids are absorbed through the intestinal mucous membrane, it is by no means inconceivable that they may undergo direct absorption into the portal vein in view of the fact that almost all of them will not be absorbed into thoracic duct lymph. The author intended to verify experimentally the allegations by BLOOM et al. just by applying paper chromatography of fatty acids, especially to determine whether or not influx into the portal vein of lower fatty acids ever takes place. It is theoretically the best method of supplementing fatty substances to selectively administer a triglyceride emulsion consisting of the commonest fatty acids which are always found in animal organs or dépot fats. In this sense, the author resorted to paper chromatography of fatty acids with regard to the study of component fatty acids in each organ or dépot fats.

II. MATERIALS AND METHODS

A. Experimental Materials

1) Fat emulsions: 20% sesame oil emulsion and 20% cod liver oil emulsion were used. These contain 7% glucose each.

2) Natural fats: Natural butter fat, cod liver oil, sesame oil, etc. were used.

3) Synthesized simple glycerides: Tricaproin, tricaprylin, tricaprin, trilaurin, triolein, etc. were used.

4) Experimental animals: Healthy adult dogs weighing about 10kg were used. In order to maintain as similar conditions as possible, each of them was fed a measured diet for more than seven days, and then fasted for 24 hours so that experiments could be made in an immediate postabsorptive state.

B. Experimental Methods

1) Method of collecting chyle

As in SHIROTANI's method with cats, an incision approximately 6cm long was made in the left supraclavicular fossa of an anesthetized dog. Then, ample exposure of the ascending thoracic duct along the inside of the left jugular vein was made and complete prevention of thoracic duct lymph influx into the blood was achieved (Fig. 1). Next, a polyethylene tube was inserted into the thoracic duct, and dripping lymph was collected in a pre-arranged collecting flask. Subsequent to this, the above-mentioned test fats were administered into the stomach by stomach tube and chyle, now cloudy as a result of fat absorption, was collected in a collecting flask.

2) Method of collecting chyle and portal blood simultaneously

As shown in Fig. 1, collection of chyle was done using a polyethylene tube after oral administration of test fats. By this procedure, complete interruption of chyle flowing into the blood stream could be achieved. At the same time, collection of portal blood as it was after two hours' lapse of intragastric administration of test fats was done. Chyle and portal blood were collected for fatty acid analysis.

3) Organs

The experimental animals were sacrificed by bleeding and the organs were immediately removed, and pulverized finely by marine sand for analysing their

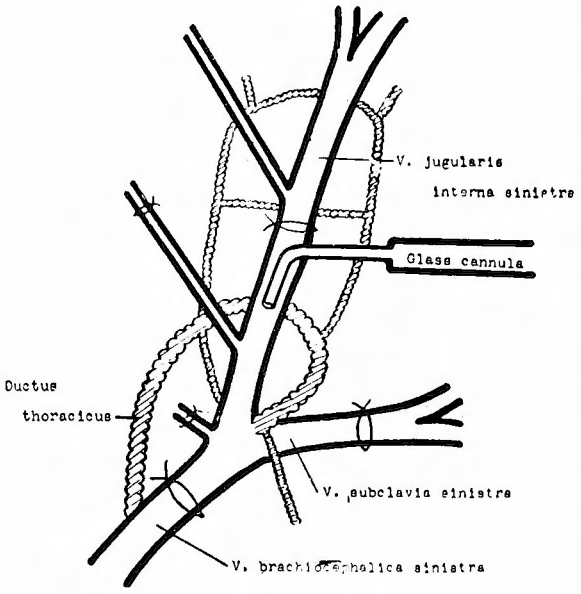


Fig. 1 Method of Collecting Chyle

component fatty acids.

4) Paper chromatography of fatty acids

Paper chromatography was done by the NODA-HIRAYAMA' method, i. e. the development was made as the *p*-bromophenacylester 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, methanol-glacial 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.

III. RESULTS

I) On the Transport Pathway of Absorbed Fats

In order to identify the transport pathway of absorbed fats through the intestinal mucous membrane, comparison was made between component fatty acids contained in the test fats which were orally administered to experimental animals and component fatty acids contained in chyle (or portal serum) which were collected when the test fats were orally administered.

1) Absorption into thoracic duct

In this experiment, the following materials were used: cod liver oil emulsion containing a comparatively large amount of highly unsaturated fatty acids, eicosenoic acid, docosenoic acid, etc., butter fat containing lower fatty acids, and sesame oil emulsion composed of higher saturated fatty acids, oleic acid, essential fatty acids and containing no lower fatty acids, eicosenoic acid, docosenoic acid, highly unsaturated fatty acids.

The fatty acids which are contained in the thoracic duct lymph of a dog in a postabsorptive state after 24 hours' fasting, as shown in Fig. 2, were myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid. But no lower fatty acids than lauric acid and highly unsaturated fatty acids were found.

A) Oral administration of cod liver oil emulsion

Fig. 3 shows the analytic results of fatty acids contained in cod liver oil emulsion (F. I.) and fatty acids extracted from chyle (L. F. I.) collected when 40g of cod liver oil emulsion was administered by stomach tube.

In cod liver oil emulsion, there are found myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, eicosenoic acid, docosenoic acid, and highly unsaturated fatty acids. Also in chyle collected when cod liver oil emulsion was orally administered, there were detected various kinds of fatty acids in the same percentage as the above. It is apparent from this fact that all fatty acids contained in cod liver oil emulsion could well be absorbed into the thoracic duct.

Especially, it can be clearly seen that docosenoic acid, highly unsaturated fatty acids, eicosenoic acid, etc. which were not detected in thoracic duct lymph in a postabsorptive state, have been well absorbed into the thoracic duct.

B) Oral administration of butter fat

Fig. 4 shows the analytic results of fatty acids contained in natural butter fat (B.) and fatty acids extracted from chyle (L. B.) collected after 40g of natural butter fat had been administered orally.

In natural butter fat are found butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, and oleic acid. Especially noteworthy is the fact that the percentage of both oleic and palmitic acids is high.

When the fatty acids extracted from chyle collected after natural butter fat had been orally administered were examined by paper chromatography, it was found that fatty acids higher than myristic acid were always in almost the same condition as the former, while fatty acids lower than lauric acid gradually showed spots which became thinner and fewer. And spots of fatty acids lower than caproic acid were never seen.

In other words, it is considered that fatty acids higher than myristic acid could be absorbed into thoracic duct lymph, but that fatty acids lower than lauric acid gradually declined in the percentage of absorption into the thoracic duct in proportion to the decrease in number of carbon atoms.

C) Oral administration of sesame oil emulsion

Fig. 5 shows the analytic results of fatty acids contained in sesame oil emulsion (F. I.) and fatty acids extracted from chyle (L. F. I.) when 40g of

Table 1. Neutralisation and Iodine Values of Component Fatty Acids of Test Fats and Lipids Collected from Chyle

Acid	Cod Liver Oil Emulsion		Sesame Oil Emulsion		Butter Fat	
	Fat fed	Chyle fat	Fat fed	Chyle fat	Fat fed	Chyle fat
Neutralisation Value	188.2	176.9	196.7	191.9	217.0	192.2
Iodine Value	135.4	106.4	109.8	104.6	34.0	66.1

sesame oil emulsion was administered by stomach tube.

Sesame oil emulsion contains myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid, especially linoleic acid and oleic acid in large quantities.

In contrast, various fatty acids extracted from chyle after oral administration of sesame oil were found in the same percentage as the former.

This indicates clearly that each of these fatty acids in sesame oil emulsion could well be absorbed into the thoracic duct.

Neutralization and iodine value of the component fatty acids contained in the afore-mentioned three test fats and the component fatty acids in chyle collected during oral administration of these test fats, are shown in Table 1.

According to the results mentioned above, it is roughly presumed that lower fatty acids gradually decline in percentage of absorption into thoracic duct lymph in proportion to the decrease in number of carbon atoms. To further clarify this problem, simple glycerides such as tricaprylin, tricaprin, and trilaurin were synthesized, and then a simple glyceride mixture containing equal amounts of them were administered orally to experimental animals in order to study again the absorption of lower fatty acids into thoracic duct lymph.

D) Oral administration of tricaprylin, tricaprin, and trilaurin

10g of each of the above simple glycerides was mixed, and 30g of this mixture was administered by tube into the stomachs of experimental animals.

Fig. 6 shows the analytic results of fatty acids contained in the synthesized simple glyceride mixture (D) and fatty acids extracted from chyle (L.) collected when the above mentioned synthesized simple glyceride mixture was administered orally. It is apparent from these results that lower fatty acids such as lauric acid, capric acid, and caprylic acid gradually decline in their percentage of absorption into the thoracic duct in proportion to the decrease in number of carbon atoms.

In regard to the absorption of lower fatty acids into the thoracic duct, the conclusion is that the fewer the carbon atoms, the lower is the absorption ratio.

In short, our direct examinations in which paper chromatography of fatty acids was used to observe the absorption of fatty acids through the intestinal mucous membrane when fats were orally administered, have gained the same results as the indirect verification method by CHAIKOFF, REISER, BOLLMAN et al. using isotopes, and the experiment by FERNANDES on a child with chylothorax.

2) Absorption into the portal vein

From the results mentioned above, the percentage of absorption of lower fatty acids into thoracic duct lymph is exceedingly low as compared with that of higher fatty acids. However, no reports have yet appeared confirming the direct observation of the absorption of lower fatty acids into the portal blood during the oral administration of fats.

The author, therefore, studied the absorption of caprylic acid and caproic acid into thoracic duct lymph when large amounts of synthesized tricaprylin and tricaproin were orally administered. In parallel, the author studied also the absorption of such fatty acids into the portal blood.

Fig. 7 shows the analytic results of fatty acids which are always found in the portal serum of dogs in the postabsorptive state. The component fatty acids in portal serum of a dog in postabsorptive state are myristic acid, palmitic acid, stearic acid, oleic acid, eicosenoic acid, linoleic acid, linolenic acid, and highly unsaturated fatty acids. However, the author could detect no fatty acids lower than lauric acid.

A) Oral administration of tricaprylin

Fig. 8 shows the analytic results of fatty acids extracted from chyle (L.) and portal serum (B.) collected after oral administration of tricaprylin. As shown in Fig. 8, caprylic acid is generally absorbed into the portal blood through the intestinal mucous membrane, but still some portion of it gets absorbed into thoracic duct lymph.

B) Oral administration of tricaproin

Fig. 9 shows the analytic results of fatty acids extracted from chyle and portal serum collected after oral administration of tricaproin. The comparison between fatty acids in chyle (L.) collected after oral administration of 40g of tricaproin to experimental animals, and fatty acids extracted from portal serum (B.) suggests that just as in the experiment on administering tricaprylin, the portal serum contains more caproic acid than chyle. That is, as caproic acid generally tends to be absorbed into the portal blood, it is possible that certain portions of it get absorbed into thoracic duct lymph.

Thus, the absorption of orally administered fats through intestinal mucous membrane varies according to the type of fatty acid. Higher fatty acids than myristic acid are generally transported into the blood stream by way of the thoracic duct, while lower ones are absorbed directly into the portal blood mostly through the intestinal mucous membrane. However, it is not necessarily proper to think that absolutely none of the fatty acids lower than myristic acid are absorbed into thoracic duct lymph, since it was discovered that certain portions of them are absorbed into thoracic duct lymph. It was also learned that lower fatty acids showed a gradual decline in the percentage of absorption into thoracic duct lymph, as the number of their carbon atoms decreased. It is considered that the difference in the transport pathway of absorbed fatty acids based upon the number of carbon atoms constituting such fatty acids, is closely associated with the degree of water-solubility and hydrolysis with lipase.

From these facts, the conclusion has been reached that for intravenous adminis-

tration of fats, triglycerides composed exclusively of as high fatty acids as possible should be emulsified into globules less than 0.5μ in diameter and be infused as an emulsion.

II) Component Fatty Acids in Various Organs

As mentioned earlier in this report, it is a matter of course that the most appropriate method for parenteral administration of fats is a glyceride emulsion consisting of the commonest fatty acids which are always found in the body.

1) Component fatty acids in dog's organs

The component fatty acids in the cerebrum, liver, kidney, spleen, muscle, large omentum, subcutaneous adipose tissue, bile, serum, lymph, etc. of a healthy adult dog in the postabsorptive state, were analysed by paper chromatography.

The analytic results are shown in Figs. 10 to 17. The commonest fatty acids in all organs were myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid.

In regard to the neuturalization values subcutaneous adipose tissue and the large omentum were highest with values of 202 to 204, containing palmitic and oleic acid in great quantities. Characteristic, however, is the fact that they contain lauric acid, but almost no highly unsaturated fatty acids.

Generally speaking, large amounts of highly unsaturated fatty acids, docosenoic acid, eicosenoic acid, etc. are found in liver and bile which give higher iodine values. Although a certain amount of highly unsaturated fatty acids can always be detected in serum too, their presence can hardly be certified in thoracic duct lymph, when in the postabsorptive state.

Table 2. Fatty Acid Composition of Lipids in Dog's Organs

		Brain	Liver	Kidney	Spleen	Muscle	Subcu-taneous	Oment-um	Bile	Serum	Lymph
Saturated Fatty Acids	Butyric	−	−	−	−	−	−	−	−	−	−
	Caproic	−	−	−	−	−	−	−	−	−	−
	Caprylic	−	−	−	−	−	−	−	−	−	−
	Capric	−	−	−	−	−	−	−	−	−	−
	Lauric	−	−	−	−	−	+	+	−	−	−
	Myristic	+	+	+	+	+	+	+	+	+	+
	Palmitic	###	###	###	###	###	###	###	###	###	###
	Stearic	##	##	+	##	+	+	+	##	##	+
	Arachidic	−	±	±	±	−	±	±	−	−	−
Unsaturated Fatty Acids	Hexadecenoic	−	−	−	−	−	+	+	−	−	−
	Oleic	###	###	###	###	###	###	###	##	##	##
	Eicosenoic	+	+	+	+	+	±	±	+	+	±
	Linoleic	##	##	##	##	##	##	##	##	##	##
	Linolenic	+	+	+	+	+	+	+	+	+	+
	Highly Unsaturated	+	##	+	+	+	±	±	##	+	±
Iodine Value		121.0	141.0	86.2	115.7	90.7	68.1	67.9	122.3	117.2	73.9

Table 3. Neutralisation and Iodine Values of Component Fatty Acids in Human Organs

Acid	Serum	Omentum	Subcutaneous
Neutralisation Value	193.6	204.4	208.9
Iodine Value	111.3	68.1	71.5

Table 2 tabulates the species and iodine value of the component fatty acids of various organs.

2) Component fatty acids in human subcutaneous adipose tissue, large omentum, and serum

The analytic results on component fatty acids in subcutaneous adipose tissue, large omentum, and serum of an adult, are shown in Table 3 and Fig. 18. The experimental results are almost the same as in the dog.

The analytic results on fatty acids in each organ, dépot fat, serum, and thoracic duct lymph of dogs and humans indicate that the safest parenteral method of supplementing fats is to administer a glyceride emulsion consisting of various fatty acids such as myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid which are invariably present in all organs almost in the same ratio. Therefore, as shown in Fig. 19, sesame oil is considered very appropriate.

IV. DISCUSSION

In our laboratory, the necessity for parenteral administration of fats has been well recognized, and efforts have been made during the past several years to prepare intravenously infusible fat emulsion. However, the problem still remains as to what fats should be chosen for such an emulsion.

In order to solve this problem, the analysis of component fatty acids in chyle and portal blood collected after various test fats had been orally administered to experimental animals, was carried out by paper chromatography. The results were in agreement with those of Bloom et al. in which isotopes were used; higher fatty acids than myristic acid are generally absorbed into the thoracic duct through the intestinal mucous membrane, while lower ones gradually decline in the percentage of absorption into the thoracic duct in proportion to the decrease in number of carbon atoms, and are absorbed mainly into the portal blood. Thus, it follows that in infusing fats directly into the cutaneous vein, triglycerides composed substantially of higher fatty acids than myristic acid should be emulsified into globules less than 0.5μ in diameter and be infused as an emulsion.

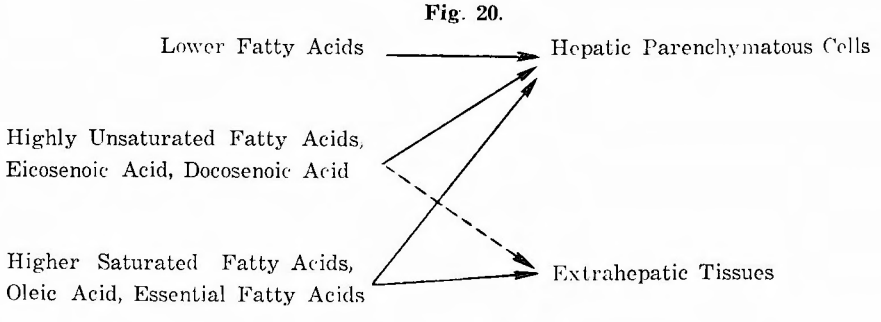
In this case, attention is called to the following fact. Even lower fatty acids do not necessarily resist absorption into the thoracic duct, but rather some portions may be absorbed into the thoracic duct.

Asada and Izukura in our laboratory have used sesame oil emulsion, cod liver oil emulsion, and synthetic triolein emulsion for oral or 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 parencymatous cells of the liver, in comparison with cases in which sesame oil emulsion was used, containing nothing but higher saturated fatty acids, oleic acid and the essential fatty acids. And KUYAMA found the same results as when he 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 further.

It is natural that in my analysis of component fatty acids of various organs, much larger amounts of highly unsaturated fatty acids, docosenoic acid, eicosenoic acid etc. were contained in liver than in extrahepatic tissues. Accordingly, the nutritional effect of fats should be decided in consideration of these two metabolic processes (Fig. 20). That is, fatty acids of the former group are a burden to the liver, markedly increase ketone body production and may cause 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. The author decided that in view of the transport pathway of absorbed fats, triglycerides consisting merely of higher fatty acids have only to be infused intravenously in the form of emulsified fat. Yet such a definition is still insufficient, so the following addition should be made: Triglycerides which contain none of the highly unsaturated fatty acids, eicosenoic acid, docosenoic acid, but merely higher fatty acids such as myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid, should be used as materials for a fat emulsion.

Fig. 20.



V. SUMMARY

Using paper chromatography of fatty acids, an attempt was made to pursue the transport pathway of absorbed fats through the intestinal mucous membrane when fats are orally administered, and simultaneously to analyse component fatty acids in various organs, serum, bile, lymph, and depót fat etc.

The following conclusions were reached:

1) There are two transport pathways of absorbed fats through the intestinal mucous membrane: absorption into the thoracic duct and absorption into the portal vein.

2) Higher fatty acids than myristic acid are generally absorbed into the thoracic duct, while lower ones are absorbed mainly into the portal vein directly.

3) In regard to lower fatty acids, the percentage of absorption into the thoracic duct gradually declines in proportion to the decrease in number of carbon atoms. Even all the lower fatty acids such as caproic, caprylic acids, do not necessarily get absorbed into the portal blood, but some are absorbed into the thoracic duct lymph too.

4) In the postabsorptive state, lower fatty acids than lauric acid cannot be found in any organ. And it was found that the fatty acids present in every normal organ, and whose percentage in every organ is almost constant, are: myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid.

5) Dépot fat contains lauric acid. However, highly unsaturated fatty acids, docosenoic acid, eicosenoic acid etc. are present in the liver, bile, and blood in considerable amounts; those present in extrahepatic tissues are far less in quantity than in the liver. The origin of highly unsaturated fatty acids, docosenoic acid, eicosenoic acid etc. contained in organs is attributable to alien fats coming from nutrients.

In closing, my hearty appreciation is extended to Dr. Yorinori Hikasa, the instructor who favored me with his tireless guidance and encouragement in this research as well as Prof. Manjiro Noda, and Mr. Osamu Hirayama, Assistant of the Biochemical Laboratory, Saikyo University, Kyoto who directed and encouraged me in paper chromatography.

EXPLANATION OF FIGURES

The spot identifications of paper chromatogram are as follows:
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, EI: Eicosenoic Acid, LE: Linoleic Acid, LN: Linolenic Acid, DO: Docosenoic Acid, HX: Hexadesenoic Acid, HU: Highly Unsaturated Fatty Acids, S: Mixture of the Derivatives of Saturated Acids from C_4 to C_{20} (Standard),

S′: Mercurated Derivative of Oleic Acid (or Oleic and Eicosenoic Acids),

S″: Mixture of Nonmercurated and Mercurated Derivatives of Oleic Acid (or Oleic and Eicosenoic Acids),

(Hg): Mercuric Compounds of the Ester Derivatives.

Fig. 2 Chromatogram of Component Fatty Acids in the Thoracic Duct Lymph Collected in Postabsorptive State (Dog).

Fig. 3 Chromatogram of Component Fatty Acids in Chyle Collected after Oral Administration of Cod Liver Oil Emulsion (Dog). F.I.: Component Fatty Acids in Cod Liver Oil Emulsion. F.I.L.: Component Fatty Acids in Chyle.

Fig. 4 Chromatogram of Component Fatty Acids in Chyle Collected after Oral Administration of Butter Fat (Dog). B.: Component Fatty Acids in Butter Fat. L.B.: Component Fatty Acids in Chyle.

Fig. 5 Chromatogram of Component Fatty Acids in Chyle Collected after Oral Administration of Sesame Oil Emulsion (Dog). F.I.: Component Fatty Acids in Sesame Oil Emulsion. F.I.L.: Component Fatty Acids in Chyle.

Fig. 6 Chromatogram of Component Fatty Acids in Chyle Collected after Oral Administration

of Synthesized Simple Glyceride Mixture (Dog). D: Component Fatty Acids in the Mixture of Simple Glycerides (Equal Amount of Trilaurin, Tricaprin, and Tricaprylin). L: Component Fatty Acids in Chyle.

Fig. 7 Chromatogram of Component Fatty Acids in Portal Serum Collected in the Postabsorptive State (Dog).

Fig. 8 Chromatogram of Component Fatty Acids in Chyle and Portal Serum Collected after Oral Administration of Synthesized Tricaprylin (Dog). L: Component Fatty Acids in Chyle. B: Component Fatty Acids in Portal Serum.

Fig. 9 Chromatogram of Component Fatty Acids in Chyle and Portal Serum Collected after Oral Administration of Synthesized Tricaproin (Dog). L: Component Fatty Acids in Chyle. B:Component Fatty Acids in Portal Serum.

Fig. 10 Chromatogram of Component Fatty Acids in Liver (Dog).

Fig. 11 Chromatogram of Component Fatty Acids in Muscle (Dog).

Fig. 12 Chromatogram of Component Fatty Acids in Kidney (Dog).

Fig. 13 Chromatogram of Component Fatty Acids in Spleen (Dog).

Fig. 14 Chromatogram of Component Fatty Acids in Brain (Dog).

Fig. 15 Chromatogram of Component Fatty Acids in Bile (Dog).

Fig. 16 Chromatogram of Component Fatty Acids in Subcutaneous Adipose Tissue (Dog).

Fig. 17 Chromatogram of Component Fatty Acids in Omentum (Dog).

Fig. 18 Chromatogram of Component Fatty Acids in Serum, Omentum majus and Subcutaneous Adipose Tissue (Human).

Fig. 19 Chromatogram of Component Fatty Acids in Sesame Oil and Cod Liver Oil.
S. O.: Component Fatty Acids in Sesame Oil. C. L. O.: Component Fatty Acids in Cod Liver Oil.

REFERENCES

1) Artom, C & Cornatzer, W. E: The Action of Choline and Fat on Lipid Phosphorylation in the Liver. J. Biol. Chem., **171**, 799, 1947.
2) Asada, S.: Histochemical Studies on the Intravenously Infused Fat Emulsion. Arch. Jap. Chir., **22**, 77, 1954.
3) Bauersfeld, E. H.: Traumatic Chylothorax from Ruptured Thoracic Duct Treated by Intravenous Injection of Aspirated Chyle. J. A. M. A. **109**, 16, 1937.
4) Berry, I. M. & Ivy, A. C.: The Tolerance of Dogs to Intravenously Administered Fatty Chyle and Synthetic Fat Emulsion. Federation Proc., **7**, 7, 1948.
5) Bloom, B., Chaikoff, I. L., Reinhardt, W. O., Entenman, C&Dauben, W. G.: The Quantitative Significance of the Lymphatic Pathway in Transport of Absorbed Fatty Acids. J. Biol. Chem., **184**, 1, 1950.
6) Bloom, B., Chaikoff, I. L., Reinhardt, W. O. & Dauben, W. G.: Participation of Phospholipids in Lymphatic Transport of Absorbed Fatty Acids. J. Biol. Chem., **189**, 261, 1951.
7) Bloom, B., Chaikoff, I. L. & Reinhardt, W. O.: Intestinal Lymph as Pathway for Transport of Absorbed Fatty Acids of Different Chain Lengths. Am. J. Physiol., **166**, 451, 1951.
8) Bloor, W. R.. The Oxidative Determination of Phospholipid (Lecithine & Cephalin) in Blood and Tissues. J. Biol. Chem., **82**, 273, 1929.
9) Bergström, S. Bergström, B. Tryding, N. & Westöö, G.: Intestinal Absorption and Metabolism of 2: 2-Dimethylstearic Acid in the Rat. Bioch. Journal, **58**, 604, 1954.
10) Collins, H, S., Kraft, L. M., Kinnet, T. D., Davidson, C. S., Young, J. & Stare, F. J.: Studies on the Tolerance of Dogs to Intravenous Administration of Fat Emulsion. J. Lab. and Clin. Med., **33**, 143, 1948.
11) Davis, R. E.: The Metabolism of Tributyrin. J. Biol. Chem., **88**, 67, 1930.
12) Eckstein, H. C.: Fat Absorption through Channels other than the Left Thoracic Duct. J. Biol. Chem., **62**, 737, 1925.
13) Eckstein, H. C.: The Influence of Diet on the Body Fat of the White Rat. J. Biol. Chem., **81**, 613, 1929.
14) Frazer, A. C.: Fat Absorption and its Relationship to Fat Metabolism. Physiol. Rev., **20**, 361,

1940.
15) Fernandes, J.: Fat Absorption Studied in a Child with Chylothorax. Thesis, Leiden.
16) Futter, J. H. and Shorland, F. B.: Studies on the Composition of Lipids of the Rabbit. Bioch. Journal, **65**, 689, 1957.
17) Hashino, H.: Experimental Studies of Fat Metabolism from the View-point of Ketone Body Formation. Arch Jap. Chir., **24**, 488, 1955.
18) Hikasa, Y., Ishigami, K., Asada, S., Zaitsu, A., Tsukada. A., and Nakata, K.: Studies on the Intravenous Administration of Fat Emulsion. J. J. S. S., **52**, 298, 1951.
19) 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.
20) Hikasa, Y.: Studies on the Intravenous Administration of Fat Emulsion Prepared by High Pressure Jet. Rev. Phys. Chem. Jap., **12**, 82, 1952.
21) Hikasa, Y., Hashino, H., Osa, H. and Takeda, S.: The Role of Watersoluble Vitamins in Fat Metabolism. Nipponrinsho., **13**, 1225, 1955.
22) Hikasa, Y., Shirotani, H., Shigenaga, M., Kuyama, T., Tan, N. and Shimada, Y.: Parenteral administration of Fats. I. Fat Metabolism in Vivo, Studied with Fat Emulsion. Arch. Jap. Chir., **27**, 396, 1958.
23) Hikasa, Y., Kuyama, S., Shigenaga, M., Chi Chien Hsü, Hanabusa, S., Tamaki, A., Matsuda, S., Onishi, H., and Tobe, T.: Parenteral Administration of Fats. Ⅱ. Clinical Application of Fat Emulsion. Arch. Jap. Chir., **27**, 736, 1958.
24) Hirayama, O., and Noda, M.: Metallic Addition Compounds of Unsaturated Fatty Acids. Ⅱ. Microanalysis of Unsaturated Fatty Acid Esters by Mercuration Method. Scientific Reports of the Saikyo University, Agriculture, **6**, 86, 1954.
25) Hirayama, O. and Noda, M.: Paper-chromatography of Fatty Acids as their Acetol Ester Derivatives. Scientific Reports of the Saikyo University, Agriculture., **7**, 43, 1955.
26) Hughes, R. H. and Wimmer, E. T.: The Absorption of Soluble, Volatile Fatty Acids. J. Biol. Chem., **108**, 141, 1935.
27) Ikeda, H.: Experimental Studies on Fat Metabolism with a Blocked Reticuloendothelial System. Arch. Jap. Chir., **26**, 215, 1957.
28) Inoue, Y., Hirayama, O. and Noda, M.: Separation and Identification of Fatty Acids. XX. 2 4-Dinitrophenylhydrazones of p-Bromophenacyl Esters as Derivatives for Characterization of Unsaturated Fatty Acids. Bull. Agr. Chem. Soc. Jap., **20**, 197, 1956.
29) Inoue, Y., Hirayama, O. and Noda, M.: Separation an Identification of Fatty Acids. XXI. Paper-chromatography of Fatty Acids as their p-Bromophenacyl Ester Derivatives. Bull. Agr. Chem. Soc. Jap., **20**, 200, 1956.
30) Izukura, T.: Histochemical Studies on Intravenously Administered Fat Emulsion. Arch. Jap. Chir., **26**, 215, 1957.
31) Kosaki, N.: Studies on Fat Metabolism after Total Pancreatectomy. Experiments in Dogs. Arch. Jap. Chir., **25**, 138, 1956.
32) Kiyasu, J. Y., Bloom, B. and Chaikoff I. L.: The Portal Transport of Absorbed Fatty Acids. J. Biol. Chem., **199**, 415, 1952.
33) Kuyama, T.: Clinical Studies on the Nutritional Effects of Intravenous Administration of Fat Emulsion. Arch. Jap. Chir., **27**, 64, 1958.
34) Lerner, S. R., Chaikoff, I. L., Entenman, C. and Dauben, W. G.: The Fate of C^{14}-Labeled Palmitic Acid Administered Intravenously as a Tripalmitin Emulsion. Proc. Soc. Exper. Biol. and Med., **70**, 384, 1949.
35) Little, J. M., Harrison, C. and Blalock, A.: Chylothorax and Chyloperitoneum. Effects of Reintroduction of Aspirated Chyle. Survery., **11**, 392, 1942.
36) Maki. Y.: unpublished.
37) Manabe, K. and Kameda, I: Homolaterality of the Portal Blood Flow in the Liver. Sogoigaku, **11**, 255, 1954.
38) Meng, H. C. and Freeman, S.: Experimental Studies on the Intravenous Injection of a Fat Emulsion into Dogs. J. Lab. and Clin. Med., **33**, 698, 1948.
39) Nishino, T.: Laboratory Studies on the Intravenous Administration of Fat Emulsion in the

Light of Tissue Metabolism. Arch. Jap. Chir., **23**, 607, 1954.
40) Oeken: Ein Fall von Zerreissung des Ductus thoracicus infolge Brustquetschung. München. med. Wochenshr., **55**, 1182, 1908.
41) Ono, K: Fat Metabolism. Saishin-Igaku, **10**, 52, 1955.
42) Osa, H.: Experimental Studies on the Intravenous Administration of a Fat Emulsion for Nutritional Purposes. Arch. Jap. Chir., **25**, 151, 1956.
43) Otani, S.: On Mechanism of Adverse Reactions Caused by Intravenous Administration of Fat Emulsion. Arch. Jap. Chir., **25**, 172, 1956.
44) Reiser, R., Bryson, M. J., Carr, M. J. and Kuiken, K. A.: The Intestinal Absorption of Triglycerides. J. Biol. Chem., **194**, 131, 1952.
45) Seno, A.: A Study of Fat Metabolism in the Isolated Perfused Liver. Arch. Jap. Chir., **24**, 179, 1955.
46) Shigenaga, M.: Experimental Studies on Fat Metabolism with Determination of Respiratory Quotient and Ketone Body Production of Tissues. Arch. Jap. Chir., **27**, 91, 1958.
47) Shirotani, H.: Histochemical Studies on Fat Metabolism by Intravenous Administration of Fatty Chyle. Arch. Jap. Chir., **26**, 38, 1957.
48) Sinclair. Robert Gordon: The Metabolism of the Phospholipids. V. The Relationship between the Amount of Fat Ingested and the Degree of Unsaturation of the Phospholipids and Neutral Fat in the Tissues of the Rat. J. Biol. Chem., **96**, 103, 1932.
49) Takahashi, K.: Experimental Studies on the Absorption of Fat from the Intestine. Tohoku-Igaku, **33**, 239, 1943.
50) Tamaki, A.: unpublished
51) Tatsumi, W.: Über die intravenöse Infusion des Fettes. Arch. Jap. Chir., **26**, 1, 1957.
52) Tobe, T.: unpublished
53) Yasuda, M.. Lipid Metabolism. J. Biol. Chem. Jap., **17**, 1, 1942.
54) Yasuda, M.: Lipid Metabolism. Nisshin-Igaku, **32**, 501, 1943.
55) Yasuda, M.: Lipids in Tissues. Igakunoayumi, **13**, 201, 1952.
56) Zilversmit, D. B., Chaikoff, I. L., and Entenman, C.: Are Phospholipids Obligatory Participants in Fat Transport across the Intestinal Wall? J. Biol. Chem., **172**, 637, 1948.
57) Zilversmit, D. B., Entenman, C. and Chaikoff, I. L.: The Measurement of Turnover of the Various Phospholipids in Liver and Plasma of the Dog and its Application to the Mechanism of Action of Choline. J. Biol. Chem., **176**, 193, 1948.

和 文 抄 録

脂酸ペーパークロマトグラフィーを応用した脂質代謝の分析化学的研究

京都大学医学部外科学教室第2講座（指導：青柳安誠教授）

丹 信 敏

脂酸ペーパークロマトグラフィー法を駆使，応用することによつて，経口的に摂取された脂質の腸粘膜からの吸収経路について追究すると同時に，生体内各種臓器中の含有脂酸，更には血清，胆汁，リンパ，貯蔵脂質中の含有脂酸の分析を行い，次のような結論に到達した．

(1) 経口的に摂取された脂質の腸粘膜からの吸収経路は2つに大別される．即ち胸管リンパ中に吸収されるものと，門脈血中へ直接吸収されるものとの2つがある．

(2) この何れの吸収経路をとるかは，脂酸の鎖の長短によつて規定されるもので，ミリスチン酸以上の高

級脂酸は專ら胸管リンパ中へ吸収されるのに反して，それ以下の低級脂酸は主として門脈血中へ直接吸収される．

(3) 併し，更に低級脂酸についてみると，低級脂酸の中でも，その炭素原子数が減ずるに従つて門脈血中への吸収率は漸次増大する．併し炭素原子数が6個あるいは8個というような低級飽和脂酸（カプロン酸，カプリル酸）であつても，その全てが門脈血中に吸収されるわけではなく，なおその幾許かは胸管リンパ中へも吸収される．

(4) 従つて脂質を乳化態として直接皮下静脈内へ注入するに当つては，出来得る限り，高級脂酸のみからなるトリ・グリセライドをその原料として選ぶべきである．併し教室先人の行つた組織顕微化学的検索成績あるいは生化学的検索成績とを併せ考えると，高級脂酸とはいうものの，高度不飽和脂酸，エイコセン酸，鯨油酸等を全く含有していてはならないもので，結局ミリスチン酸，パルミチン酸，ステアリン酸，オレイン酸，リノール酸，リノレン酸のみからなるトリ・グリセライドをその原料として選ぶべきである．

(5) そのような意味では，われわれが斯る目的に使用しつつあるゴマ油は，その原料脂質として合理的なものということが出来る．

(6) 各臓器を通じてみる時，ラウリン酸以下の低級脂酸は Postabsorptive state に於ける限り，何れの臓器組織内にも認められない．そして生体内各臓器の何れにも存在し，而も各臓器が常に略々一致した含有比率を示している脂酸は，ミリスチン酸，パルミチン酸，ステアリン酸，オレイン酸，リノール酸，リノレン酸であつた．

(7) 貯蔵脂質はラウリン酸を比較的多く含有しているため，その中和価が高い．これに反して高度不飽和脂酸は肝臓，胆汁，血清中に比較的多く含有されて居り，肝外組織中のそれが含有量は肝臓等に較べれば問題にならない程少量である．而して高度不飽和脂酸は生体内で合成されることはあり得ないから，少なくともそれら臓器中に含有される高度不飽和脂酸の由来は外来脂質に求めざるを得ない．

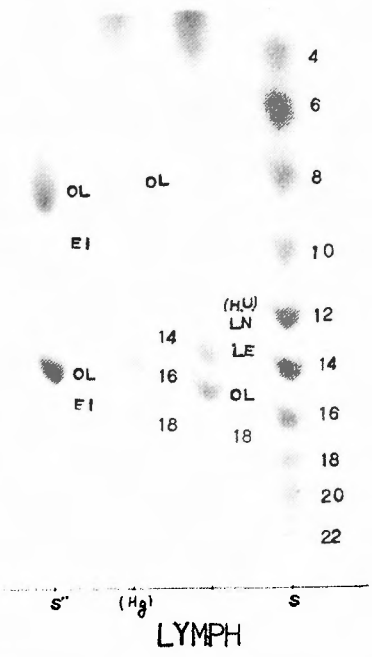


Fig. 2

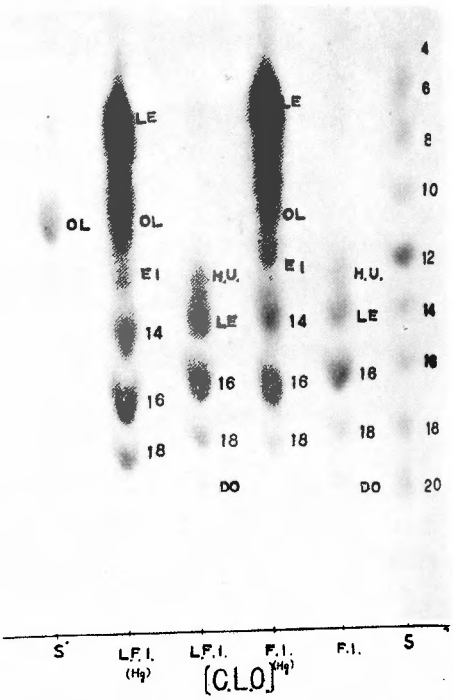


Fig. 3

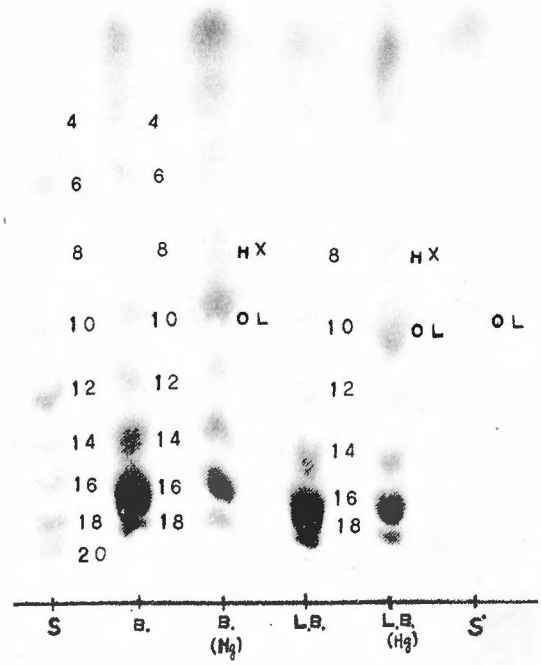
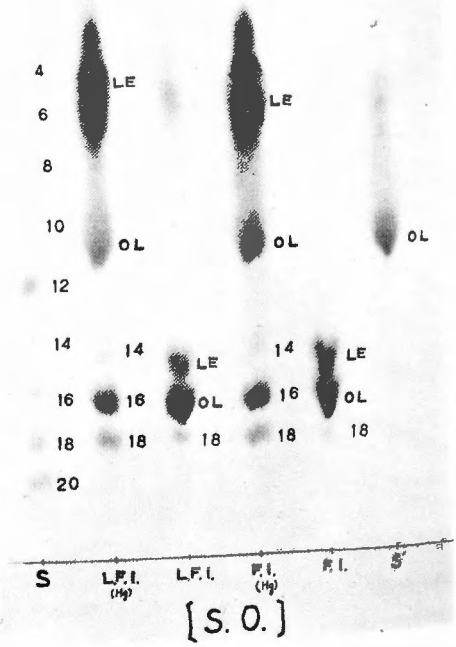


Fig. 4



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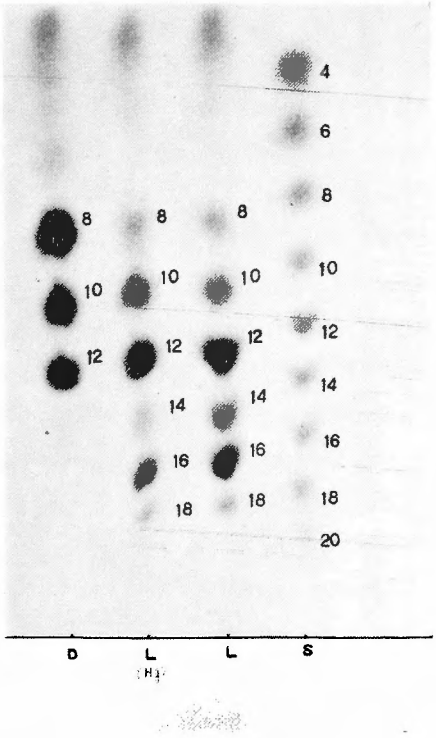

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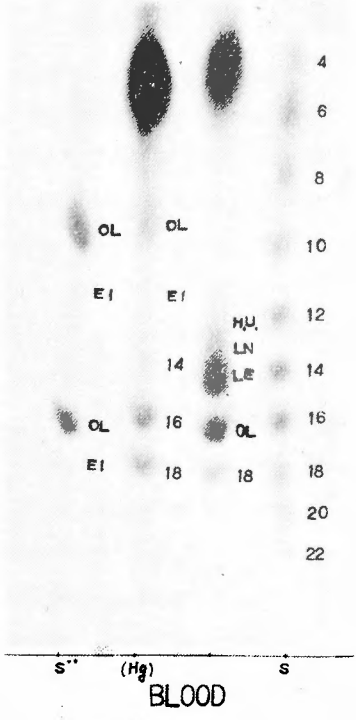

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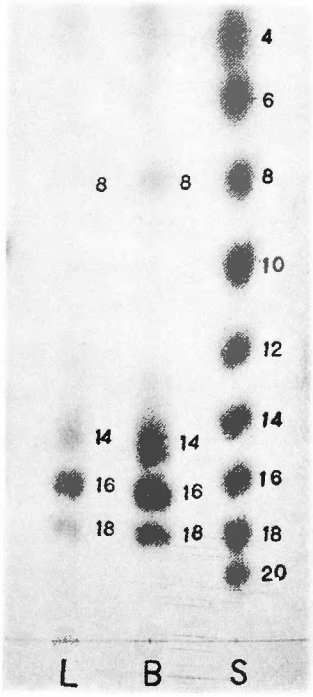

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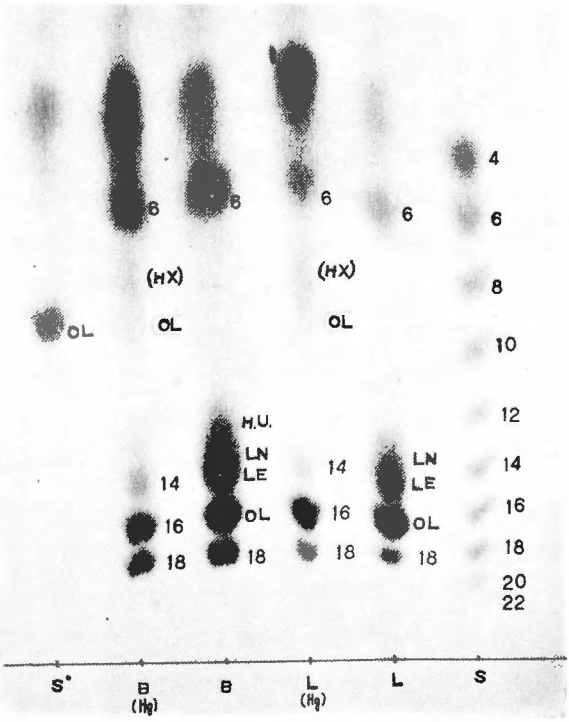

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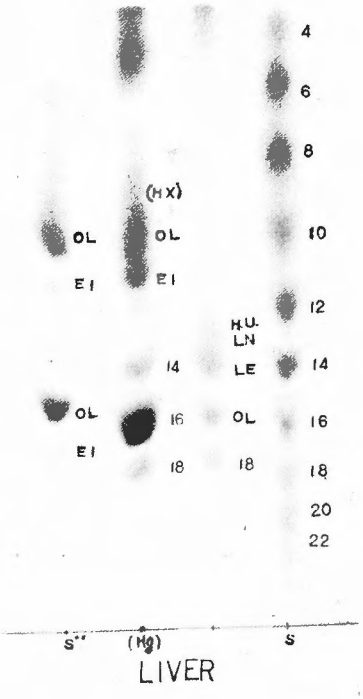

Fig. 10

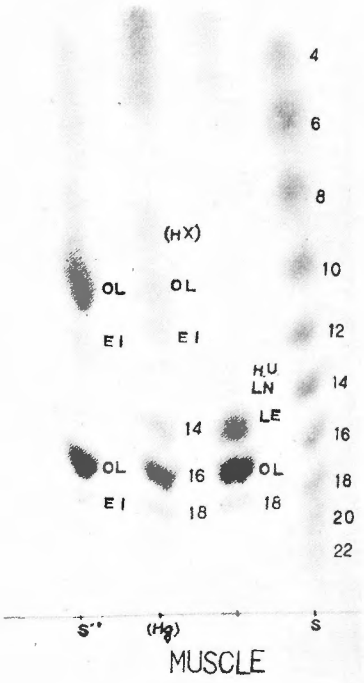

Fig. 11

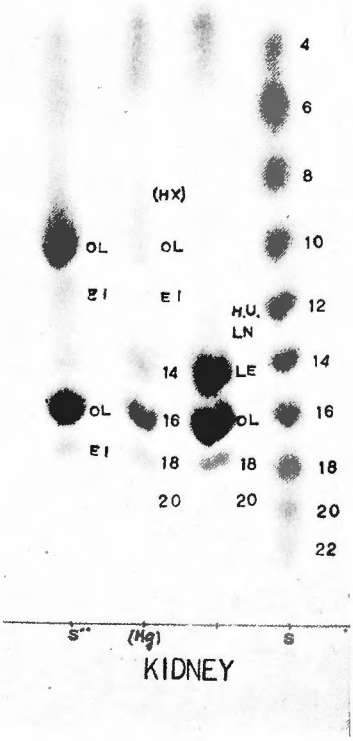

Fig. 12

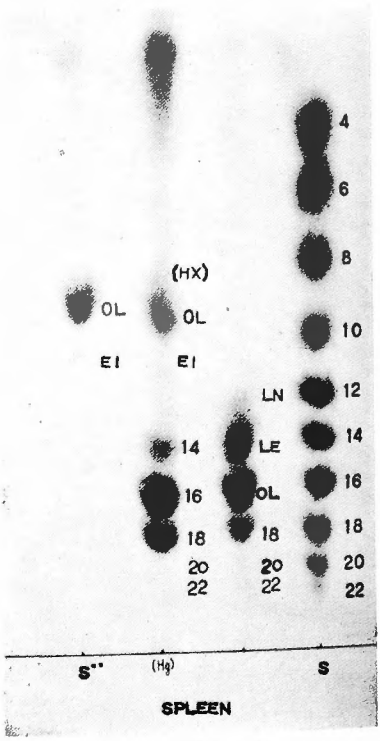

Fig. 13

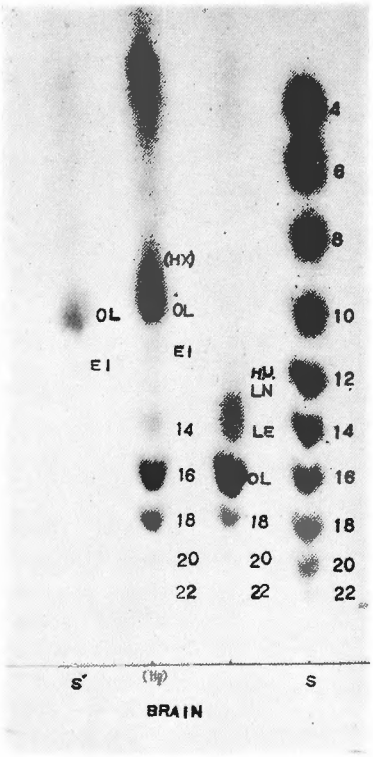

Fig. 14

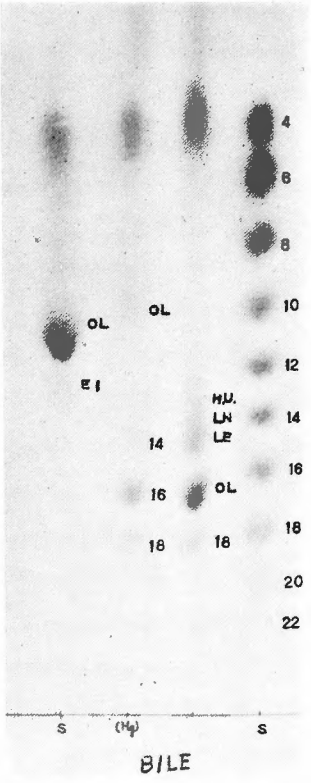

Fig. 15

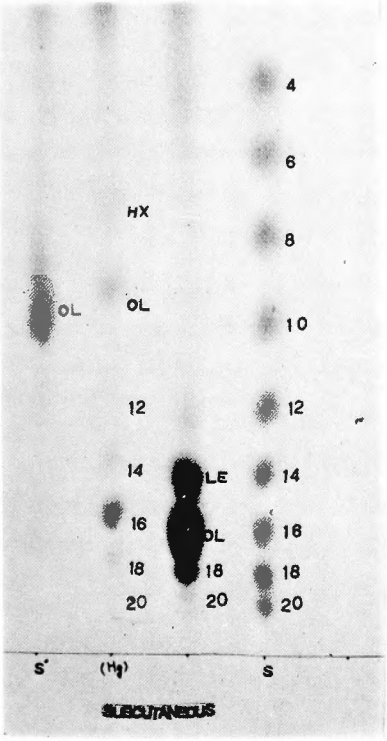

Fig. 16

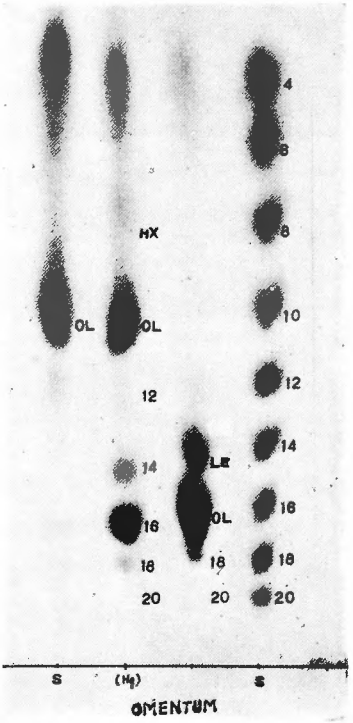

Fig. 17

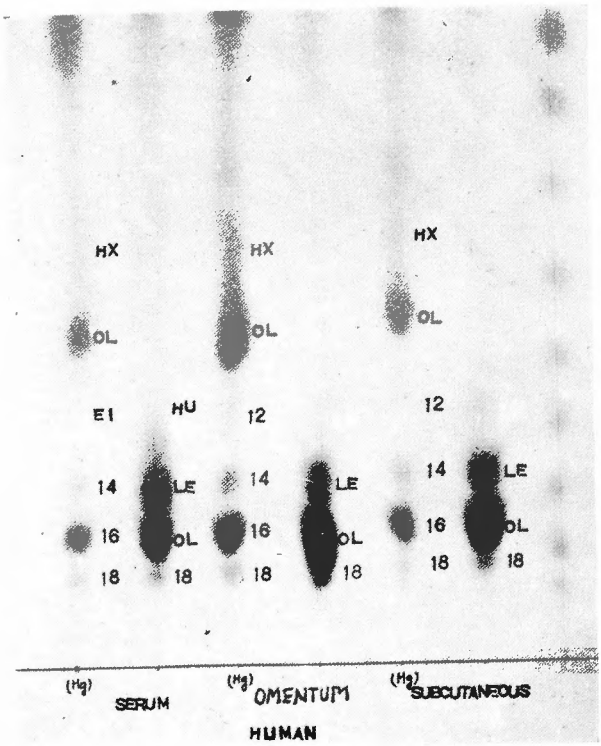
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Fig. 18

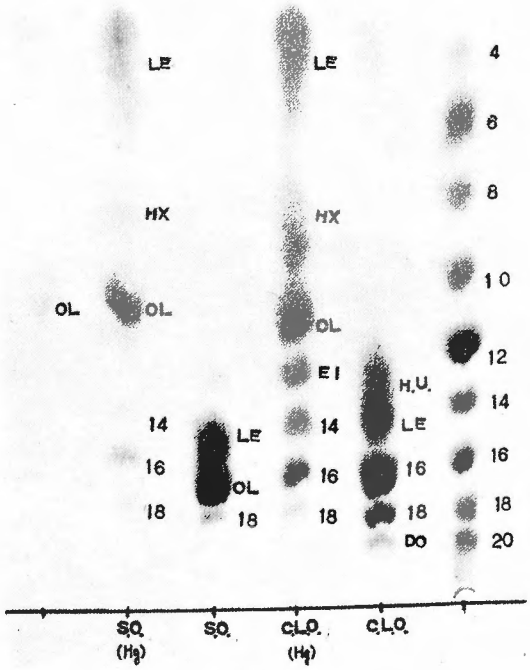
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Fig. 19