STUDIES ON THE CONJUGATED FATTY ACIDS PART III FAT ABSORPTION AND DISTRIBUTION STUDY IN FISH-1. APPLICATION OF THE CONJUGATED FATTY ACIDS FOR THE RESEARCH ON THE FAT METABOLISM OF THE CARP, CYPRINUS CARPIO LINNE

STUDIES ON THE CONJUGATED FATTY ACIDS
PART III

FAT ABSORPTION AND DISTRIBUTION STUDY IN FISH—1. APPLICATION OF THE CONJUGATED FATTY ACIDS FOR THE RESEARCH ON THE FAT METABOLISM OF THE CARP, *CYPRINUS CARPIO* LINNÉ

By

Yasuhiko TSUCHIYA and Mitsu KAYAMA

Department of Fisheries, Faculty of Agriculture, Tohoku University, Sendai, Japan

(Received Jan., 13, 1958)

Introduction

The studies on the fat of the fish are important works not only from the commercial and industrial standpoint of the fisheries, but also for the research of the fat metabolism from the physiological ground.

Although, to the present, the eminent invesigations on the fatty acid composition and on the unsaponifiable matter of the aquatic oil had been systematically clarified by the leading investigators, Tsujimoto, Hilditch and Lovern et al., they reported generally on the oil composition of the caught fish, such as the herring, cod, sardine, saury, shark and trout, in season. However, experiments on the fat metabolism of fish in the aquarium are few so far as the authors are aware, because of the difficulty of their experimental treatment. Tsujimoto's feeding experiment (1) on the eel must be reconsidered.

The rats and mice are mostly used as the experimental animals in the fat test. Even if the rats are used as experimental animals for the study of the fat metabolism, we could not analogize the results for the fish itself.

In this work the authors tried the intraintestinal intubation technique for the first time with the catheter made of polyethylene to provide the test fats for the carp, and traced the fat absorption and distribution in the carp, with the lapse of time, measuring the ultraviolet absorption of the conjugated fatty acids with Beckman spectrophotometer, as the series of the studies on the conjugated fatty acids.

Experimental and Results

1. *Preliminary examination of the rats.*

If the conjugated fatty acids are used for the study of fat metabolism, it must be considered that the acids, whether toxic or not, are absorbed through the same route as the common fatty acids. Thus, firstly, the nutritional value of the conjugated fats (2) and their distribution (3) in the rats were reexamined, comparing with the previously reported data.

The conjugated cod liver oil.

The cod liver oil removed the unsaponifiable matter was isomerized with the nickel-on-carbon catalyst as previously reported by the authors(4). As the contrast group the cod liver oil with no unsaponifiable matter was provided. The oil characteristics of both the conjugated and nonconjugated cod liver oils are shown in Table 1.

Table 1. General characteristics of the conjugated and nonconjugated cod liver oil (vitamin A-free) used for fat component of rat diet.

	N_D^{30}	d_4^{30}	I.V., H.I.V.	S. V.	Spectral absorption $E_{1cm}^{1\%}$				
					235mμ	270mμ	316mμ	346mμ	374mμ
Nonconjugated	1.4774	0.9174	172.4	183	9.9	2.0	1.3	0.8	0.2
Conjugated	1.4803	0.9212	167.2	185	94.8	53.4	22.0	7.7	2.7

Basal diet.

The basal diet employed in the present study is shown in Table 2.

Table 2. Composition of diet for the rat.

Component	Per cent
Polished rice powder	74
Casein (extracted with ether)	10
Dried yeast	3
McCollum & Simmonds' Salt mixture No. 115	3
Fat	10

Each rat received 10g of diet and one drop of the shark liver oil (vitamin A 2,970 I.U.) per day.

The test animals were two to three months old Wistar strain male and female rats. Preceeding the experiment, ten rats were fed for several days with the basal diets with the exception of the test and contrast fats. After the rats reached a constant weight, five animals for the test and another five for the contrast group were equally separated, according to the sex and weight. Each animal was fed on 10 g diets containing 10 per cent of fats and one drop of the shark liver oil (2,970 I. U. vitamin A).

The weights gained during 30 days feeding test are summarized in Table 3.

Table 3. Growth response of rats on the conjugated and nonconjugated cod liver oil diet in 30days. (From May 16 to June 15, 1956)

	Rat No.	Blood series	Sex	Body weight gained in 30 days (g)			Mean weight gained (g)
				Initial	Last day	Gained	
Nonconjugated group	1	A	♂	62	136	74	
	2	B	♂	73	142	69	
	3	A	♀	64	141	77	69.6
	4	A	♀	66	136	70	
	5	B	♀	83	141	58	
Conjugated group	6	B	♂	72	141	69	
	7	B	♂	83	152	69	
	8	A	♀	60	121	61	64.0
	9	B	♀	77	137	60	
	10	B	♀	76	137	61	

The differences of growth between the test and contrast group were analized with *t* test. The significance between the test group fed on the conjugated cod liver oil and the contrast group provided with the nonconjugated cod liver oil is not admissed at 5 per cent level and 10 per cent level, but at the 15 per cent level the difference may be judged significant.

The growth curves of the rats are graphically illustrated in Fig. 1.

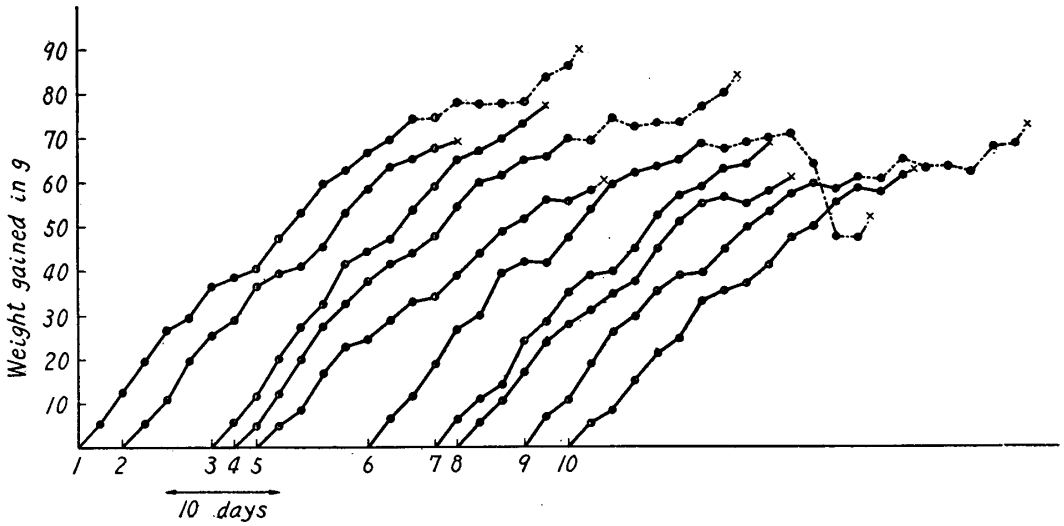

Fig. 1. Growth curves of rats. Curves numbered 1, 2, 3, 4 and 5 represent the rats of the nonconjugated group and 6, 7, 8, 9 and 10 those of the conjugated.

•-·-• Linseed oil dose to nos. 1 and 4, Chinese tung oil to nos. 6 and 9.

Numbers 1, 2, 3, 4 and 5 are rats fed on the diet with 10 per cent of the

nonconjugated cod liver oil and nos. 6, 7, 8, 9 and 10 are those fed on the diet containing the same amounts of the conjugated oil. Numbers 2, 3 and 7,8 were killed on one day stock diets with no oil after 30 days feeding test, and nos. 5 and 10 were sacrificed on two days, that is, at 24 and 48 hours respectively after the last dosage of the contrast and test diets without starvation. Numbers 1, 4 and 6, 9 are sacrificed after ten days on stock diet with no oil and successively five days on linseed oil for nos. 1, 4 and the same days on tung oil for nos. 6, 9.

Before the autopsy the rats had been lightly anaesthetized with ether. The liver, spleen, kidney, adrenals, large and small intestine, stomach, pancreas, adipose, heart, lung and brain were collected. All the tissues are ground with anhydrous sodium sulphate and the oils were extracted with ether for one day. The ether extracts were filtered, and the filtrates were made up to the proper volume, and used for the spectrophotometric measurements. The value $E^{1\%}_{1cm}$ is calculated on the oil of each tissue, by weighing the oil remaining after removing the solvent in vacuo.

The absorption curves of the tissues are illustrated in Fig. 2.

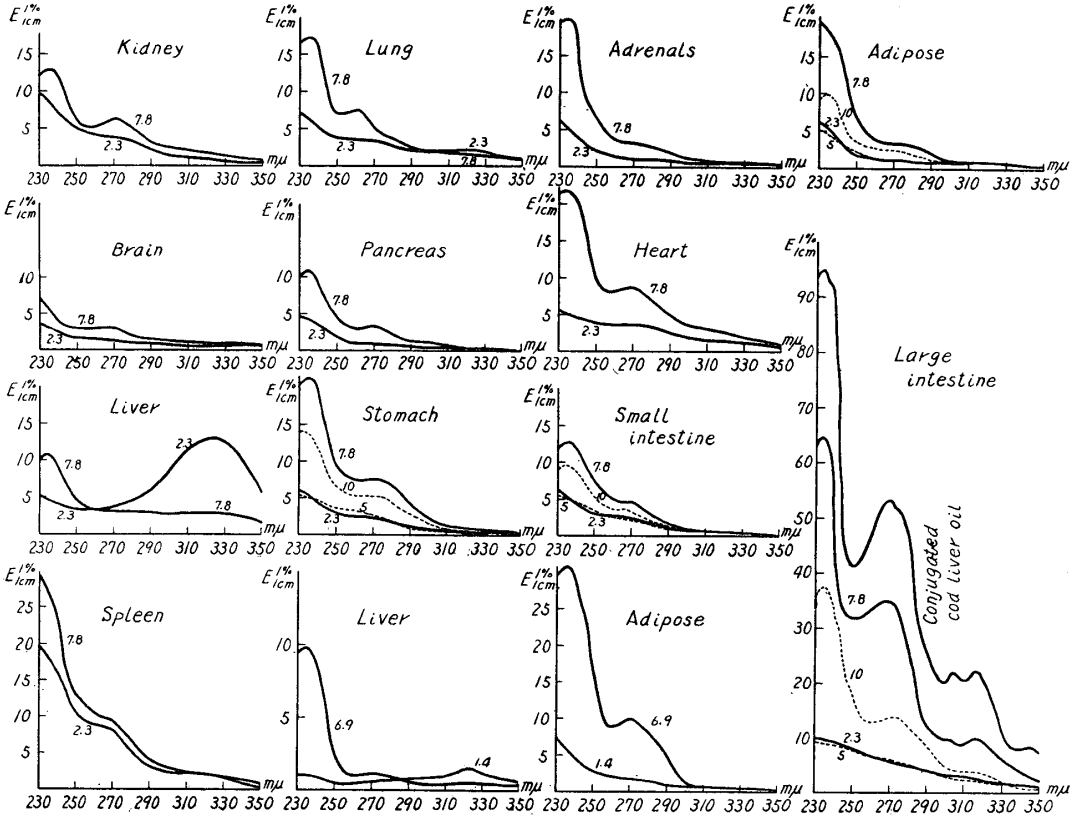

Fig. 2. Spectral absorption curves of lipids extracted from the tissues of ten experimental rats. Curves numbered 2, 3 and 7, 8 represent rats sacrificed on one day, and 5 and 10 on two days after the last feeding on control and test diet during thirty days. Numbers 1, 4 and 6, 9 respectively show the curves of the linseed oil and Chinese tung oil feeding for five days following the ten days fat-free diet.

Full interpretation of the curves is impossible at this time. It should be pointed out, however, that the animal very quickly changes the higher conjugated fatty acids, causing a loss of the band at their maximum absorption, and a rise in absorption at the less conjugated fatty acids' maximum. This fact shall be reconsidered in the discussion together with the experiments on the fish. Some interesting differences among the tissues are at once apparent. According to the changes of the absorption curves with which the 270 mμ band disappears and 235 mμ appears during 2 days, the following two groups are apparently recognized. The liver, kidney, adrenals, and spleen fall rapidly on the one hand, and the large and small intestine, stomach, adipose, lung, and heart suffer less change on the other. The brain shows a small response to the conjugated acid feeding.

From the results of the preliminary examination on the rats, it has been concluded that the toxic or antimetabolic effect of the conjugated acids on the growth is very small even if there is some. And the similar absorption and distribution patterns, comparing them with the natural fatty acids, have been observed as previously reported in rats (3). So it became possible that the method employed here is also applicable for the test on the fat metabolism of the fish as an indicator.

2. *Application of the conjugated fatty acids for the research on fat metabolism of the fish.*

Test of the intraintestinal tube for the fish.

In the case where the conjugated fatty acids are directly applied for the fat metabolism study of the fish, the question arises as to how the conjugated fatty acids or oils could be dosed to the fish without loss. Up to the present time, there are some methods of mixing the test oil with solid diet and using a capsule, and by intra-peritoneal injection technique. The author adopted the technique of the stomach tube which had been used in the rats, since the method seems to have some advantages, comparing with the other methods, in the point of natural digestibility and of exact dosage.

Thereby, in the first place, we instilled the Chinese tung oil with Nélaton's catheter No. 2 connected with a needle and syringe through the mouth to the intestine.

First 0.5 *ml* of blue ink was tentatively poured into the intestine of about 30 g weight carp to see whether the ink would leak from the mouth or gill. Blue ink came out into the water rather from the anus than from the mouth or gill after a while. Moreover, it was observed that poured ink had extended all over the intestine in the abdomen after vivisection. Thus the possibility of the method by using the stomach tube became hopeful in the fish. When 0.5 *ml* of the Chinese tung oil were instilled, neither decease of fish nor vomiting

of oil from mouth or gill were observed, and the excretion of faeces was inspected in the course of the time.

Carp is a representative fish with no stomach in its digestive organ and the intestine is mainly divided into three parts, namely, pre-, meso-, and post-intestines. The digestive organ starts from the oral cavity, and there are pharyngeal teeth at the back of the pharynx, and leads straight to the pre-intestine. The intestine rotates once at the junction between the pre- and meso-intestines, then the meso- and post- intestines turn around in the abdomen to the anus. The intraintestinal tube can be extended without force to the junction of the pre- and meso-intestine. When the tube touches the junction between the pre- and meso-intestines, it is pulled a little and then is poured an adequate amount of oil into the pre-intestine with the syringe.

At the beginning time of the experiment Nélaton's catheter was tentatively used for the intubation of the Chinese tung oil. But Nélaton's catheter is too laborious to pass it through the pharyngeal teeth owing to its flexibility, for it is made of soft India rubber. Still more there is also a fear of solubility of the rubber by the oil. Due to this the indwelling catheter for infant feeding made of polyethylene instead of Nélaton's catheter was used, because it is not so flexible. It is thinner and more elastic than the rubber tube, and was thus adopted, as a suitable one for the intestinal intubation of the test oil to the carp as shown in Plate 1.

Fat absorption and distribution experiment in the carp.

The following main experiments on the fat absorption and distribution were studied in the carp. The one year carps, which had hached out in May, 1955, were used as the test animal. About 100 g carp caught from a breeding-pond were kept in an aquarium one by one without feeding. During the experiments in the summer season the basins were left at the room temperature of 25±2~3°C, as the season became cooler the water temperature in the basins was regulated at 25°C.

0.3 *ml* of the Chinese tung oil were delivered well into the pre-intestine with the syringe connected polyethylene tube. After the required period of time had elapsed, the animal was sacrificed. First the blood was collected from the caudal part, and the internal organs were removed and separated into the spleen, kidney, gall-bladder, mesenterial fat, gonad, hepatopancreas, pre-intestine, meso- and post-intestines, ventral muscle and dorsal muscle respectively.

The tissues were ground with anhydrous sodium sulphate and the fats were extracted with ether in 50 *ml* and 25 *ml* flask after one day standing in a dark place. The ether extracts were filtered and the filtrates were made up to 25 *ml* and 10 *ml* in the measuring flask, then used for the spectrophotometric studies with Beckman spectrophotometer. After the spectrophotometric measurement,

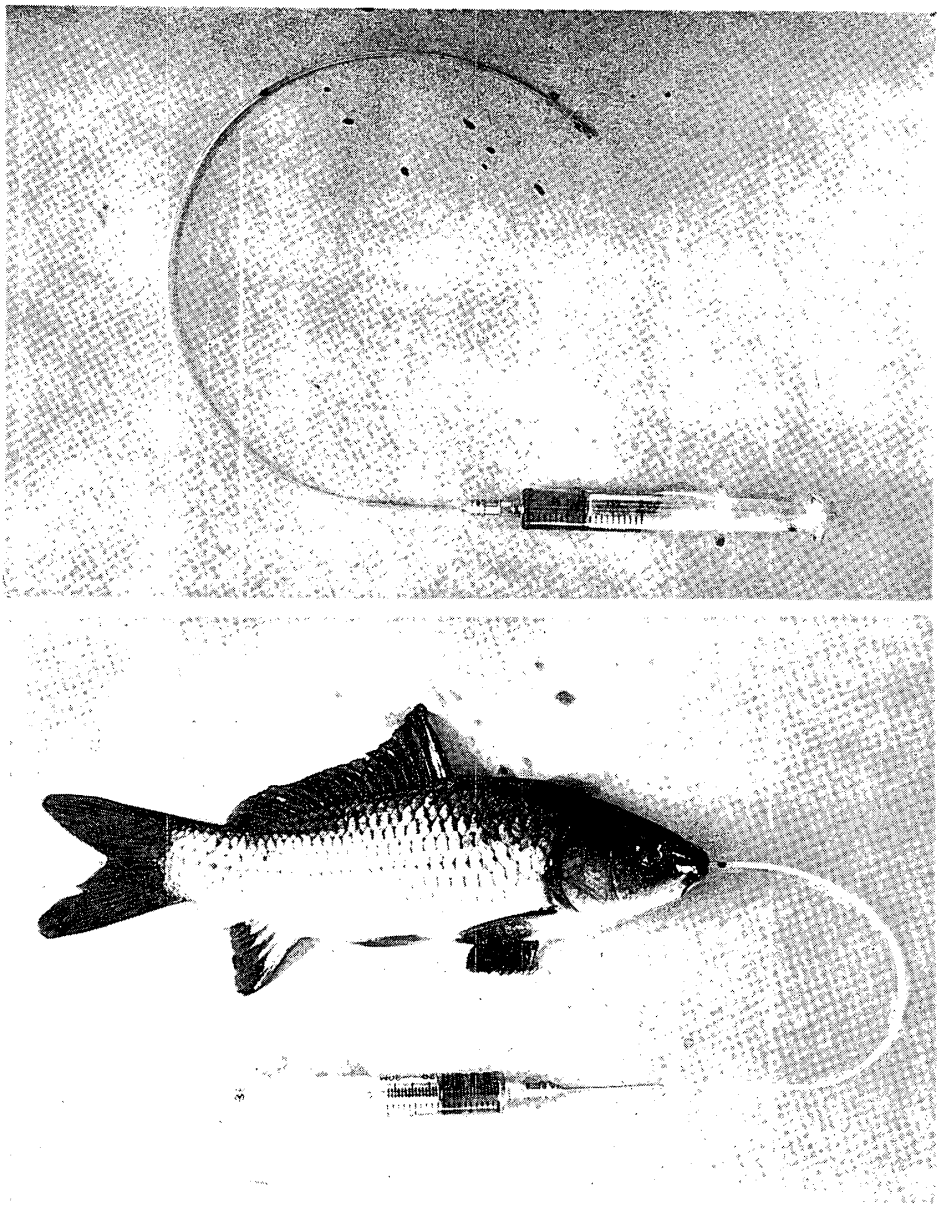

Plate 1.

Upper plate shows the polyethylene tube (indwelling catheter for infant feeding) connected with syringe. Distance from top to black marked point on the catheter is 10 cm.

Lower plate shows the intraintestinal intubation of the test oil to the carp. It is convenient to use gauze or some net to cover the surface of fish body to prevent a fish from jumping and escaping in the treatment.

the solvent ether was removed and the oil was weighed. The values of $E^{1\%}_{1cm}$ of oils are calculated. The results of the absorption curves are summarized in Fig. 3.

With the lapse of 1, 2, 4 and 8 hours, the absorption curves of the tissues generally increase, on the contrary they decrease in the course of 16, 24, 48

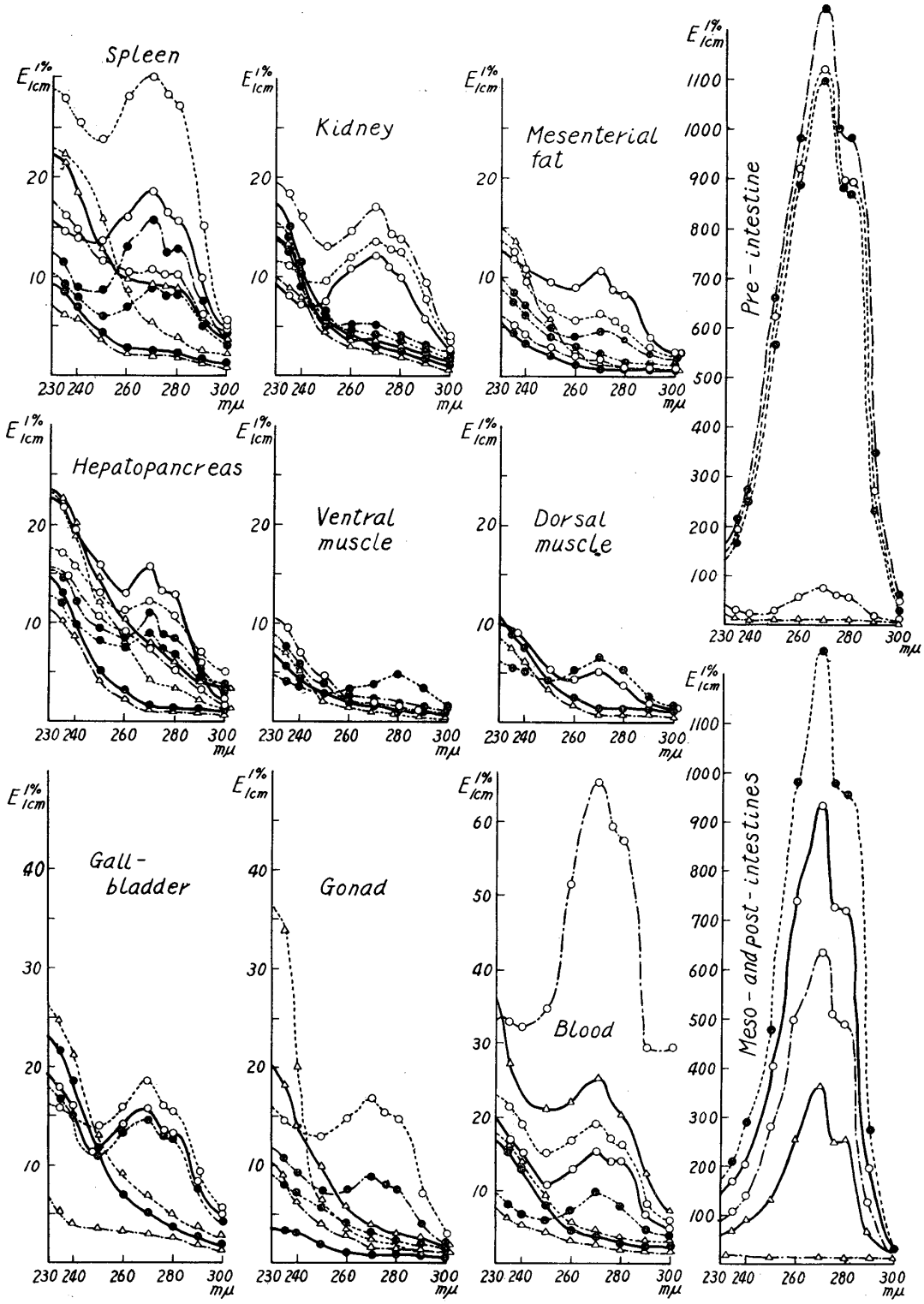

Fig. 3. Spectral absorption curves of lipids extracted from the tissues of carps with the lapse 1, 2, 4, 8, 16, 24, 48 and 72 hrs. after intraintestinal intubation of 0.3 *ml* Chinese tung oil with indwelling catheter for infant feeding.
·····●····· 1hr., -·—●—·- 2hrs., ——○—— 4hrs., ·····○····· 8hrs., -·—○—·- 16hrs., ——△—— 24hrs., ·····△····· 48hrs., -·—△—·- 72hrs., and ——●—— blank, respectively.

and 72 hours. The maximum absorptions were recognized at 4 hours in the hepatopancreas and mesenterial fats, while at about 8 hours in the spleen, gall-bladder, kidney, gonad and dorsal muscle. After 2 to 3 days the state returns to the initial or blank absorption. Also when the tung oil was fed to the carp, the maximum appearance of the absorption curves of the tissue oils was

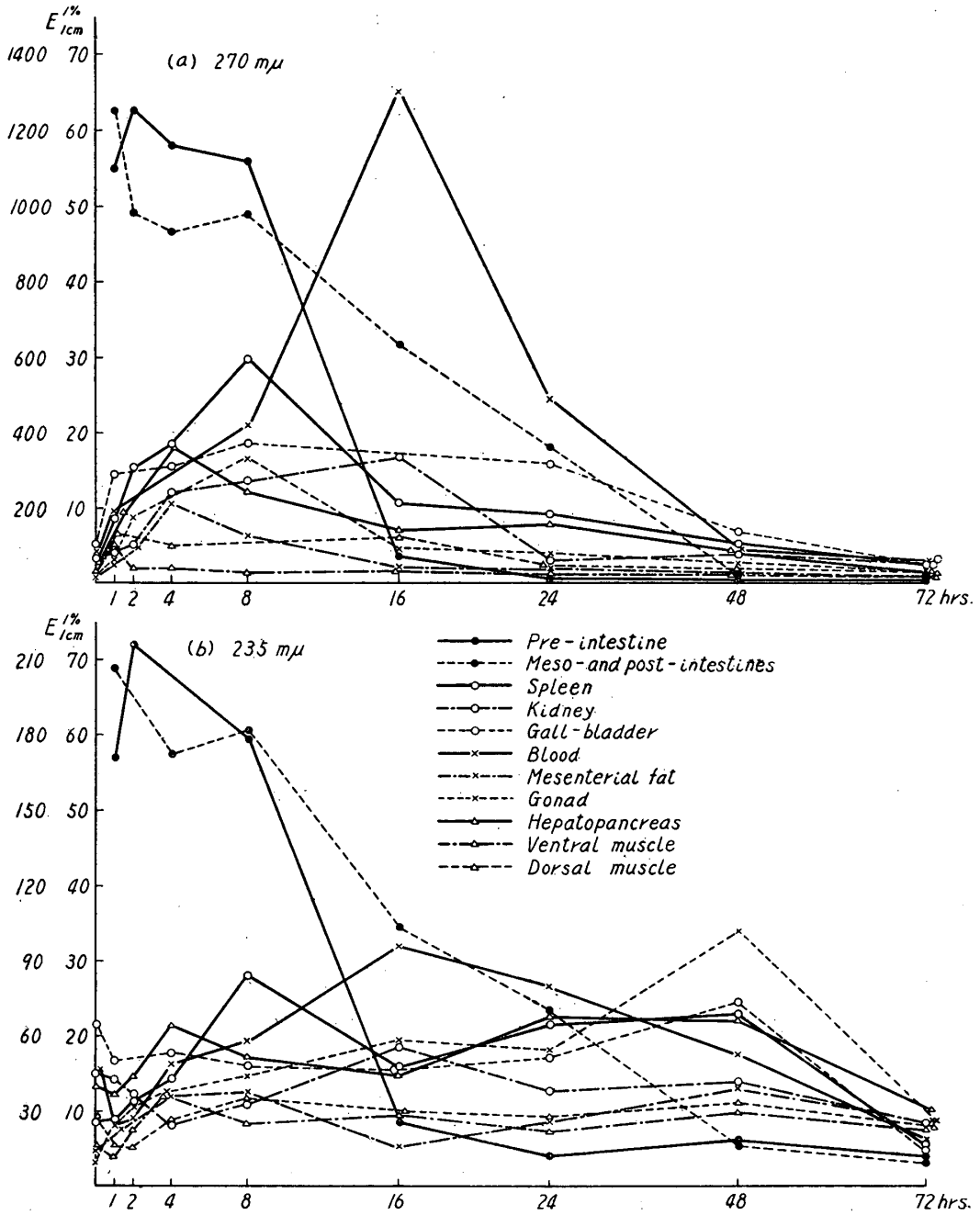

Fig. 4. Change of spectral absorption at 270 mμ (a) and 235 mμ (b) with special reference to time 1, 2, 4, 8, 16, 24, 48 and 72 hrs. in the carp.
Left $E^{1\%}_{1cm}$ scale of ordinate is for the intestine.

comparatively similar to the fed oils in only the earlier period. Then very significant responses were observed, causing a loss of the band at 270 mμ and a rise in absorption at 235 mμ, especially, in these tissues, hepatopancreas, kidney, spleen, blood and gonad. In either case of pre- or meso- and post-intestines, the absorption curves decrease wholly less and less with the lapse of the time without a large change of absorption.

To make clear the fat absorption rate, the absorption maxima at 270 mμ for the conjugated triene and at 235 mμ for the conjugated diene are pursued with the course of the time after the intubation of the tung oil. Fig. 4 illustrates the time and $E^{1\%}_{1cm}$ curves of each tissue.

It takes 4 to 8 hours to attain the maximum absorption in such tissues, hepatopancreas, spleen, and mesenterial fat as shown in the curves of Fig. 4a. While in the curve of the blood, gonad, and kidney, it takes 10 and more hours, owing to the later circulation and reservation. Moreover it is interesting to see the curves of Fig. 4b at 235 mμ. In such 'active' tissues, hepatopancreas, spleen, and kidney, the maxima at 235 mμ increase gradually with the loss of the maxima at 270 mμ in the later hours. Especially, it seems to be very important data that the absorption curves have disappeared in the pre-, meso- and post-intestines after two to three days.

Discussion

Whether the conjugated fatty acids are toxic or whether they follow a distribution pattern similar to that of the natural fatty acids have been glanced over in the preliminary examination in the rats, feeding on the conjugated cod liver oil for 30 days. However, attention must be given to that the physiological action of the conjugated fatty acids seems to act very slightly as anti-metabolite against the vitamin A, not against the essential fatty acids. That has come recently under our notice from long term feeding experiment in the rats. Even though there are some antagonistic relations between the conjugated fatty acids and vitamin A, the application of the conjugated fatty acids for the research on fat absorption and distribution is very useful, because of the required little dosage once for all. The latter problem must be clarified from the experiments in the fish using ^{14}C labelled fatty acids identical with natural fatty acids except radioisotopic. These investigations shall be reported in later papers.

The transformation of the absorption curve, that is, rapid loss at 270 mμ band and a new maximum at 235 mμ in the animal tissues both of the rats and fish, is based upon the conversion of trienoic conjugated acid to dienoic conjugated acid. Although such physiological transformation is now obscure, the change may be due to the enzymatic oxidation or hydrogenation and to the

addition of radicals in some form in the tissues. It offers an interesting problem not only in the case of the conjugated fatty acids, but also in the naturally occurring polyenoic fatty acids, how they are metabolized in the interior of body.

As seen in Fig. 4, the disappearance of the maximum absorption in the intestine of the fish in two to three days after the intubation of the Chinese tung oil with intraintestinal tube involves the following important meaning.

A culturist of carp practises the so-called IKE KOMI or IKE SHIME in Japanese to clean up the contents in the intestine of carp, segregating it in a corf without feeding during one to two days, before marketing. Considering our results of feeding the Chinese tung oil to the carp, it is supposed that IKE KOMI is a reasonable way and takes adequate days for the pretreatment before the transportation of the carp for marketing.

The uses of the conjugated fatty acids for the studies of fat metabolism in the fish are very good methods. The conjugated oils or fatty acids are cheaper than radioisotope and there is no dangerous contamination for the worker in this technique. But the changing of the maximum peak with a loss at higher wavelength and a rapid rise at less wavelength owing to the oxidation, hydrogenation or some other mechanism is a fault of the method for this purpose.

Summary

As the first attempt, the intraintestinal intubation technique was applied to provide the test oil for the study on the fat metabolism of the carp with a catheter made of polyethylene in the aquarium. The conjugated fatty acids were used as a tracer, measuring their peculiar absorption in the ultraviolet region with the Beckman spectrophotometer.

Preceeding to the main experiment on the fish, it was examined whether the conjugated fatty acids are toxic and whether metabolized through the way similar to the natural fatty acids in the rats, using the conjugated cod liver oil isomerized with nickel-on-carbon catalyst and the Chinese tung oil as the test materials. The following two conclusions are summarized in the case of the rat.

1. Even though there are some antimetabolic action of the conjugated fatty acids in the long term feeding, the acids can be used in the fat metabolism study as a tracer for the animal, because the test needs only a small amount and a trial only once.

2. The conjugated fatty acids follow a distribution pattern similar to that of the natural fatty acids comparing with the previously published data.

The method of using the conjugated fatty acids for tracing of the fat metabolism is applied to the fish. In order to intubate the test oil into the

intestine, both Nélaton's catheter No. 2 and indwelling catheter for infant feeding are used in the experiment with the carp. The latter tube is made of polyethylene and is elastic and thinner so that it is very suitable for such a project. The Chinese tung oil is composed chiefly of triglyceride of eleostearic acid with three double bonds in the form of conjugation, of it 0.3 *ml* were poured into the pre-intestine of the carp with the polyethylene tube connecting to a syringe. The absorption rate and distribution have been followed by spectrophotometric analysis with the lapse of time. The change of the absorption maxima with a loss of the band at 270 mμ and with a rapid rise in absorption at 235 mμ have been observed in the tissues, especially in the spleen, kidney, and hepatopancreas.

The data of the disappearance of absorption band in the pre-, meso- and post-intestines of the carp after two to three days have experimentally testified a custom of fish culturist who practises the so-called IKE KOMI or IKE SHIME in Japanese, before marketing to clean up the contents in intestine, separating carps in corf without feeding during one to two days.

Here we express our hearty thanks to Prof. H. Ariyama and his coworkers, Department of Living Science, for their kind advice on rat feeding, to Prof. S. Nishida, Department of Animal Husbandry, for his presentation of Wistar rats, and to Assistant Prof. T. Kariya, Department of Fisheries, Faculty of Agriculture, Tohoku University, for his discussion on the results with fish.

This research fund was partly defrayed by a grant from the Ministry of Education.

References

1) Tsujimoto, M. (1931). Rept. Govern. Chem. Ind. Res. Inst., Tokyo, **26**, 10 (in Japanese).
2) (a) Melnick, D. (1954). J. Am. Oil Chem. Soc., **31**, 63.
   (b) Kaneda, T., H. Sakai, S. Ishii, and K. Arai (1955). Bull. TOKAI Reg. Fish. Res. Lab., No. 12 (in Japanese).
3) (a) Miller, E. S. and G. O. Burr (1937). Proc. Soc. Exp. Biol. Med., New York, **36**, 726.
   (b) Miller, E. S., R. H. Barnes, J. P. Kass, and G. O. Burr (1939). *ibid.*, **41**, 485.
   (c) Barnes, R. H., E. S. Miller, and G. O. Burr (1939). *ibid.*, **42**, 45.
   (d) Reiser, R. and M. J. Bryson (1951). J. Biol. Chem., **189**, 87.
   (e) Reiser, R. (1951). Arch. Biochem. Biophysics, **32**, 113.
   (f) Mead, J. F., A. B. Decker, and L. R. Bennett (1951). J. Nutrition, **43**, 485.
   (g) Mead, J. F., D. L. Fillerup, A. B. Decker, and L. R. Bennett (1952). *ibid.*, **46**, 499.
   (h) Clément, G. et P. May (1953). J. Physiol., Paris, **45**, 79.
4) Tsuchiya, Y. and M. Kayama (1957). Tohoku J. Agr. Res., **7**, 277.