EXPERIMENTAL STUDIES ON FAT METABOLISM IN VIVO WITH SPECIAL REFERENCE TO DETERMINATION OF ALPHA-KETOGLUTARIC ACID

by

Toshimichi Fukata

From the 2nd Surgical Division, Kyoto University Medical School
(Director: Prof. Dr. Yasumasa Aoyagi)
Received for publication May 22, 1959

I. INTRODUCTION

A fat emulsion which can be given intravenously was prepared in our laboratory.[20,21] Since then, fat metabolism in vivo has been examined histochemically by Asada[1] and Izukura[28], and biochemical studies have been conducted by Ikeda[27], Nakata[46], Seno[56] and Kuyama[35]. These studies in our laboratory clearly demonstrate that the fat globules (triglycerides) infused intravenously are first phagocytized by the alveolar phagocytes and the reticuloendothelial cells of the liver and the spleen, and are changed from glycerides into phospholipides in these cells. The phospholipides thus produced enter not only into the hepatic parenchymatous cells, but also into the extrahepatic tissues in the form of lipoprotein, and undergo further metabolism.

Furthermore, Hashino[19] in our laboratory proved, by means of perfusion experiments of the isolated liver and by intravenous administration experiments, that fat can be oxidized at least to the stage of ketone bodies in the isolated liver as well as in the body and that the production of ketone bodies is chiefly performed in the liver. Shigenaga[57] in our laboratory attempted to determine, by the use of Warburg's apparatus, the respiratory quotient and the rate of ketone body production in various tissues following intravenous administration of fat emulsion. As a result, he has demonstrated that the liver is the chief organ for the production of ketone bodies, and has reached the conclusion that extrahepatic tissues have the function of oxidizing ketone bodies produced in the liver.

In the present paper, the author has concentrated upon examination of the phases of production of α-ketoglutaric acid (hereinafter referred to as K. G. A.) in rat tissues following the intravenous administration of fat emulsion by paper partition chromatography, in order to determine whether the fat globules (triglycerides) infused intravenously can be introduced into the T. C. A. cycle, the final stage of fatty acid oxidation.

II. MATERIALS AND METHODS

1) Experimental Animals: Healthy male rats weighing approximately 150g were used. After being fed the standard diet for about one week, they were fasted for 12 hours prior to the experiment.

2) Fat Emulsion: 20% sesame oil emulsion, containing 14% glucose was used.

3) Used Drugs:

1. methionine (*l*-methionine)
2. riboflavin (riboflavin-5'-phosphate)
3. nicotinic acid (niacin amide)
4. vitamin C (*l*-ascorbic acid)
5. pantothenic acid (calcium pantothenate)

4) Infusion Method: 14% glucose solution, the fat emulsion, or mixtures in three different proportions of the vitamins and the fat emulsion were infused intravenously as shown in Table I.

Table I *Combinations of Fat Emulsion and Various Drugs Used in the Experiments*

Group	Drugs					
A	14% Glucose	—	—	—	—	—
B	Fat emulsion	—	—	—	—	—
C	Fat emulsion	*l*-Methionine	Riboflavin	Niacin amide	*l*-Ascorbic acid	—
D	Fat emulsion	*l*-Methionine	Riboflavin	Niacin amide	—	Calcium pantothenate
E	Fat emulsion	*l*-Methionine	Riboflavin	Niacin amide	*l*-Ascorbic acid	Calcium pantothenate

5) Tissue Samples: The experimental animals were sacrificed by cardiac puncture, and 4～5 ml of blood was collected. 1 ml of this blood was used for the determination. The liver, the diaphragm and the kidney were quickly removed and immediately frozen between two blocks of dry ice according to the method described by CAVALLINI and FRONTALI.[4]

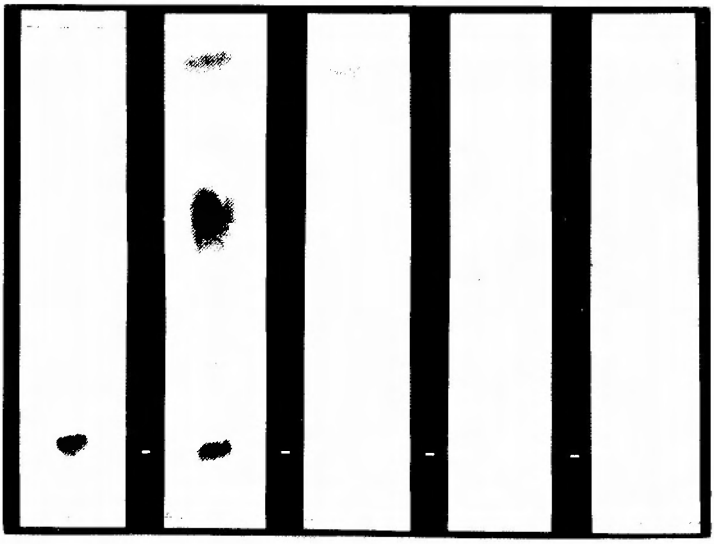


Fig. 1 Chromatograms of keto acid 2 : 4-dinitrophenylhydrazones (Solvent, *n*-butanol/ethanol/sodium bicarbonate). The filter papers are arranged in order from left to right: pure α-ketoglutaric acid 2 : 4-dinitrophenylhydrazone; blood; liver; diaphragm; and kidney. The white marks between the filter papers show the position of the spot due to α-ketoglutaric acid phenylhydrazone on the paper.

6) Procedures of Measurement: The determination of K. G. A. was made with paper chromatography.[5,6,10~12,17,32,51,55,58,60]

The method used was an improvement on that of CAVALLINI and FRONTALI[4] devised by TOKUSHIGE, MORIWAKI, KATSUKI and TANAKA.[66] A sample chromatogram is shown in Fig. 1.

The ketone bodies were measured by the method of KOIDE, MUKOYAMA and MORITA[33] which is an improvement of GREENBERG and LESTER's method.[15]

III. RESULTS

Using healthy male rats in the post-absorptive state after fasting for 12 hours, the K. G. A. levels in blood and tissues prepared under nembutal anesthesia were estimated and compared with those prepared without the use of anesthetic. The results of this investigation, as shown in Table II, show that the differences are

Table II *Changes in the Levels of α-Ketoglutaric Acid According to the Methods of Preparation of Experimental Tissues*

	under anesthetic	without anesthetic
Blood (mg/dl)	1.01	1.02
Liver (mg/hg)	0.18	0.22
Diaphragm (mg/hg)	0.67	0.69
Kidney (mg/hg)	0.37	0.38

almost negligible. The values given in the table are the means of 5 anesthetized samples and 10 non~anesthetized samples. The results obtained are in agreement with EL HAWARY and THOMPSON's conclusion[10]. Experimental tissues were therefore prepared without the use of anesthetic in the present investigation.

(1) Group A (Infusion of 14% Glucose Solution Alone)

This experiment was performed to afford a control to the subsequent infusion experiment of the fat emulsion (Group B). The standard dose for the intravenous administration of fat emulsion has been settled as 0.3g of fat per 100g of body weight in the following infusion experiments, according to the data of SHIGENAGA's[57] investigations. In Group A, accordingly, 1.5 ml of 14% glucose solution per 100g was infused intravenously into rats.

Tissues were prepared in the manner stated above at certain definite intervals after the infusion, and the levels of K. G. A. in blood, liver, diaphragm and kidney were determined. The results are shown in Table III. The levels of K. G. A. and ketone bodies in the blood reached the maximum one hour after the infusion, and then gradually decreased. An almost identical tendency was observed in the K. G. A. levels of various tissues. The increase of K. G. A. one hour after infusion was greatest in the diaphragm, followed in order by that in the blood, the liver and the kidney. The difference, however, between the rates of increase of K. G. A. levels in the liver and the kidney was insignificantly small.

(2) Group B (Infusion of Fat Emulsion Alone)

To this group, 20% sesame oil emulsion containing 14% glucose, was given

Table III *α-Ketoglutaric Acid Levels in Various Tissues and Concentration of Ketone Bodies in Blood Following Intravenous Infusion of Glucose Solution* (Each value shows the mean of three samples.)

Group A		Remarks		Time after infusion 0	1 hr.	3 hr.	6 hr.	9 hr.	12 hr.	15 hr.	18 hr.
14% Glucose	α-Ketoglutaric acid	Blood	Mean (mg/dl)	1.02	1.28	1.13	1.13	1.10	1.12	0.99	1.04
			Change (%)	0	+26	+11	+11	+8	+10	−3	+2
		Liver	Mean (mg/hg)	0.22	0.25	0.23	0.20	0.23	0.22	0.18	0.20
			Change (%)	0	+14	+5	−9	+5	0	−18	−9
		Diaphragm	Mean (mg/hg)	0.69	0.88	0.83	0.76	0.72	0.70	0.67	0.69
			Change (%)	0	+28	+20	+10	+4	+1	−3	0
		Kidney	Mean (mg/hg)	0.38	0.40	0.40	0.40	0.38	0.40	0.37	0.36
			Change (%)	0	+5	+5	+5	0	+5	−3	−5
	Ketone bodies	Blood	Mean (mg/dl)	0.45	0.58	0.51	0.47	0.47	0.45	0.46	0.50
			Change (%)	0	+29	+13	+5	+5	0	+2	+11

Table IV *α-Ketoglutaric Acid Levels in Various Tissues and Concentration of Ketone Bodies in Blood Following Intravenous Infusion of Fat Emulsion* (Each value shows the mean of three samples.)

Group B		Remarks		Time after infusion 0	1 hr.	3 hr.	6 hr.	9 hr.	12 hr.	15 hr.	18 hr.
Fat emulsion	α-Ketoglutaric acid	Blood	Mean (mg/dl)	1.02	1.12	1.10	1.16	1.26	1.17	1.08	1.05
			Change (%)	0	+10	+8	+14	+24	+15	+6	+3
		Liver	Mean (mg/hg)	0.22	0.20	0.25	0.24	0.26	0.20	0.22	0.20
			Change (%)	0	−9	+14	+9	+18	−9	0	−9
		Diaphragm	Mean (mg/hg)	0.69	0.56	0.79	0.99	1.19	0.90	0.73	0.72
			Change (%)	0	−19	+14	+43	+72	+30	+6	+4
		Kidney	Mean (mg/hg)	0.38	0.39	0.42	0.47	0.50	0.50	0.41	0.39
			Change (%)	0	+3	+11	+24	+32	+32	+8	+3
	Ketone bodies	Blood	Mean (mg/dl)	0.45	0.59	0.84	0.95	1.07	0.90	0.79	0.87
			Change (%)	0	+30	+86	+111	+137	+100	+76	+93

intravenously at the rate of 1.5 ml per 100g. The results are shown in Table IV. The level of K. G. A. in the blood, the diaphragm and the kidney increased gradually until it reached the maximum after 9 hours. After that it decreased gradually. Furthermore, the changes in the concentration of ketone bodies in the blood were similar to those in the K. G. A. level, and agreed well with the results obtained by HASHINO.[19]

These experiments with Groups A and B and previous investigations in our laboratory, show clearly that intravenously infused fat is first phagocytized by alveolar phagocytes and reticuloendothelial cells of the liver and the spleen and the glycerides are changed into phospholipides in these cells. These phospholipides enter

not only into the hepatic parenchymatous cells, but also into the extrahepatic tissues in the form of lipoprotein, and undergo further metabolism. That is, the fat infused intravenously breaks down to acetyl-CoA in the Fatty Acid cycle and, in the presence of oxaloacetic acid, enters into the T. C. A. cycle.

(3) Groups C, D and E (Simultaneous Infusions of Various Vitamins and the Fat Emulsion)

What effects occur in the catabolic process of the intravenously infused fat, if various vitamins needed for fat. metabolism are simultaneously employed with the fat emulsion? To make this point clear, the author investigated the changes in the levels of K. G. A. in blood and tissues, when various vitamins, namely, methionine, riboflavin, nicotinic acid, vitamin C and pantothenic acid, were infused in different combinations as follows: Into rats of Group C, 3 mg of methionine, 3 mg of riboflavin, 6 mg of nicotinic acid and 6 mg of vitamin C per 100g of body weight were injected intravenously together with the standard dose of fat emulsion. Into rats of Group D, 3 mg of methionine, 3 mg of riboflavin, 6 mg of nicotinic acid and 6 mg of pantothenic acid per 100g of body weight were injected with the fat emulsion in the same way. And into rats of Group E, 3 mg of methionine, 3 mg of riboflavin, 6 mg of nicotinic acid, 6 mg of vitamin C and 6 mg of pantothenic acid per 100g of body weight were injected. The results of these experiments are presented in Tables V, VI, VII and Fig. 2.

Levels of K. G. A. in the blood, the diaphragm and the kidney, as well as the concentration of ketone bodies in the blood, increased gradually after the infusion, and reached the maximum after 6 hours. After that, they decreased gradually. It is clear that the reduction of time from 9 to 6 hours before reaching the maximum was caused by the simultaneously infused vitamins. Levels of K. G. A. in the blood, the diaphragm and the kidney after 6 hours were greater in Group C than in the other two groups, and little difference was seen between Groups D and E. In regard

Table V *α-Ketoglutaric Acid Levels in Various Tissues and Concentration of Ketone Bodies in Blood Following Simultaneous Infusion of Methionine, Riboflavin, Niacin Amide and Ascorbic Acid with Fat Emulsion* (Each value shows the mean of three samples.)

Group C	Remarks			Time after infusion							
				0	1 hr.	3 hr.	6 hr.	9 hr.	12 hr.	15 hr.	18 hr.
Fat emulsion + *l*-Methionine + Riboflavin + Niacin amide + *l*-Ascorbic acid	α-Ketoglutaric acid	Blood	Mean (mg/dl)	1.02	1.15	1.37	1.45	1.30	1.07	1.05	1.05
			Change (%)	0	+13	+34	+42	+27	+5	+3	+3
		Liver	Mean (mg/hg)	0.22	0.21	0.24	0.20	0.22	0.20	0.20	0.21
			Change (%)	0	−5	+9	−9	0	−9	−9	−5
		Diaphragm	Mean (mg/hg)	0.69	0.62	1.33	1.36	1.28	0.91	0.69	0.74
			Change (%)	0	−10	+93	+97	+86	+32	0	+7
		Kidney	Mean (mg/hg)	0.38	0.45	0.49	0.54	0.47	0.42	0.40	0.41
			Change (%)	0	+18	+29	+42	+24	+11	+5	+8
	Ketone bodies	Blood	Mean (mg/dl)	0.45	0.52	0.60	0.84	0.71	0.62	0.50	0.53
			Change (%)	0	+16	+33	+86	+58	+38	+11	+17

Table VI *α-Ketoglutaric Acid Levels in Various Tissues and Concentration of Ketone Bodies in Blood Following Simultaneous Infusion of Methionine, Riboflavin, Niacin Amide and Calcium Pantothenate with Fat Emulsion* (Each value shows the mean of three samples.)

Group D	Remarks			Time after infusion							
				0	1 hr.	3 hr.	6 hr.	9 hr.	12 hr.	15 hr.	18 hr.
Fat emulsion +	α-Ketoglutaric acid	Blood	Mean (mg/dl)	1.02	1.18	1.30	1.31	1.23	1.00	1.05	1.02
			Change (%)	0	+16	+27	+28	+21	−2	+3	0
l-Methionine +		Liver	Mean (mg/hg)	0.22	0.26	0.22	0.23	0.19	0.20	0.22	0.21
			Change (%)	0	+18	0	+5	−14	−9	0	−5
Riboflavin +		Diaphragm	Mean (mg/hg)	0.69	0.69	0.81	1.24	0.90	0.75	0.70	0.68
			Change (%)	0	0	+17	+80	+30	+9	+1	−1
Niacin amide +		Kidney	Mean (mg/hg)	0.38	0.39	0.43	0.44	0.38	0.36	0.39	0.35
Calcium pantothenate			Change (%)	0	+3	+13	+16	0	−5	−3	−8
	Ketone bodies	Blood	Mean (mg/dl)	0.45	0.56	0.88	1.18	0.91	0.64	0.71	0.50
			Change (%)	0	+24	+96	+162	+103	+43	+57	+12

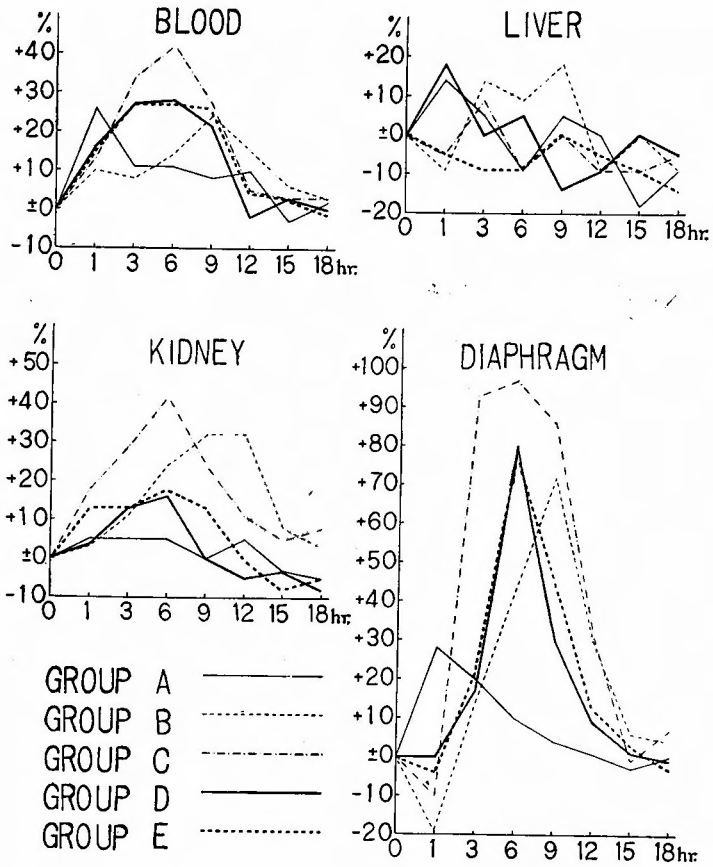
Table VII *α-Ketoglutaric Acid Levels in Various Tissues and Concentration of Ketone Bodies in Blood Following Simultaneous Infusion of Methionine, Riboflavin, Niacin Amide, Ascorbic Acid and Calcium Pantothenate with Fat Emulsion* (Each value shows the mean of three samples.)

Group E	Remarks			Time after infusion							
				0	1 hr.	3 hr.	6 hr.	9 hr.	12 hr.	15 hr.	18 hr.
Fat emulsion +	α-Ketoglutaric acid	Blood	Mean (mg/dl)	1.02	1.17	1.30	1.30	1.29	1.06	1.05	1.01
			Change (%)	0	+15	+27	+27	+26	+4	+3	−1
l-Methionine +		Liver	Mean (mg/hg)	0.22	0.21	0.20	0.20	0.22	0.21	0.20	0.19
			Change (%)	0	−5	−9	−9	0	−5	−9	−14
Riboflavin +		Diaphragm	Mean (mg/hg)	0.69	0.66	0.84	1.22	1.00	0.78	0.71	0.67
Niacin amide +			Change (%)	0	−4	+22	+77	+45	+13	+3	−3
l-Ascorbic acid +		Kidney	Mean (mg/hg)	0.38	0.43	0.43	0.45	0.43	0.38	0.35	0.36
			Change (%)	0	+13	+13	+18	+13	0	−8	−5
Calcium pantothenate	Ketone bodies	Blood	Mean (mg/dl)	0.45	0.51	0.50	0.57	0.53	0.50	0.43	0.49
			Change (%)	0	+13	+11	+27	+18	+11	−4	+8

to ketone bodies, the increase was most marked in Group D, and was less apparent in Group C, and least in Group E. These results of the present investigations are consistent with the observations of HASHINO[19], who had carried out biochemical studies on the concentration of ketone bodies in rabbits, using the same mixtures of fat emulsion and vitamins as in Groups C and E. It may be noted here that the delay in the appearance of maximum values in his experiments was due to the use of a herbivorous animal.

To state the above considerations in different words, the infusion caused a marked increase in the K. G. A. level in Group C, but the increase in the concentration of

Fig. 2 Changes in α-ketoglutaric acid levels in blood, liver, diaphragm and kidney.



ketone bodies was not so notable. In Group D, on the contrary, the concentration of ketone bodies obviously increased, but the increase in the K. G. A. level was only slight. Concentrations of both K. G. A. and ketone bodies in Group E showed hardly any increase (Fig. 2).

In view of the results so far achieved, it would be safe to say that vitamin C is involved in the reaction in which citric acid is converted to K. G. A., whereas pantothenic acid is involved in the further reaction in which K. G. A. is converted to succinic acid. The citric acid in the above reaction is produced by condensation of oxaloacetic acid with acetyl-CoA, which has been produced through fatty acid oxidation (Fig. 3). Therefore, it is quite reasonable that no accumulation of K. G. A. and ketone bodies is observed and the function of the T. C. A. cycle is most smooth in Group E, to which vitamin C and pantothenic acid have been administered simultaneously with the fat emulsion.

(4) Control Experiment

We have seen that the levels of both K. G. A. and ketone bodies in Groups C, D and E reached the maximum 6 hours after the infusion of the fat emulsion. In order to ascertain beyond any doubt that these increases are due solely to the infused

Fig. 3 Acetoacetic and citric acid in relation to a 2-carbon fragment (Acetyl-CoA).

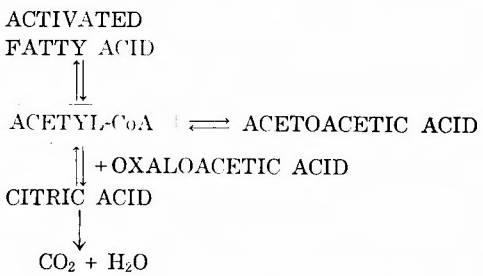


Table VIII *Control Experiment*

Group		Group C′		Group D′		Group E′	
Drugs		14% Glucose + *l*-Methionine + Riboflavin + Niacin amide + *l*-Ascorbic acid		14% Glucose + *l*-Methionine + Riboflavin + Niacin amide + Calcium pantothenate		14% Glucose + *l*-Methionine + Riboflavin + Niacin amide + *l*-Ascorbic acid + Calcium pantothenate	
α-Keto-glutaric acid	Blood	Mean (mg/dl)	1.03	Mean (mg/dl)	1.01	Mean (mg/dl)	1.01
		Change (%)	+1	Change (%)	−1	Change (%)	−1
	Liver	Mean (mg/hg)	0.20	Mean (mg/hg)	0.20	Mean (mg/hg)	0.19
		Change (%)	−9	Change (%)	−9	Change (%)	−14
	Diaph-ragm	Mean (mg/hg)	0.79	Mean (mg/hg)	0.69	Mean (mg/hg)	0.71
		Change (%)	+14	Change (%)	0	Change (%)	+3
	Kidney	Mean (mg/hg)	0.41	Mean (mg/hg)	0.34	Mean (mg/hg)	0.38
		Change (%)	+8	Change (%)	−11	Change (%)	0
Ketone bodies	Blood	Mean (mg/dl)	0.48	Mean (mg/dl)	0.47	Mean (mg/dl)	0.45
		Change (%)	+7	Change (%)	+5	Change (%)	0

fat emulsion, the author carried out a control experiment by using 14% glucose solution, instead of sesame oil emulsion, with various vitamins in Control Groups C', D' and E'. The results are shown in Table VIII. In regard to levels of K. G. A. and ketone bodies, no great difference was seen between these groups. Besides, the values thus attained did not differ so much from the results in rats in the post-absorptive state after fasting 12 hours. Accordingly, it may be argued that the increase of K. G. A. and ketone body levels after infusion is due chiefly to the oxidation of infused fat.

IV. DISCUSSION

The general view of fat metabolism in vitro, which is biochemically supported today, is briefly as follows: Activated fatty acid breaks down into 2-carbon fragments, viz. acetyl-CoA after repeated β-oxidations[8,14,31,42,44]. One part of acetyl-CoA enters directly into KREBS' T. C. A. cycle[34], and is dissolved into carbon dioxide

and water (Direct oxidation). The other part of acetyl-CoA reacts, in pairs, in the liver by the action of condensing enzyme to form acetoacetic acid, which is diffused into the blood stream and is carried to the extrahepatic tissues, and then is dissolved and activated into acetyl-CoA again. This acetyl-CoA enters at last into the T. C. A. cycle, and is oxidized completely to carbon dioxide and water like the former (Indirect oxidation). [2,3,7,9,13,19,30,36,38,41,43,45,67] It has also been made clear that all enzyme systems catalizing the reactions of fatty acid oxidation are contained in mitochondria. [37,39,40,54]

Now the problem is whether these considerations are also appliciable to fat metabolism in vivo. Histochemical and biochemical studies on this problem have been continued for several years in our laboratory, using the fat emulsion which was prepared in our laboratory. The results so far obtained by the research of our predecessors indicate, roughly, that the lower fatty acids, highly unsaturated fatty acids and unsaturated fatty acids having more than 20 carbon atoms are the ones which undergo, for the most part, indirect oxidation. On the other hand, higher saturated fatty acids, oleic acid and the essential fatty acids are the ones which in general undergo direct oxidation. In other words, acids of the former group not only impose a heavy burden on the liver, but also produce ketone bodies to a marked degree, and those of the latter impose scarcely any burden on the liver and produce hardly any ketone bodies.[63,65] In addition to this, our predecessors have also shown that for a desirable combustion of fatty acids, even of those that undergo a fairly economical, direct oxidation, the simultaneous infusion of glucose and various vitamins, especially riboflavin, nicotinic acid, vitamin C and pantothenic acid is very necessary.[1,19,22~28,35,46~48,50,56,57,59,62~64]

So far we have judged that the fat infused in the form of emulsion is ultimately metabolized to carbon dioxide and water by the T. C. A. cycle. Our conjecture, however, has been formed on the indirect bases of the results of measurements of the respiratory quotient and the production rate of ketone bodies in various tissues[57] and the changes in the concentration of ketone bodies in blood[19] following the intravenous administration of the fat emulsion.

By measuring the level of K. G. A., a member of the T. C. A. cycle, in the liver and extrahepatic tissues (diaphragm and kidney), the author has confirmed beyond doubt that the intravenously infused fats (triglycerides) are oxidized and finally enter the T. C. A. cycle to undergo the final oxidative processes. These measurements have also supported the view that the simultaneous infusion of the above-mentioned vitamins is necessary to expedite the metabolic processes and to secure maximum nutritional effects of the infused fat.

In this connection, the author investigated the particular roles played by vitamin C and pantothenic acid in the T. C. A. cycle, by estimating the levels of K. G. A. in various tissues and the concentration of ketone bodies in blood following intravenous administration of the fat emulsion under different conditions (Groups C, D and E). There is no great difference, so far as the levels of K. G. A. in various tissues are concerned, between the simple infusion of the fat emulsion (Group B) and the

simultaneous infusion of the fat with methionine, riboflavin and nicotinic acid. The simultaneous infusion of these vitamins, however, reduces the length of time in which the increment of K. G. A. reaches the maximum. Moreover, when vitamin C is added to methionine, riboflavin and nicotinic acid (Group C), the levels of K. G. A. in various tissues show a marked increase. And yet, when pantothenic acid is infused with the fat emulsion in addition to these vitamins (Group E), the levels of K. G. A. in tissues do not increase much. In other words, the levels of K. G. A. increase most with the simultaneous infusion of such vitamins as riboflavin, nicotinic acid and vitamin C (Group C). Riboflavin and nicotinic acid are here necessary for the function of the Fatty Acid cycle, and vitamin C is necessary for the activation of aconitase,[18,61] which plays an important role in the mutual transformation of citric acid, *cis*-aconitic acid and iso-citric acid. It may well be considered that the comparatively slight increase of K. G. A. in Group E is due to the transformation of K. G. A. into succinic acid. To give an explanation in greater detail: K. G. A. is enabled to enter smoothly the further metabolic process to be transformed to succinic acid by the action of pantothenic acid, since K. G. A. thereby undergoes oxidative decarboxylation by coenzymes like co-carboxylase, D. P. N. and activated CoA to form succinic acid.[16,29,49,52,53] From the view-point of K. G. A. production, the changes in its level in Group E are similar to those in the group (Group D) to which methionine, riboflavin, nicotinic acid and pantothenic acid are administered simultaneously with the fat emulsion. But in regard to ketone bodies in blood, it can be readily recognized that there is a distinct difference between these two groups. That is to say, ketone body production in Group E is less than that in Group D.

To summarize what has been said. From the view-point of K. G. A. production, it might seem on the surface that the metabolic process can be sufficiently facilitated only with the simultaneous infusion of methionine, riboflavin, nicotinic acid and pantothenic acid (Group D). But, it should be noted that without vitamin C, acetyl-CoA produced in fatty acid oxidation, does not smoothly undergo the mutual transformation of citric acid, *cis*-aconitic acid and iso-citric acid which is performed by the action of aconitase, and that, instead of going through the T. C. A. cycle, it is exploited for the production of ketone bodies, and results in the slight increase of K. G. A. in Group D, as we have already seen. On the contrary, in Group E to which methionine, riboflavin, nicotinic acid, vitamin C and pantothenic acid are simultaneously given with the fat emulsion, K. G. A. produced by the action of vitamin C, is continually transformed into succinic acid by the action of pantothenic acid, although the levels of K. G. A. in these two Groups, D and E, seem more or less alike. Hence, it must be said that a marked difference in metabolic process lies between the two groups.

V. SUMMARY

The author determined the level of α-ketoglutaric acid in blood, liver, diaphragm and kidney and the concentration of ketone bodies in blood, at various intervals after the intravenous infusion of the sesame oil emulsion invented in our laboratory.

Simple infusion of the fat emulsion, as well as its simultaneous infusion with methionine, riboflavin, nicotinic acid, vitamin C and pantothenic acid was investigated in these studies.

The following conclusions were reached:

1. The fat infused in the form of emulsion is evidently oxidized in the parenchymatous organs, and ultimately metabolized to carbon dioxide and water by the function of the T. C. A. cycle.

2. Fat metabolism in vivo can be expedited by the infusion of methionine, riboflavin, nicotinic acid, vitamin C and pantothenic acid simultaneously with the fat emulsion.

3. In the T. C. A. cycle, vitamin C facilitates the reaction until the production of α-ketoglutaric acid from acetyl-CoA, and pantothenic acid acts upon the production of succinic acid from α-ketoglutaric acid.

The author wishes to express sincere gratitude to Dr. Y. HIKASA for his helpful suggestions and kind guidance throughout the present investigation.

The results of this investigation as described in the preceding pages were made public at the 44th annual meeting of THE GASTRO-ENTEROLOGICAL SOCIETY OF JAPAN in 1958.

BIBLIOGRAPHY

1) Asada, S.: Histochemical studies on the intravenously infused fat emulsion. Arch. Jap. Chir., **22**, 77, 217, 1953, Acta Med. Univ. Kioto, **31**, 171, 1954.
2) Baldwin, E.: Dynamic aspects of biochemistry, 1952.
3) Buchaman, J. M., Sakami, W. & Gurin, S.: A study of the mechanism of fatty acid oxidation with isotopic acetate. J. Biol. Chem., **169**, 411, 1947.
4) Cavallini, D. & Frontali, N.: Quantitative determination of ketoacid by paper partition chromatography. Biochim. Biophys. Acta, **13**, 439, 1954.
5) Cavallini, D., Frontali, N. & Toschi, G.: Determination of keto-acids by partition chromatography on filter-paper. Nature, **163**, 568, 1949.
6) Cavallini, D., Frontali, N. & Toschi, G.: Keto-acid content of human blood and urine. Nature, **164**, 792, 1949.
7) Chaikoff, I. L., Goldman, D. S., Brown, G. W. Jr., Dauben, W. G. & Gee, M.: Acetoacetate formation in liver, I. From palmitic acid-1-C^{14}, 5-C^{14} and 11-C^{14}. J. Biol. Chem., **190**, 229, 1951.
8) Dakin, H.: Oxidation and reduction in the animal body. London, Longmans, Green u. Co., 1912.
9) Drysdale, G. R. & Lardy, H. A.: Fatty acid oxidation by a soluble enzyme system from mitochondria. J. Biol. Chem., **202**, 119, 1953.
10) El Hawary, M. F. S. & Thompson, R. H. S.: Separation and estimation of blood keto acids by paper chromatography. Biochem. J., **53**, 340, 1953.
11) Friedemann, T. E. & Haugen, G. E.: Pyruvic acid, II. The determination of keto acids in blood and urine. J. Biol. Chem., **147**, 415, 1943.
12) Frohman, C. E., Orten, J. M. & Smith, A. H.: Chromatographic determination of the acids of the citric acid cycle in tissues. J. Biol. Chem., **193**, 277, 1951.
13) Grafflin, A. L. & Green, D. E.: Studies on the cyclophorase system, II. The complete oxidation of fatty acid. J. Biol. Chem., **176**, 95, 1948.
14) Green, D. E.: Fatty acid oxidation in soluble systems of animal tissues. Biolog. Rev. (Cambridge), **29**, 330, 1954.
15) Greenberg, L. A. & Lester, D.: A micromethod for the determination of acetone and ketone bodies. J. Biol. Chem., **154**, 177, 1944.
16) Gergely, J., Hele, P. & Ramarkishman, C. V.: Succinyl and acetyl coenzyme A deacylase. J. Biol. Chem., **198**, 323, 1952.

17) Goy, K. F.: Über das quantitative Vorkommen von α-Ketoglutarsäure im Blut bei verschiedenen pathologischen Zuständen im Vergleich mit anderen α-Ketosäuren und der Citronensäure. Hoppe-Seyl. Z., **294**, 128, 1953.
18) Hara, M. & Suda, M.: Acetone body formation and mitochondrial enzyme system activity. Sympos. Enzym. Chem., **9**, 66, 1954.
19) Hashino, H.: Experimental studies of fat metabolism from the view-point of ketone body formation. Arch. Jap. Chir., **24**, 488, 1955.
20) Hikasa, Y.: Studies on the intravenous infusion of fat emulsion. J. Jap. Surg. Soc., **51**, 394, 1950.
21) Hikasa, Y., Asada, S., Zaitsu, A., Tsukada, A. & Nakata, K.: Studies on the intravenous administration of fat emulsion. Arch. Jap. Chir., **21**, 1, 1952.
22) Hikasa, Y., Kuyama, T. & Ôtani, S.: Clinical application of fat emulsions. Arch. Jap. Chir., **25**, 396, 1956.
23) Hikasa, Y., Kuyama, T., Shigenaga, M., Hsü, C. C., Hanafusa, S., Tamaki, A., Matsuda, S., Ônishi, H. & Tobe, T.: Parenteral administration of fats. Ⅱ. Clinical application of fat emulsion. Arch. Jap. Chir., **27**, 736, 1958.
24) Hikasa, Y., Shirotani, H., Shigenaga, M., Kuyama, T., Tan, N. & Shimada, Y.: Parenteral administration of fats. Ⅰ. Fat metabolism in vivo, studied with fat emulsion. Arch. Jap. Chir., **27**, 396, 1958.
25) Hikasa, Y., Takeda, S., Osa, Y. & Hashino, H.: The role of watersoluble vitamines in fat metabolism. Nippon-Rinsho, **13**, 1225, 1955.
26) Hsü, C. C.: unpublished. (Arch. Jap. Chir.)
27) Ikeda, H.: Experimental studies on fat metabolism with a blocked reticuloendothelial system. Arch. Jap. Chir., **26**, 355, 1957.
28) Izukura, T.: Histochemical studies on intravenously administered fat emulsion. Arch. Jap. Chir., **26**, 215, 1957.
29) Kaufman, S., Gilvary, C., Cori, O. & Ochoa, S.: Enzymatic oxidation of α-ketoglutarate and coupled phosphorylation. J. Biol. Chem., **203**, 869, 1953.
30) Kennedy, E. P. & Lehninger, A. L.: The products of oxidation of fatty acids by isolated rat liver mitochondria. J. Biol. Chem., **185**, 275, 1950.
31) Knoop, F.: Der Abbau aromatischer Fettsäuren im Tierkörper. Beitr. Chem. Physiol. u. Path., **6**, 150, 1905.
32) Koepsell, H. J. & Sharpe, E. S.: Microdetermination of pyruvic and α-ketoglutaric acids. Arch. Biochem. Biophy., **38**, 443, 1952.
33) Koide, Y., Mukôyama, H.& Morita, T.: Colorimetry of acetone body in blood and urine. Seikagaku (J. Jap. Biochem. Soc.), **25**, 306, 1953.
34) Krebs, H. A.: The intermediary stages in the biological oxidation of carbohydrate. Adv. Enzymol., **3**, 191, 1943.
35) Kuyama, T.: Clinical studies on the nutritional effects of intravenous administration of fat emulsion. Arch. Jap. Chir., **27**, 64, 1958.
36) Lehninger, A. L.: Fatty acid oxidation and the Krebs tricarboxylic acid cycle. J. Biol. Chem., **161**, 413, 1945.
37) Lehninger, A. L.: A quantitative study of the products of fatty acid oxidation in liver suspension. J. Biol. Chem., **164**, 291, 1946.
38) Lehninger, A. L.: The oxidation of higher fatty acids in heart muscle suspension. J. Biol. Chem., **165**, 131, 1946.
39) Lehninger, A. L. & Kennedy, E. P.: Oxidation of fatty acids and tricarboxylic acid cycle intermediates by isolated rat liver mitochondria. J. Biol. Chem., **179**, 957, 1949.
40) Leloir, L. F. & Munoz, J. M.: Fatty acid oxidation in liver. Biochem. J., **33**, 734, 1939.
41) Lynen, F.: Functional group of coenzyme A and its metabolic relations, especially in the fatty acid cycle. Fed. Proc., **12**, 683, 1953.
42) Lynen, F.: Der Fettsäurecyclus. Angew. Chem., **67**, 463, 1955.
43) Lynen, F.: Die Beteiligung des Coenzyms A bei Biosynthesen. Seikagaku (J. Jap. Biochem. Soc.), **30**, 1, 1958.

44) Lynen, F. & Ochoa, S.: Enzymes of fatty acid metabolism. Biochim. Biophys. Acta, **12**, 299, 1953.
45) Lynen, F., Wessely, L., Wieland, O. & Rueff, L.: Zur β-oxydation der Fettsäure. Angew. Chem., **64**, 687, 1952.
46) Nakata, K.: Experimental studies on fat metabolism in the lung. Arch. Jap. Chir., **23**, 445, 1954.
47) Nishino, T.: Laboratory studies on the intravenous administration of the fat emulsion in the light of tissue metabolism. Arch. Jap. Chir., **23**, 607, 1954.
48) Noda, F.: unpublished. (Arch. Jap. Chir.)
49) Ochoa, S.: Enzymic mechanisms in the citric acid cycle. Adv. Enzymol., **15**, 183, 1954.
50) Osa, H.: Experimental studies on the intravenous administration of a fat emulsion for nutritional purposes. Arch. Jap. Chir., **25**, 154, 1956.
51) Sakurai, S. & Sugae, K.: Determination of α-ketoglutaric acid by column chromatography. J. Biochem. Tokyo, **42**, 367, 1955.
52) Sanadi, D. R., Gibson, D. M. & Ayengar, P.: Guanosine triphosphate, the primary product of phosphorylation coupled to the break down of succinyl coenzyme A. Biochim. Biophys. Acta, **14**, 434, 1954.
53) Sanadi, D. R. & Littlefield, J. W.: Studies on α-ketoglutaric oxidase, Ⅲ. Role of coenzyme A and diphosphopyridine nucleotide. J. Biol. Chem., **201**, 103, 1953.
54) Schneider, W. C. & Potter, V. R.: Intracellular distribution of enzymes. The distribution of oxalacetic oxidase activity in rat liver and rat kidney fractions. J. Biol. Chem., **177**, 893, 1949.
55) Seligson, D. & Shapiro, B.: Alpha-keto acids in blood and urine studied by paper chromatography. Analyt. Chem., **24**, 754, 1952.
56) Seno, A.: A study of the fat metabolism in the isolated perfused liver. Arch. Jap. Chir., **24**, 179, 1955.
57) Shigenaga, M.: Experimental studies on fat metabolism with determinations of respiratory quotient and ketone body production of tissues. Arch. Jap. Chir., **27**, 91, 1958.
58) Shimizu, T.: The determination of α-ketoglutaric acid in blood and urine. J. Biochem. Tokyo, **37**, 421, 1950.
59) Shirotani, H.: Histochemical studies on fat metabolism by intravenous administration of fatty chyle. Arch. Jap. Chir., **26**, 38, 1957.
60) Smith, M. J. H. & Taylor, K. W.: The separation of α-keto acids in blood and urine by paper chromatography. Biochem. J., **55**, XXX, 1953.
61) Suda, M., Takeda, Y., Sujishi, K. & Tanaka, T.: Metabolism of tyrosine. Ⅲ. Relation between homogentisicase, ferrous ion and *l*-ascorbic acid in experimental alcaptonuria of guinea pig. J. Biochem. Tokyo, **38**, 297, 1951.
62) Takeda, S. Experimental studies on the effect of riboflavin following the intravenous administration of fat emulsion. Arch. Jap. Chir., **25**, 221, 1956.
63) Tan, N.: Analytic chemical studies on fat metabolism by application of paper chromatography of fatty acids. Arch. Jap. chir., **28**, 1178, 1959,
64) Tatsumi, W.: Klinische Beobachtungen über die intravenöse Infusion des Fettes. Arch. Jap. Chir., **26**, 1, 1957.
65) Tobe, T.: Experimental investigation of various fats as to their nutritional value. Arch. Jap. Chir., **28**, 184, 1959.
66) Tokushige, M., Moriwaki, T., Katsuki, Y. & Tanaka, S.: Quantitative determination of ketoacids by paper partition chromatography. unpublished. (J. Chem. Soc. Jap.)
67) Weinhouse, S., Millington, R. H. & Volk, M. E.: Oxidation of isotopic palmitic acid in animal tissues. J. Biol. Chem., **185**, 191, 1950.

和文抄録

α-ケトグルタール酸を中心としてみた生体内脂質代謝過程の実験的研究

京都大学医学部外科学教室第2講座　(指導：青柳安誠教授)

深田齊迪

著者は教室創製のゴマ油乳剤を単独或はメチオニン，リボフラビン，ニコチン酸，ビタミンC，パントテン酸等と併用してラッテに経静脈性に注入し，血液，肝臓，横隔膜，腎臓に於けるα-ケトグルタール酸量の逐時的測定並に血中ケトン体濃度の測定を行い，次のような結論に達した．

1. 注入されたゴマ油乳剤含有脂酸は明らかに実質臓器内で酸化せられ終極的にはクエン酸回路内に導入される．

2. メチオニン，リボフラビン，ニコチン酸，ビタミンC，パントテン酸等のビタミン類をゴマ油乳剤と併用することにより生体内脂質代謝過程はより一層円滑化される．

3. クエン酸回路に於いて，ビタミンCはα-ケトグルタール酸生成迄の反応を円滑にし，パントテン酸はα-ケトグルタール酸よりコハク酸生成の段階に作用する．