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## Mitochondrial Swelling and Uncoupling Activity of Long-Chain Fatty Acids

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# Mitochondrial Swelling and Uncoupling Activity of Long-Chain Fatty Acids\*

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## Abstract

The effect of various fatty acids on the swelling-contraction and oxidative phosphorylation of mitochondria from rat liver and Ehrlich ascites tumor cell have been studied and the results are as follows: 1. The swelling of rat liver mitochondria is induced by fatty acid. The extent of this uncoupling action is in the descending order of myristate, laurate, palmitate, stearate and behenate in saturated fatty acid and linoleate, linolenate, ricinoleate and oleate in the unsaturated fatty acid. This swelling action is stronger with unsaturated fatty acids than that of saturated ones and cis form is stronger than trans form. 2. The uncoupling oxidative phosphorylation of rat liver mitochondria is also observed with these fatty acids and the activities are proportional to the degree of the swelling action. 3. The degree of swelling of rat liver mitochondria is proportional to the concentration of oleate and is inhibited by anaerobiosis and respiratory inhibitor except amytal. 4. The mitochondria swollen by fatty acid can be recontracted reversibly by ATP, Mg<sup>++</sup> and bovine serum albumin. 5. The swelling action of sodium oleate is the strongest on mitochondria from rat liver, followed by those from the liver of Ehrlich ascites tumor bearing mouse, Ehrlich ascites tumor cells and solid Ehrlich tumor cells. 6. Sodium oleate inhibits the incorporation of <sup>32</sup>p into ATP, ADP, GTP and UDPG in mitochondria.

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## MITOCHONDRIAL SWELLING AND UNCOUPLING ACTIVITY OF LONG-CHAIN FATTY ACIDS.

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In 1960 SCHOLEFIELD<sup>1</sup> reported the inhibition of phosphorylation in the cancer cells by fatty acids and in 1956 LEHNINGER<sup>2</sup> found uncoupling oxidative phosphorylation and swelling action<sup>3</sup> of fatty acids and of endogenous uncoupling factor such as unsaturated long-chain fatty acids, on mitochondria. In 1956 PRESSMAN and LARDY<sup>4</sup> reported that various fatty acids stimulated the DNP-stimulated latent ATPase activity of mitochondria. In spite of these findings the relationship among the oxidative phosphorylation, mitochondrial swelling and latent ATPase has remained unclarified. For the purpose to elucidate this point, the authors carried out a series of follow-up studies using various fatty acids. As the result, it has been found that there is an interesting relationship among these uncoupling oxidative phosphorylation, latent ATPase and swelling of mitochondria, and that the mitochondrial swelling induced by fatty acid is inhibited by various respiratory inhibitors especially azide and anaerobiosis.

### MATERIALS AND METHODS

Mitochondria from rat liver and Ehrlich ascites tumor cells (6 days after transplantation) were prepared according to HOGEBROOM and SCHNEIDER's method<sup>5</sup>. These mitochondria were resuspended in 0.25 M sucrose solution (1 g tissue equivalent mitochondria of rat liver cells per 1 ml of sucrose and 5 g tissue equivalent mitochondria of Ehrlich ascites tumor cells per 1 ml of sucrose) as stock mitochondrial suspensions. Lauric, myristic, palmitic, behenic, elaidic, oleic, linoleic, linolenic and arachidic acids obtained from Tokyo Kasei Co. were purified and served as the reagents. ATP, ADP and antimycin A were obtained from Shigma Chem. Co., azide, KCN from Katayama Kagaku Co. and amytal from Yamanouchi Seiyaku Co. These reagents were all dissolved with 0.15 M

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KCl—0.02 *M* Tris-HCl buffer (pH 7.4) solution to keep certain ionic strength in order to maintain the mitochondrial function. Fatty acids were also diluted with the same solution in the form of Na-salt.

The mitochondrial swelling was measured by the method of LEHNINGER<sup>5</sup>. Stock mitochondria were washed 3 times with 0.25 *M* sucrose. Incubation mixture was composed of 4.75~4.4 ml of 0.25 *M* sucrose — 0.02 *M* Tris — HCl buffer (pH 7.4) or 0.15 *M* KCl — 0.02 *M* Tris — HCl buffer (pH 7.4), 0.2—0.5 ml of 1 *mM* fatty acid (final concentration 0.04~0.1 *mM*) and 0.1—0.05 ml of stock mitochondria. The incubation was conducted at 37°C for 60 minutes. The incubation mixture was introduced rapidly to Beckman spectrophotometer and the extinction was measured at 520 *mμ* for the period of 60 minutes from the start, at the interval of 5 minutes. In this instance the concentration of fatty acids was adjusted to suit the purpose of each experiment.

Inhibitory effect on mitochondrial swelling induced by fatty acid was tested by respiratory inhibitors, such as 1 *mM* amytal, 10  $\gamma$  / 5 ml antimycin A, 5 *mM* azide and 1 *mM* KCN, as the final concentration, and by the anaerobiosis.

The changes in the absorption at 520 *mμ* were observed to examine reversible contracton of the swollen mitochondria induced by fatty acid<sup>6</sup> with addition of 5 *mM* ATP, 5 *mM* Mg<sup>++</sup> and 3 mg of bovine serum albumin (BSA) to the incubation mixture.

The uncoupling oxidative phosphorylation by fatty acid was studied by Warburg manometric method and by TAKAHASHI's method<sup>7</sup> for the determination of  $\Delta$ 10P fraction of mitochondria. The vessels contained 1.92 ml of stock mitochondrial suspension (1g tissue equivalent mitochondria suspended in 5 ml of 0.25 *M* sucrose solution), 0.30 ml of 0.4 *M* of sodium succinate and 0.3 ml of Krebs-Ringer phosphate solution in main chamber, 0.2 ml of 20 % KOH in center well, and 0.28 ml of 0.4~1 *mM* sodium oleate in the side arm. The gas phase was air and incubation temperature was 38°C. For the estimation of the incorporation of <sup>32</sup>P into  $\Delta$ 10 P fraction, the incubation mixture contained 2 ml of 0.15 *M* KCl — 0.02 *M* Tris buffer (pH 7.4), 2 ml of stock mitochondrial suspension, 0.6 ml of 1~4 *mM* of sodium oleate (in control system 0.6 ml of physiological saline solution) and 0.3 ml of Krebs-Ringer phosphate containing the labeled phosphate 10  $\mu$ c. The medium was incubated for 30 minutes at 20°C in air environment. After the incubation, the reaction mixture was centrifuged at 0°C for 10 minutes at 14000  $\times$  g, washed 3 times with 0.25 *M* sucrose solution to eliminate the contamination of absorbed<sup>32</sup>P. The acid soluble phosphate compounds in mitochondria were extracted with 3 ml of 5 per cent trichloro-acetic acid for 30 minutes at 0°C and centrifuged for 10 minutes at 1700  $\times$  g. One ml of the supernatant was used for the quantitative measurement and for counting radioactivity of phosphate. Another one ml of the supernatant was hydrolysed

with one ml of 1.5 *N* H<sub>2</sub>SO<sub>4</sub> for 10 minutes at 100°C and the quantity and radioactivity of <sup>32</sup>P were estimated by the TAKAHASHI's method<sup>7</sup>.

On the other hand, oxygraphic method was employed to estimate the oxygen consumption and phosphorylation of rat liver mitochondria. The oxygraphy was constructed by one of the authors, K. UTSUMI, which is a slightly modified form of HAGIHARA's<sup>8</sup>. The incubation mixture consisted of 0.05 *M* sucrose, 0.02 *M* KCl, 0.02 *M* K-phosphate, and 0.1 *mM* EDTA (pH 7.5). Two ml of the incubation mixture was introduced to the sample cell of oxygraphy and then 0.2 ml of stock mitochondrial suspension was added to the incubation mixture (state I and II). After 1 minute 0.02 ml of 1 *M* sodium succinate was again added (state IV) and 1 minute later 0.02 ml of 10 *mM* of ADP was added (state III). After reversing to state IV, 0.04 ml of 4 *mM* of sodium salt of fatty acid (0.08 *mM* in final concentration) was added and after lapse of 1 minute 0.02 ml of 10 *mM* of ADP was again added. Then the effects of fatty acids on the oxygen consumption and oxidative phosphorylation were estimated by the ratio both before and after the treatment of fatty acids of respiration and oxidative phosphorylation in the presence of succinate.

The incorporation of <sup>32</sup>P into the acid soluble organic phosphate compound fraction of mitochondria was observed to prove the effect of fatty acid on the phosphorylation of mitochondria. The incubation mixture contained 3 ml of mitochondrial suspension (5 g tissue equivalent of mitochondria per 3 ml of 0.25 *M* sucrose solution), one ml of Krebs-Ringer phosphate containing 100  $\mu$ C of <sup>32</sup>P, 1 ml of 0.2 *M* sodium succinate and 5 ml of 1 *mM* sodium oleate (oleate was replaced by the KCl-Tris solution in the control system). After incubation for 30 minutes at 25°C the acid soluble compounds were extracted with 5 per cent perchloric acid, neutralized with 1 *N* KOH and absorbed to Dowex 1 ( $\times$  4, 200–400 meshes) of formate type and eluated with formic acid and ammonium formate according to the method of TERADA<sup>9</sup>.

## RESULTS

*Effect of various fatty acids on the mitochondrial swelling of rat liver and Ehrlich ascites tumor cells:* Generally, it is well known that fatty acids act as the uncoupler of oxidative phosphorylation and the damaging reagent of biological membrane structure. These facts suggest that the fatty acid acts as swelling-inducing reagent. As shown in Fig. 1 the swelling action of various fatty acids on the mitochondria of rat liver cells fluctuates according to the number of carbon chain in saturated fatty acid. The strongest activity is observed in carbon<sub>14</sub> myristate at the concentration of 0.1 *mM*. The swelling activity of C<sub>12</sub> laurate and C<sub>16</sub> palmitate are lower than that of myristate and the lowest are of C<sub>18</sub> stearate and C<sub>22</sub> behenate. On the other hand, in the un-

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saturated fatty acids, the strength of their swelling action is in the descending order of linoleic, linolenic, richinoleic and oleic acids (Table 1). In this case, however, elaidic acid which is in the trans form of oleic acid shows the lower rate of activity than that of oleic acid. These differences appear at the period of 5 minutes incubation at 30°C, the rate of swelling is not much different among them at the period of 10 minutes. This means that there is a difference in the kinetics of the swelling action between trans- and cis- forms. On the whole the swelling action of these unsaturated fatty acids is stronger than that of saturated fatty acids (Fig. 1 and Table 1).

The kinetics of mitochondrial swelling induced by fatty acid is changed by the concentration of fatty acid and incubation mixture. As shown in Figs. 2 and 3 the degree and initial velocity of mitochondrial swelling are increased by the increment of oleic acid concentration and the swelling is inhibited by sucrose.

Table 1. Effect of unsaturation of C<sub>18</sub> fatty acids (sodium salts) on the mitochondrial swelling. The test system is as in Fig. 1 and expressed the rate of swelling by fatty acid to spontaneous one.

Reagent	Unsaturation	Rate of swelling			
		After 5 minutes		After 10 minutes	
		0.1 mM	0.0025%	0.1 mM	0.0025%
Stearic	None	1.84	2.5	9.8	8.0
Oleic	9, cis	50.2	41.2	83.8	69.8
Elaidic	9, trans	10.3	8.5	60.0	49.2
Richinoleic	9,	56.1	40.6	76.7	63.8
Linoleic	9, 13, cis	92.1	76.3	93.4	77.6
Linolenic	9, 13, 17, cis	80.4	67.0	91.8	68.0

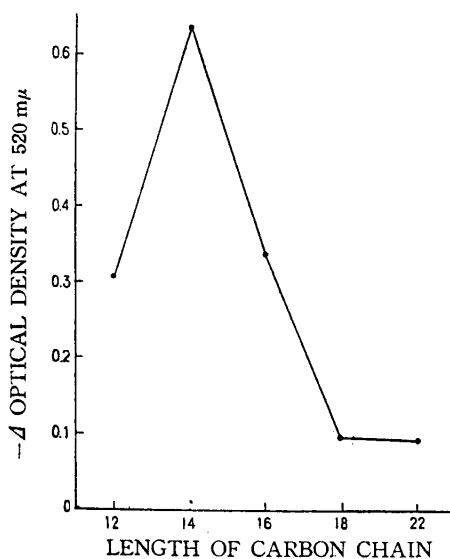


Fig. 1. The mitochondrial swelling action of saturated fatty acids (sodium salts) as function of carbon chain length. A medium of 5.0 ml of 0.15 M KCl-0.02 M Tris pH 7.4 was added to each tube, containing 0.1 mM of fatty acid. The changes of optical density were measured at 520 mμ at 37°C 20 minutes after the addition of washed rat liver mitochondria derived from 50 mg whole liver. The data are estimated with  $-\Delta$  optical density at 520 mμ.

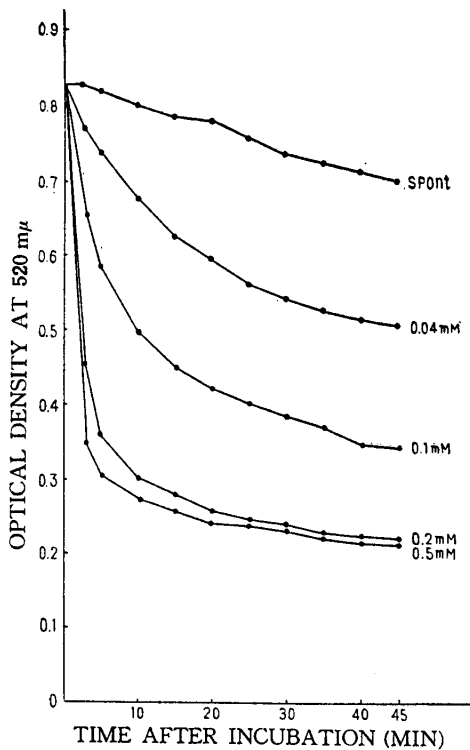


Fig. 2. Effect of various concentrations of sodium oleate on the swelling of mitochondria. The medium consisted of 5.0 ml of 0.15 *M* KCl-0.02 *M* Tris, pH 7.4. The amount of oleate shown was added, and optical absorbancy changes were measured at 520  $m\mu$  at 20°C after the addition of washed rat liver mitochondria derived from 100 mg whole liver.

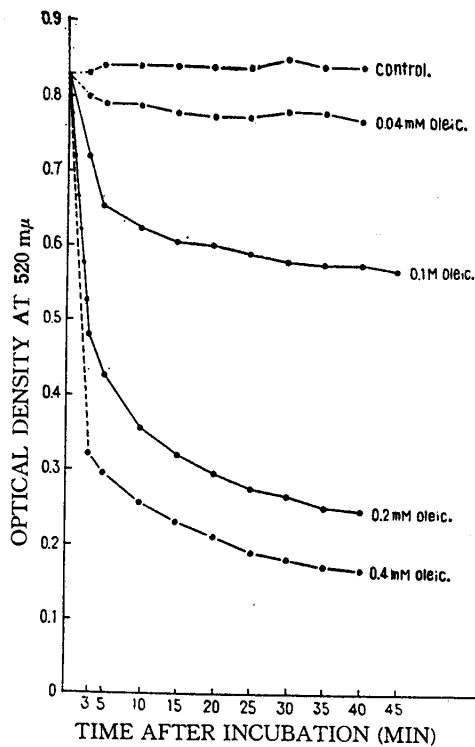


Fig. 3. Effect of various concentrations of sodium oleate on the swelling of mitochondria. The medium consisted of 5.0 ml of 0.25 *M* sucrose-0.02 *M* Tris, pH 7.4. The amount of oleate shown was added, and the reaction followed at 20°C as shown in Fig. 2

The minimum concentrations of oleic acid are found to be 0.04 *mM* in KCl solution but 0.1 *mM* in the sucrose solution as shown by LEHNINGER<sup>5</sup>. He<sup>6</sup> reported that the swollen mitochondria induced by various swelling agents can be recontracted by  $Mg^{++}$ , BSA and ATP. This recontraction of mitochondria is controlled by the presence or absence of contracting factor<sup>10</sup> on mitochondria. After the contracting factor is reduced by some swelling agents such as G-SH, cystein and Co A, recontraction does not occur by adding  $Mg^{++}$ , BSA and ATP. In the case of fatty acid induced swelling, however, the recontraction does occur and also the fatty acid induced swelling is inhibited by BSA and ATP. (Fig. 4)

In 1957 COOPER<sup>11</sup> reported the rate of swelling varies due to tissues which mitochondria are prepared from, and ARCOS<sup>12,13</sup> in 1960 stated that rat liver mitochondrial swelling induced by various agents was reduced in the process of

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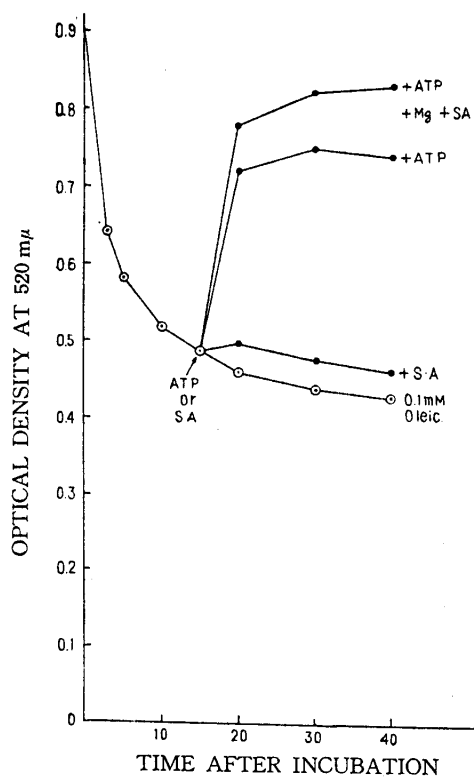


Fig. 4. Requirements for reversal of swelling induced by sodium oleate. The incubation mixture contained of 5.0 ml of 0.15 *M* KCl- 0.02 *M* Tris (pH 7.4), 0.1 *mM* sodium oleate and 100 mg tissue equivalent mitochondria. Additions were 5 *mM* ATP, 5 *mM* MgCl<sub>2</sub> and 3 mg per ml of bovine serum albumin. The reaction followed at 20°C.

carcinogenesis by DAB. In the present experiments the swelling action of oleic acid on various cell mitochondria was examined and the data were shown in Fig. 5. The swelling action is found to decrease in the order of the mitochondria from mouse liver, Ehrlich ascites tumor bearing mouse liver, Ehrlich ascites tumor cells and solid tumor of Ehrlich. The order of mitochondrial swelling seems to be parallel to their respiratory activity of each cell. In this respect, the inhibition of swelling of rat liver mitochondrial induced by various respiratory substrates has been examined using respiratory inhibitors such as amytal, azide, antimycin A, KCN, and anaerobiosis have been confirmed to inhibit the substrate inducing swelling (Table 2), which agrees with the reports of many investigators<sup>14,15</sup>. Oleic acid inducing swelling of rat liver mitochondria is also inhibited by anaerobiosis and by the respiratory inhibitors except amytal.



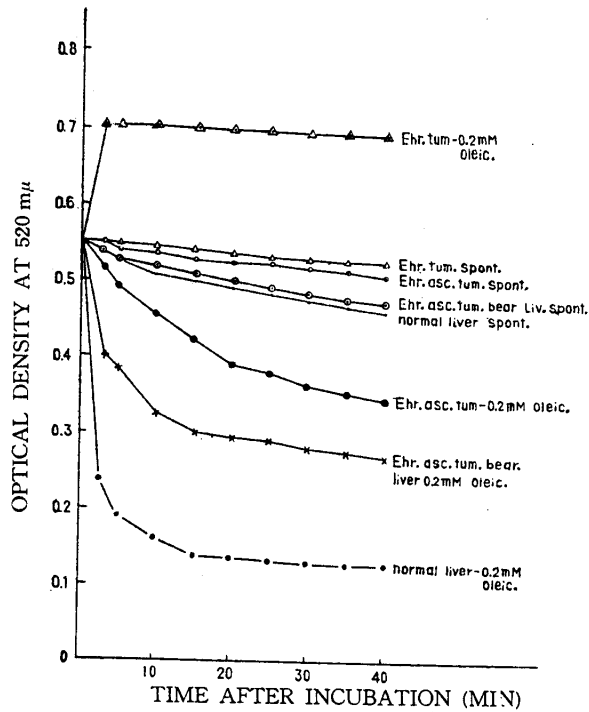


Fig. 5. Effect of sodium oleate (0.2 mM) on the mitochondrial swelling of mouse liver (50 mg), Ehrlich tumor bearing mouse liver (50 mg) and Ehrlich ascites tumor cells (250 mg) (solid and ascites). The details as in Fig. 2.

Table 2. Effects of respiratory inhibitors on the swelling of mitochondria by various substrates. The medium consisted of 0.15 M KCl-0.02 M Tris, pH 7.4 and 50 mg tissue equivalent rat liver mitochondria. Substrates and inhibitors present at zero time and incubated at 20°C 30 minutes. The data indicate the rate of absorbancy change to initial absorbancy at 520 mμ.

Substrate	Non	Amytal (1×10 <sup>-3</sup> M)	Ant. A (10γ/5ml)	Azide (5×10 <sup>-3</sup> M)	CN <sup>-</sup> (10 <sup>-3</sup> M)	Anaerob.
Non	89.0	79.5 (A)	89.0	92.0 (I)	92.0 (I)	
B-OH (3×10 <sup>-3</sup> )*	73.1 (A)	95.2 (I)	79.5 (I)	88.5 (I)	86.2 (I)	
Succinate (∞)	85.2 (A)	86.7 (I)	98.0 (I)	96.2 (I)	85.0	
Glutam. (∞)	70.0 (A)	74.2 (I)	80.8 (I)	83.6 (I)	77.0 (I)	
α-KG* (∞)	64.9 (A)		81.3 (I)			
Oleate(8×10 <sup>-5</sup> M)	26.1 (A)	16.0 (A)	28.2 (I)	41.2 (I)	27.6 (I)	29.5 (I)

\* B-OH...β hydroxybutylate α-KG...α-ketoglutarate A...Activation I...Inhibition

*Effect of fatty acid on the oxidative phosphorylation of mitochondria:*  
As has been reported previously<sup>19</sup>, the uncoupling oxidative phosphorylation of the cell is brought about by fatty acids and it also occurs in mitochondria, i. e.

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the respiratory activity of mitochondria is increased by the addition of 0.04 mM of sodium oleate but incorporation of  $^{32}\text{P}$  into  $\Delta 10\text{P}$  fraction of mitochondria is inhibited severely as shown in Fig. 6 and Table 3. Mitochondrial respiration is released in the presence of oleic acid but after the lapse of 15 minutes at  $38^\circ\text{C}$ , the fall off is observed by the denaturation of mitochondria as shown in the cell level experiment<sup>19</sup>. This phenomenon is observable only in the intact mitochondria having the respiratory control<sup>34</sup>.

By oxygraphic measurement of respiration and oxidative phosphorylation,

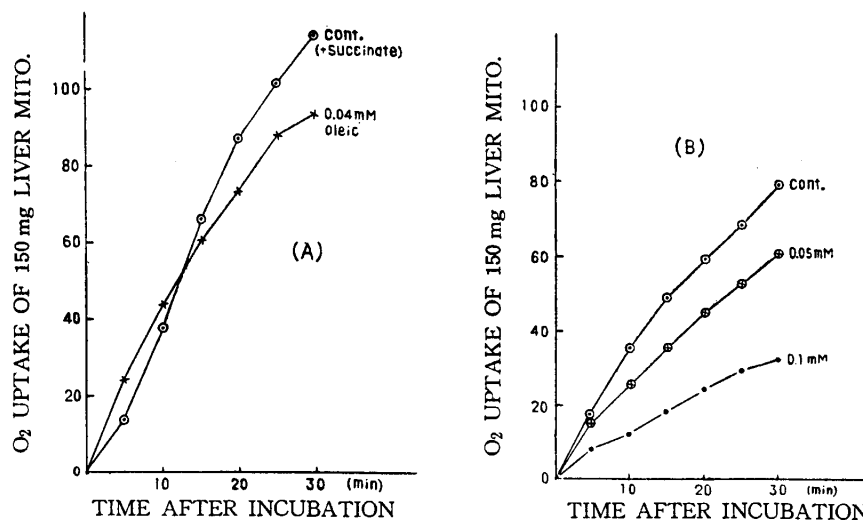


Fig. 6. Effect of sodium oleate on the respiration of rat liver mitochondria. Detail of incubation system as in the text.

(A) mitochondria was aged 3 hours at  $0^\circ\text{C}$  in 0.25 M sucrose solution.

(B) mitochondria was aged 3 hours at  $0^\circ\text{C}$  in 0.15 M KCl solution.

Table 3. Effect of sodium oleate on the  $^{32}\text{P}$  incorporation into  $\Delta 10\text{P}$  fraction of rat liver mitochondria. Detail of incubation mixture as in the text.

	Ratio of RA of Pi	Ratio of RA of $\Delta 10\text{P}$	Ratio of SA of Pi	Ratio of SA of $10\text{P}$
Control	100	100	100	100
0.4 mM oleate	82.5	5.9	106	4.9
0.1 mM oleate	65.7	38.9	92	83.4

RA: relative activity      SA: specific activity

the stimulated respiration under the existence of succinate and uncoupling phosphorylation by adding of fatty acids are observed (Table 4 and Fig. 7). Parallel relationship can be observed between uncoupling oxidative phosphorylation and swelling action of mitochondria. (Fig. 1 and Fig. 7)

Table 4. Effect of various fatty acids (sodium salts) on the respiration and oxidative phosphorylation of rat liver mitochondria. Succinate level oxygen consumption and oxidative phosphorylation are expressed by  $m\mu$  atom/min/100 mg tissue equivalent of mitochondria and P/O before and after the treatment of fatty acid (0.05 mM) by the method of oxygraphy.

Reagent (0.05mM)	Before treatment		After treatment	
	O <sub>2</sub> consumption ( $m\mu$ atom)	P/O	O <sub>2</sub> consumption ( $m\mu$ atom)	P/O
Lauric acid	30.0	1.54	45.0	1.05
Myristic acid	30.0	1.54	50.0	0.02
Palmitic acid	30.0	2.00	27.5	1.67
Stearic acid	30.0	1.67	15.0	1.54
Behenic acid	27.5	1.82	17.5	1.82
Oleic acid	30.0	1.67	50.0	0.01
Eleidic acid	30.0	1.82	32.5	1.54

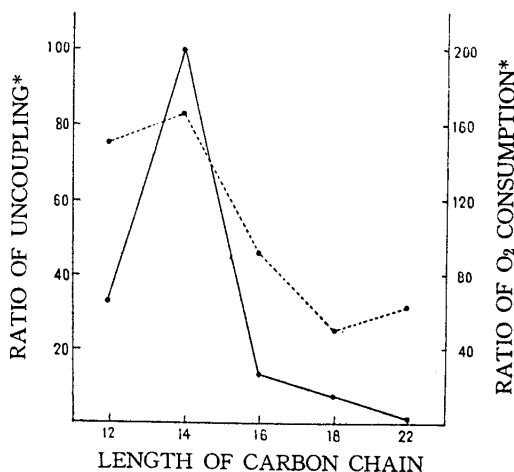


Fig. 7. Effect of various saturated fatty acids (sodium salts) on the respiration and phosphorylation of rat liver mitochondria. The medium contained as described in the text. (0.08 mM of sodium oleate and 100 mg tissue equivalent of mitochondria).

\* Ratio of uncoupling .....  $\left( 1 - \frac{p/o \text{ of after treatment}}{p/o \text{ of before treatment}} \right) \times 100$  -----

\* Ratio of O<sub>2</sub> consumption .....  $\frac{O_2 \text{ up take of after treatment}}{O_2 \text{ up take of before treatment}} \times 100$  .....

*The inhibition of <sup>32</sup>P incorporation into Δ10P fraction of mitochondria by sodium oleate*: From the data of uncoupling oxidative phosphorylation by fatty acid, the effect of oleic acid on the <sup>32</sup>P incorporation into acid soluble organic phosphate compounds is examined to reveal which fraction

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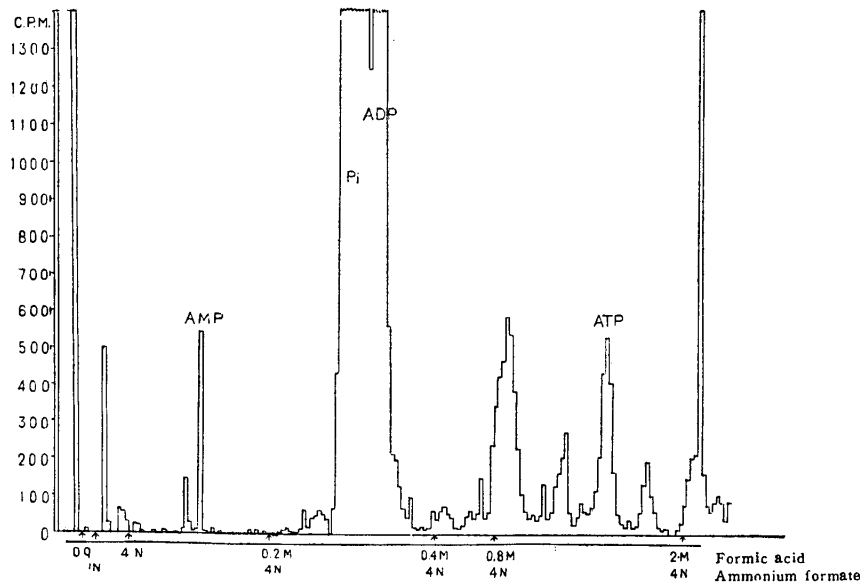


Fig. 8. Incorporation of  $^{32}\text{P}$  into acid soluble phosphorous compounds of rat liver mitochondria (500 mg) after incubation 30 minutes at  $25^\circ\text{C}$ . The incubation mixture consisted with 3 ml of mitochondrial suspension, 1 ml of KRP solution (containing  $100\ \mu\text{c}$  of  $^{32}\text{P}$ ), 1.0 ml of 0.2M sodium succinate and 5.0 ml of 0.15 M KCl-0.02M Tris buffer solution (pH 7.4).

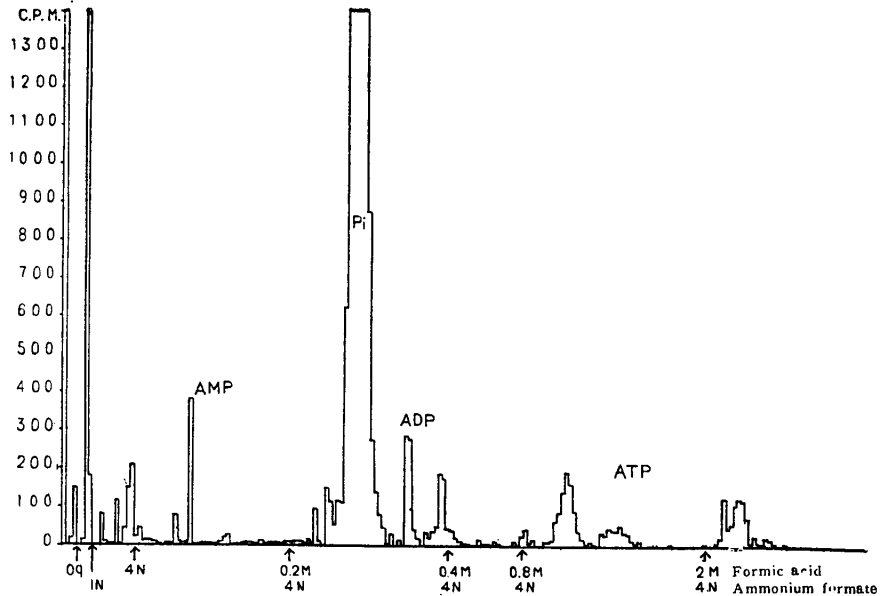


Fig. 9. The Effect of sodium oleate on the incorporation of  $^{32}\text{P}$  into acid soluble phosphorous compounds of rat liver mitochondria. Incubation mixture containing 0.5 mM sodium oleate.

is suppressed by fatty acid in the mitochondria. As shown in Figs. 8 and 9 the typical inhibition of  $^{32}\text{P}$  incorporation into ATP, ADP, GTP and UDPG are observed.

#### DISCUSSION

The mitochondria as the energy producing machine in the cell have been studied by many authors about the relationship between the structure and function. These experiments have drawn attention of many investigators on metabolic control mechanism related to the morphological changes of mitochondria. In this respect interesting results are reported<sup>2,5,6,14-25</sup> concerning the relation among the respiration, oxidative phosphorylation and swelling of mitochondria, especially noteworthy one is the recent report of PACKER<sup>4</sup> in which he found that the swelling-shrinkage of cancer cell is altered with the initial burst of respiration by adding of glucose (Crabtree effect). Namely, the metabolic control may be regulated by permeability of mitochondria according to swelling and shrinkage, which is caused by ADP, inorganic phosphate (Pi) and electron transport.

It is important to study the effect of fatty acid on the mitochondrial function on the following reasons: the fatty acid acts as uncoupler, swelling agent and stimulator of latent ATPase activity. In this experiment it has been clarified that the extent of uncoupling action is parallel to that of mitochondrial swelling action of fatty acid as a function of the carbon chain length. This finding is similar to the stimulation of latent ATPase activity by various fatty acids as reported by PRESSMAN and LARDY<sup>4</sup> and to the surface activity of each fatty acid. The above mentioned data suggest that the effect of fatty acid on the mitochondrial function could be decided by solubility of the acid into the lipoprotein of membrane structure and by damage to the functional structure of membrane. Thus the swelling of mitochondria could be induced. The swelling of mitochondria, therefore, means the structural change of membrane and will induce the loosened or uncoupled oxidative phosphorylation. It also means the stimulation of latent ATPase activity, which is a reversal process of the equation of phosphorylation proposed by LEHNINGER<sup>25</sup> and others. Even the swelling action is parallel to the uncoupling of mitochondria, an attention may be called on the difference in the incubation mixture in these cases: i. e. the swelling test is examined in the KCl solution using the mitochondria washed 4 times and uncoupling test is in the sucrose solution using the mitochondria after a single washing. When the swelling test is conducted in the sucrose solution with the mitochondria washed once, contraction occurs rather than swelling by adding of fatty acids. This fatty acid inducing contraction will be reported in a later paper. The medium used for testing the intensity of swelling and uncoupling, however, are

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more effective than the sucrose medium. Moreover, the true P/O ratio would not be estimated by the method of oxygraphy because the difference between the added exogenous ADP and the endogenous ADP formed by stimulation of latent ATP-ase activity by fatty acid are in distinguishable. The experiments are being conducting to test whether the stimulation of ATPase activity or uncoupling action by fatty acids cause the decrease of P/O ratio.

The mitochondria swollen by various swelling agents are recontracted reversibly by ATP,  $Mg^{++}$  and bovine serum albumin. This finding suggests that mitochondria contain the mechanoprotein as actomyosin in muscle. To support this idea OHNISHI<sup>26</sup> found in 1962 the actomyosin-, actin- and myosin-like proteins extracted from mitochondria and clarified that these proteins show cross reactions with actin and myosin prepared from skeletal muscle. In 1960 RACKER<sup>27</sup> also reported the coupling factor containing the latent ATPase activity. The mitochondrial membrane consists of structural protein, lipids and elementary particles<sup>8</sup>. From these findings it may be assumed that the mitochondrial structural protein contains the mechanoprotein may be orientated in some arrangement to an easily contractable state.

CORWIN<sup>14</sup> and others reported that respiratory inhibitors showed inhibitory action on the mitochondrial swelling induced by respiratory substrates, and the same results were likewise observed in this experiment. This means that the electron transport is one of the factors inducing the swelling of intact mitochondria. The inhibitory actions of azide and anaerobiosis on mitochondrial swelling induced by fatty acid suggest that the fatty acid may play a role as the respiratory substrate.

Cancer cell mitochondria showing low rate of swelling may be consisted of low saturated fatty acid as lipid component<sup>29,30</sup>. One of the physical properties of the lipoprotein is that it controls the rate of the swelling of mitochondria. These findings may be correlated to the regulation mechanism of cancer cell metabolism.

Recently a reversible uncoupling of oxidative phosphorylation has been demonstrated by SLATER<sup>20,18</sup>, HULSMANN<sup>18</sup> and PRESSMAN<sup>4</sup>: uncoupled of oxidative phosphorylation by oleic acid is reversed by the addition of serum albumin. These phenomena were also observed by our group (unpublished) but only at a low concentration of fatty acid. The mechanism of the reversible uncoupling reaction can be explained by the binding of serum albumin with fatty acid. In view of the reversible uncoupling concerned with the regulation of cell metabolism, unsaturated fatty acids are considered to be the regulator of cell metabolism in natural system. Namely, the usual endogenous uncoupling factor or endogenous respiratory inhibitor contains some isoctane soluble fatty acids perhaps bounded to protein as cytoplasmic component as an inactive form. Then the regulation mechanism of cancer cell metabolism, differing from normal

one, may be aroused by the endogenous uncoupling factor and according to the data of specific lipid metabolism of cancer cells<sup>31,2</sup>.

#### SUMMARY

The effect of various fatty acids on the swelling-contraction and oxidative phosphorylation of mitochondria from rat liver and Ehrlich ascites tumor cell have been studied and the results are as follows :

1. The swelling of rat liver mitochondria is induced by fatty acid. The extent of this uncoupling action is in the descending order of myristate, laurate, palmitate, stearate and behenate in saturated fatty acid and linoleate, linolenate, richinoleate and oleate in the unsaturated fatty acid. This swelling action is stronger with unsaturated fatty acids than that of saturated ones and *cis* form is stronger than *trans* form.

2. The uncoupling oxidative phosphorylation of rat liver mitochondria is also observed with these fatty acids and the activities are proportional to the degree of the swelling action.

3. The degree of swelling of rat liver mitochondria is proportional to the concentration of oleate and is inhibited by anaerobiosis and respiratory inhibitor except amytal.

4. The mitochondria swollen by fatty acid can be recontracted reversibly by ATP,  $Mg^{++}$  and bovine serum albumin.

5. The swelling action of sodium oleate is the strongest on mitochondria from rat liver, followed by those from the liver of Ehrlich ascites tumor bearing mouse, Ehrlich ascites tumor cells and solid Ehrlich tumor cells.

6. Sodium oleate inhibits the incorporation of  $^{32}P$  into ATP, ADP, GTP and UDPG in mitochondria.

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