*Acta Medica Okayama*

*Volume* 17, *Issue* 6 1963 *Article* 1

1963

# Relation between mitochondrial swelling induced by inorganic phosphate and accumulation of $P^{32}$ in mitochondrial Pi fraction

Kozo Utsumi*

*Okayama University,

# Relation between mitochondrial swelling induced by inorganic phosphate and accumulation of $P^{32}$ in mitochondrial Pi fraction*

Kozo Utsumi

**Abstract**

1. Rat liver mitochondria are swollen by inorganic phosphate in the medium of slightly hypotonic sucrose solution containing respiratory substrate and the mitochondrial swelling is inhibited or turned to shrink by ADP, respiratory inhibitor, anaerobiosis and uncoupler of oxidative phosphorylation. This mitochondrial swelling is not inhibited by the inhibitor of phosphorylating respiration such as oligomycin and tributyltin chloride. 2. Rat liver mitochondria are swollen by ATP in the presence of antimycin A, inorganic phosphate and 0.1 mM of CaCl2 and such a swelling is inhibited by oligomycin. 3. Accumulation of a small amont of $P^{32}$ in acid soluble Pi fraction of rat liver mitochondria proceeds even in the medium containing neither ATP nor Ca++ but is inhibited by respiratory inhibitor, ATP, ADP and uncoupler of oxidative phosphorylation. The accumulation of $P^{32}$ in mitochondria, however, is not inhibited by oligomycin. 4. The accumulation of $P^{32}$ is induced by ATP in the presence of antimycin A and Ca++(O.l mM) and such an accumulation of $P^{32}$ is inhibited by oligomycin. 5. It is suggested that the Pi-induced swelling of mitochondria is correlated to the accumulation of inorganic phosphate and both of them are tightly coupled to the initial step in the process of oxidative phosphoryaltion.

*PMID: 14168970 [PubMed - indexed for MEDLINE] 

Acta Med. Okayama 17, 259—271 (1963)

# RELATION BETWEEN MITOCHONDRIAL SWELLING INDUCED BY INORGANIC PHOSPHATE AND ACCUMULATION OF $P^{32}$ IN MITOCHONDRIAL *Pi* FRACTION

Kozo UTSUMI

*Department of Biochemistry, Cancer Institute of Okayama University Medical School, Okayama*

*Recieved for publication, November 25, 1963*

Studies on the swelling-shrinkage of the isolated mitochondria are of a great help in the elucidation of the mechanism of oxidative phosphorylation. The mitochondria suspended in the medium of KCl- or sucrose-Tris HCl buffer (pH 7.4) are swollen by various agents[1-11] and the swelling is inhibited by some respiratory inhibitors[6,11-16]. The facts alluded that the swelling of mitochondria is closely correlated to electron transport. Recently PACKER[13,14,17,18], UTSUMI *et al.*[37] and CORWIN *et al.*[12] found that the swelling-shrinkage of mitochondria is closely correlated to the oxidative phosphorylation and suggested that the state of shrinkage or swelling of mitochondria can be determined by the availability of high energy intermediates. In some cases, it is recognized that the swelling of isolated mitochondria is brought about by the active or passive transport of salt ions of surrounding medium[3,19,20]. The active transports of inorganic phosphate (Pi) and calcium ion ($Ca^{++}$), which correlate the high energy intermediate of oxidative phosphorylation, were clarified by BRIERLEY *et al.*[21,22], VASINGTON *et al.*[23,24] and LEHNINGER *et al.*[25,26]. In this case the transported phosphate accumulated in a form of calcium phosphate and magnesium phosphate. Then the accumulation of ions are inhibited by respiratory inhibitors or uncouplers of oxidative phosphorylation. These experiments suggest the possibility that the reversible changes of mitochondrial volume would be due to the active transport of ions into mitochondria from surrounding medium.

Thus, the studies have been carried out on the relationships among the reversible swelling-shrinkage, respiration, phosphorylation of rat liver mitochondria and accumulation of Pi in mitochondria.

## MATERIALS AND METHODS

### *Materials :*

Rat liver mitochondria were isolated by the modified method of Hogeboom's[27] using the homogenizing medium composed of 0.25 M sucrose solution containing 40 $\mu$M EDTA and 50 $\mu$M Tris-HCl buffer (pH 7.4). After decapita-

tion the rat liver was homogenized in ten volumes of homogenizing medium for 2 minutes at 0-4 °C by Potter-Elvehjem glass homogenizer and teflon homogenizer, and then centrifuged at 50x g for 7 minutes to eliminate the cell and nucleus. The supernatant of this homogenate was superimposed to equal volume of 0.34 M sucrose solution containing 40 $\mu$M of EDTA and 50 $\mu$M of Tris-HCl buffer (pH 7.4) and centrifuged at 700x g for ten minutes to separate all the nuclear fraction. After centrifugation of the supernatant at 5,000x g for ten minutes, opalescent supernatant and pink partially sedimented layer were discarded. The precipitate, mitochondria, were washed twice with the homogenizing medium (over 50 volumes of the precipitate). The 1 g tissue equivalent mitochondria were suspended in 1 ml of 0.25 M sucrose solution containing 50 $\mu$M Tris-HCl buffer (pH 7.4) as a stock mitochondrial suspension.

Adenosine-5'-diphosphate (ADP), adenosine triphosphate (ATP) and antimycin A were obtained from Sigma Chemical Co. Tributyltin chloride (TBTC) was donated by Prof. HAGIHARA (University of Osaka) and oligomycin by Dr. MINAKAMI (University of Tokyo).

*Measurements :*

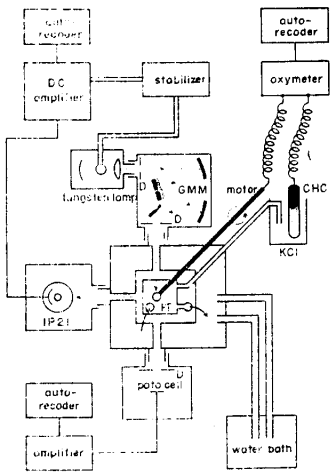



Fig. 1 Diagrammatic drawing of the apparatus of 90° light-scattering containing oxygen electrode. GMM; grating mirror monochromator, 1P21; phototube, D; diaphragm, Pt; rotating platinum electrode, motor; synchronous motor, CHC: calomel half cell, oxymeter: oxymeter constructed by the method of HAGIHARA[28].

The volume changes of mitochondria were measured by the absorption at 520 m$\mu$ and 90° light-scattering at 520 m$\mu$ with the apparatus designed and constructed by the author and were recorded by the autorecorders as illustrated in Fig. 1 and Photograph 1. The relationships between the changes of wet weight and absorption at 520 m$\mu$ of mitochondria are shown in Table 1. The change of 0.1 optical density at 520 m$\mu$ corresponded to the change of 61 $\mu$moles of water per mg protein of mitochondria. Moreover, the changes of 90° light-scattering of mitochondria were proportional to the changes of these absorption at 520 m$\mu$ and the former could be amplified easily to arbitrary unit. Therefore, very small changes of mitochondrial volume related to the physiological function could be detected by the changes of 90° light-scattering (Fig. 2). Oxygen consumption and oxidative phosphorylation were measured by rotating platinum electrode by a modified method of HAGIHARA[20] in the cell of

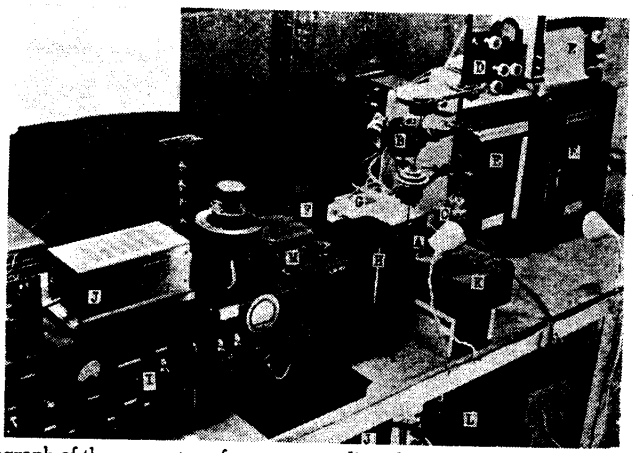


Photo. 1 Photograph of the apparatus of an autorecording electrophotcmeter and 90° light-scattering photometer with oxygen electrode. A; cell, B; synchronous motor, C; calomel half cell, D; oxymeter, E; autorecoder, F; tungsten lamp, G; grating mirror monochromator, H; phototube, I; DC amplifier, J; stabilizer, K; photo-cell, L; water bath.

Table 1 Relationships between the optical density and wet weight of mitochondria.

Rat liver mitochondria (0.5 g tissue equivalent) were added to 50 ml of 0.15 M KCl-0,02 M Tris buffer (pH 7.4) containing Na-oleate ranging from 0,001 to 0.005 per cent and were incubated at 25°C for 40 minutes. The changes of optical density after incubation at 520 mμ were measured using 1.0 cm cell. The mitochondria were collected by centrifugation and wet weight and dry weight were determined.

| Condition | Optical density at 520 mu | Wet weight (mg) | Dry weight (per cent) | Absorbed water (μmoles) |
|---|---|---|---|---|
| Initial | 1.352 | 31.5 | 27.9 | |
| Swollen by oleate (0.001%) | 1.120 | 40.0 | 17.8 | 455.0 |
| 〃 (0.002%) | 1.012 | 43.1 | 14.8 | 667.0 |
| 〃 (0.003%) | 0.920 | 48.0 | 13.7 | 828.0 |
| 〃 (0.004%) | 0.700 | 54.9 | 10.2 | 1370.0 |
| 〃 (0.005%) | 0.650 | 57.5 | 9.5 | 1525.0 |

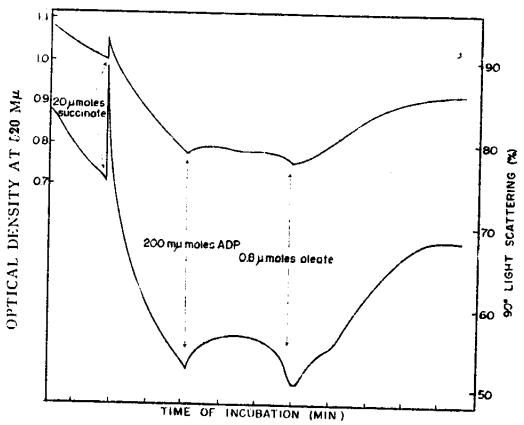



Fig. 2 Relation between the changes of optical absorption at 520 mμ and changes of 90° light-scattering of mitochondria. Mitochondria were incubated in 2 ml of 0,05 M sucrose, containing 40 μmoles KCl, 40 μmoles K-phosphate buffer (pH 7.4) and 80 mμmoles EDTA at 25°C. Arrows show the addition of reagents, Upper curve, optical density at 520 mμ. Lower curve, per cent of 90° light-scattering. An upward deflection of the trace corresponds to shrinkage and the downward to swelling.

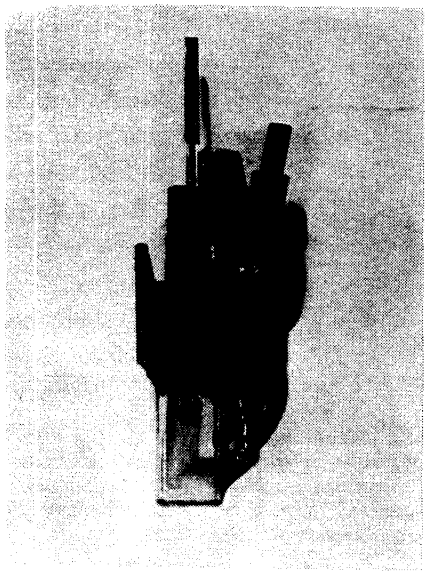


Photo. 2 Photograph of a cell for the measurements of extinction, 90° light-scattering and oxygen consumption of mitochondria.

this apparatus (Photo. 2). The total volume of the cell was 2 ml. Then the swelling-shrinkage and oxygen consumption of mitochondria (about 1.5 mg protein) were recorded simultaneously by autorecorders in the medium consisted of 0.1 —0.05 M sucrose, 20 mM KCl, 5 mM Tris-HCl buffer (pH 7.4), and 40 $\mu$M EDTA in final volume of 2 ml. The details of respective experimental conditions were given in text and legends to figures and tables.

*Accumulation of inorganic phosphate*

The accumlation of $P^{32}$ in mitochondrial Pi fraction was estimated by the method of AZZON and ERNSTER[29]. Incubation mixture contained the following substances at the designated concentration in total volume of 3 ml: 0.1 M sucrose, 10 mM Tris-HCl buffer (pH 7.4), 20 mM KCl, 3 mM Na-succinate, 1 mM $MgCl_2$, 3 mM K-phosphate buffer (pH 7.4 containing 10 $\mu$c of $P^{32}$) and about 4 mg protein of mitochondria. Incubation was carried out at 25°C with shaking. After incubation, 1 ml aliquots of reaction mixture were removed and rapidly filtered by suction through a pad of celite held on filter paper of funnel to collect the mitochondria. The celite retained mitochondria were washed twice immediately under suction with 1 ml portions of ice cold incubation mixture without Pi. The celite pad was extracted with 8 per cent of perchloric acid and the amount and the radioactivity of inorganic phosphate on the extract were measured by the method of TAKAHASHI[30]. Mitochondrial protein was determined by the method of KJELDAHL[41].

## RESULTS

*Relation between the swelling-shrinkage and oxidative phosphorylation:*

In the medium of 0.05 M sucrose, 20 mM KCl, 20 mM K-phosphate buffer (pH 7.4) and 40 $\mu$M EDTA, the rat liver mitochondria consumed the oxygen about 5 $\mu$M/minute/mg protein of mitochondria and showed a small degree of swelling. By addition of 10 mM Na-succinate (state 4[31]) the respiration was increased (10 $\mu$M oxygen/minute) and the accelerated swelling was observed followed by momentary shrinkage of mitochondria (Fig. 3). By adding ADP

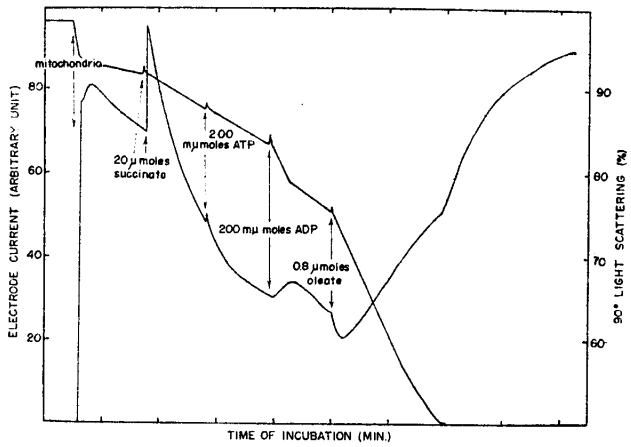



Fig. 3 Swelling and shrinkage changes of rat liver mitochondria accompaning the exhausion of oxygen in various states of mitochondria. Rat liver mitochondria (1.8 mg protein) incubated in 2 ml of 0,05 M sucrose containing 40 μmoles KCl, 40 μmoles K-phosphate and 80 mμmoles EDTA at 25°C. The upper trace refers to the oxygen concentration in the medium and the lower trace to the swelling-shrinkage by 90° light-scattering. Arrows show the addition of substances.

(state 3[31]) the respiration was increased (about 40 μM oxygen/minute) and the mitochondrial shrinkage occured. But by reversing to state 4 after phosphorylation of ADP to ATP, the respiration was decreased (about 13 μM oxygen/minute)

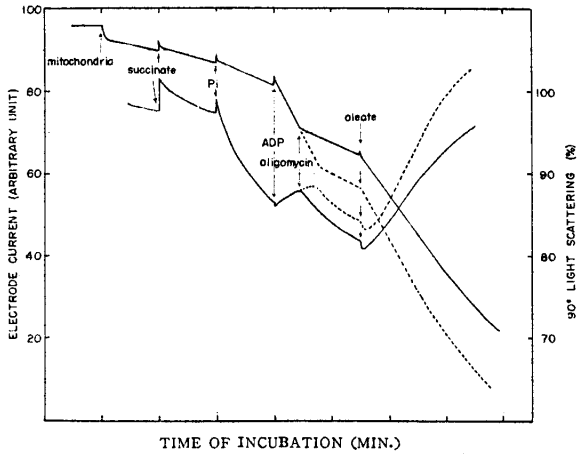
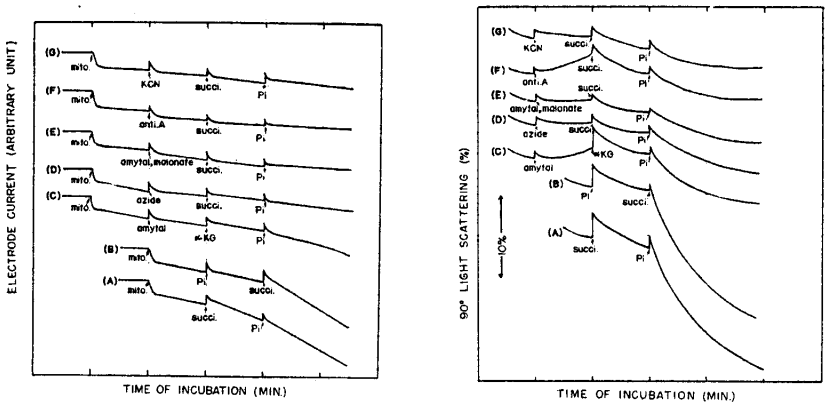



Fig. 4 Effect of oligomycin on the mitochondrial swelling at state 3. Rat liver mitochondria (1.9 mg protein) were incubated in 2 ml of 0.1 M sucrose containing 40 μmoles KCl, 80 mμmoles EDTA, and 10 μmoles Tris-HCl buffer (pH 7.4) at 25°C. The amount of additions were as follows: Na-succinate; 6 μmoles, K-phosphate; 4 μmoles, ADP; 400 mμmoles, oligomycin; 5.6 μg, and Na-oleate; 0.4 μmoles. Other conditions were as shown in Fig. 3. Dotted lines show the oxygram and 90° light-scattering by no abbition of oligomycin.

and swelling again occured. In this state, the mitochondrial swelling was stopped or turned to shrinkage by the addition of respiratory inhibitors such as antimycin A (2.5 $\mu$g/ml), KCN (1 mM), malonate (1 mM) and azide (1 mM), and uncouplers of oxidative phosphorylation such as oleic acid (0.4 mM)[16,32,33] and 2,4-dinitrophenol (DNP, 10 $\mu$M)[34] as shown in Fig. 3 but not by thyroxine (0.1 mM) and arsenate (1 mM). This type of shrinkage was observed by anaerobiosis. Of course, the degree of mitochondrial shrinkage induced by oleic acid was varied extensively by the amount of oleic acid. This swelling-shrinkage was slightly inhibited by ATP (3 mM). In this instance, the mitochondrial shrinkage did not occur by adding the inhibitors of phosphorylating respiration such as oligomycin[34] and TBTC[3] (Fig. 4).

*Mitochondrial swelling by inorganic phosphate :*

Mitochondria were swollen by Pi without decrease of the ability for phosphorylation (Figs. 5 A and 6 A). In the medium of 0.1 M sucrose, 20 mM KCl,




Figs. 5 and 6 Effects of Na-succinate, $\alpha$-ketoglutarate ($\alpha$-KG) and respiratory inhibitors such as amytal, azide, malonate, antimycin A and KCN on the Pi-induced swelling of rat liver mitochondria. Fig. 5 shows the oxygen consumption and 6 shows the 90° light-scattering of mitochondria. The amounts of additions were as follows: Na-succinate and $\alpha$-KG; 6 $\mu$moles, amytal; 4 $\mu$moles, azide; 3 $\mu$molea, malonate; 6$\mu$moles, antimycin A; 4 $\mu$g and KCN; 2 $\mu$moles. Other conditions were same as in Fig. 4.

5 mM Tris-HCl buffer (pH 7.4) and 40 $\mu$M EDTA, rat liver mitochondria showed a small degree of swelling and of respiration, and anaccelerated swelling and respiration were observed by adding of Na-succinate (3 mM) followed by addion of Pi (2 mM) without change of mitochondrial ability for phosphorylation (Figs. 5 B and 6 B). Moreover, even under the presence of respiratory substrate in the medium, the Pi induced swelling was reduced or inhibited by respiratory inhibitors such as antimycin A, KCN, malonate, azide and amytal

(Figs. 5 C, D, E, F, and G and 6 C, D, E, F, and G) or uncouplers of oxidative phosphorylation, oleic acid and DNP (Fig. 7 A and B), but not by the inhibitors of phosphorylating respiration such as oligomycin and TBTC (Fig. 8).

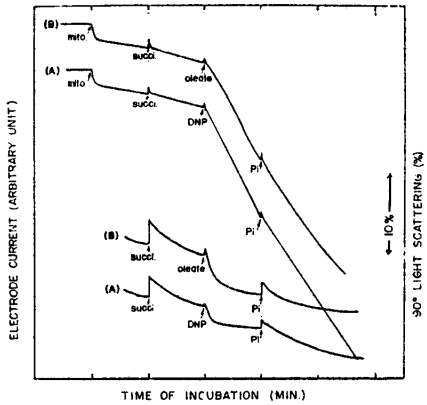



Fig. 7 Effect of uncoupler of oxidative phosphorylation on the mitochondrial swelling induced by Pi. The upper trace refers to the oxygen consumption and the lower one to the swelling-shrinkage by 90° light-scattering. The amounts of additions were as follows: DNP; 68 mμmoles, Na-oleate; 0.6 μmoles. Other conditions were same as in Fig. 4.

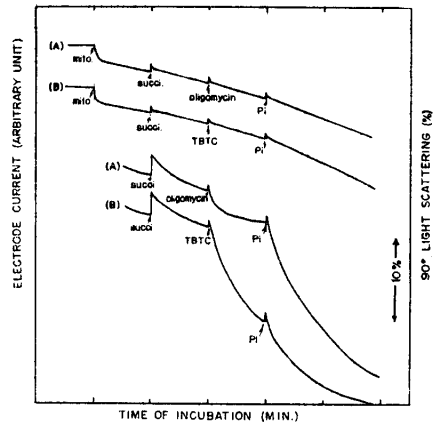
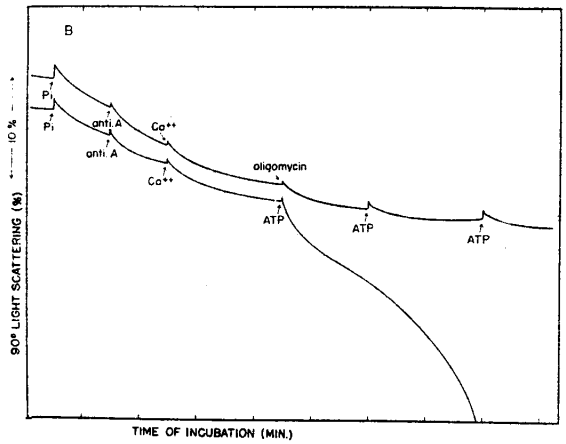



Fig. 8 Fffect of the inhibitor of phosphorylating respiration on the Pi-induced swelling of mitochondria. The amounts of additions were as follows: oligomycin; 5.6 μg, TBTC; 1.4 mμmoles. The upper trace refers to the oxygen consumption and lower trace to the 90° light-scattering. Other conditions were same as in Fig. 4.




Eig. 9 Swelling of rat liver mitochondria induced by ATP in the presence of Pi, $Ca^{++}$ and antimycin A and its inhibition by oligomycin. The mitochondria were incubated in 2 ml of 0.1 M sucrose solution containing 40 μmoles KCl, 80 mμmoles EDTA, 2 μmoles $MgCl_2$ and 10 μmoles Tris-HCl buffer (pH 7.4) at 25°C. The amounts of additions were as follows: Pi; 2 μmoles, antimycin A; 4 μg, $CaCl_2$; 0.2 μmoles, oligomycin; 5.6 μg and ATP; 6 μmoles. Other conditions were same as in Fig. 4.

Namely the Pi induced swelling correlated the phosphorylating intermediate related to the initial step of oxidative phosphorylation. The Pi induced swelling was accelerated by adding $Ca^{++}$ (0.1 mM). This swelling was drastic (adout 70 per cent decrease in scattering) and the swollen mitochondria lost the ability for oxidative phosphorylation. In the presence of antimycin A in the medium containing $Ca^{++}$ (0.1 mM) the Pi induced swelling was not brought about but did occur by addition of ATP (3 mM). This Pi induced swelling by ATP was inhibited by inhibitor of phosphorylating respiration such as oligomycin and TBTC as shown in Fig. 9.

*Accumulation of $P^{32}$ in mitochondria :*

Table 2 shows the results of Pi accumulation in Pi fraction of mitochondria

Table 2 Requirement for Pi-accumulation by rat liver mitochondria.
Rat livermitochondria (4.3 mg protein) were incubated in 3 ml of 0.1 M sucrose containing 30 μmoles of Tris-HCl buffer (pH 7.4), 9 μmoles of Na-succinate, 3 μmoles of $MgCl_2$, 9 μmoles of K-phosphate buffer (pH 7.4 containing 10 μc of $P^{32}$). Incubation was carried out at 25°C. Other additions are described in table. Counts of accumulated $P^{32}$ into mitochondrial Pi fraction were measured by the method of AZZONE and ERNSTER[29] and TAKAHASHI[30].

| Incubation system addition (+) or omission (−) | Incubation time (min.) | Counts of $^{32}P$ accumulated in mitochondria (c. p. m.) | Pi content of mitochondria (mμmoles/mg protein) |
|---|---|---|---|
| Complete | 1 | 1805 | — |
| 〃 | 3 | 2597 | 150 |
| 〃 | 10 | 2746 | — |
| 〃 | 20 | 2688 | — |
| −$Mg^{++}$ | 3 | 1370 | 150 |
| −succinate | 3 | 1986 | 70 |
| +DNP (90 mμmoles) | 3 | 371 | 70 |
| +antimycin A (2μg/ml) | 3 | 611 | 93 |
| +KCN (3 μmoles) | 3 | 872 | 70 |
| +oligomyein (2. 8μg/ml) | 3 | 2946 | 190 |
| +ATP (9 μmoles) | 3 | 1643 | 100 |
| + ADP (9 μmoles) | 3 | 1117 | 115 |

under the various conditions. In the medium of 0.1 M sucrose, 10 mM Tris-HCl buffer (pH 7.4), 1 mM $MgCl_2$, 20 mM KCl, 3 mM Na-succinate and 3 mM K-phosphate (pH 7.4 containing 10 μc of $P^{32}$), the amount of accumulated $P^{32}$ in mitochondria was slightly reduced by omitting the $Mg^{++}$ or succinate and was not changed by the incubation over 3 minutes. But it was inhibited by addition of DNP, KCN or antimycin A and was not inhibited but increased by oligomycin or TBTC. In the presence of ATP and or ADP in the medium

the Pi accumulation was decreased. The Pi accumulation was accelerated by $Ca^{++}$ (0.1 mM) as indicated in Table 3. In this case Pi accumulation was also

Table 3 Accumulation of $P^{32}$ in Pi fraction of rat liver mitochondria by ATP in the presence of $Ca^{++}$ and antimycin A and its inhibition by oligomycin. The mitochondria (4.8 mg protein) were incubated in 3 ml of 0.1 M sucrose solution containing 60 μmoles of KCl, 30 μmoles of Tris-HCl buffer (pH 7.4), 3 μmoles of $MgCl_2$, 9 μmoles of Na-succinate and 9 μmoles of K-phosphate buffer (pH 7.4 containing 10 μc of $P^{32}$). Incubation was carried out at 25°C. Other additions are described in table. Counts of accumulated $P^{32}$ were measured by the method of AZZONE and ERNSTER[28].

| Incubation system addition (+) or omission (−) | Counts of $^{32}P$ accumulated into mitochondria (c. p. m.) |
|---|---|
| Complete | 15509 |
| −$Ca^{++}$ | 3275 |
| −succinate | 3190 |
| +antimycin A (2 μg/ml) | 1466 |
| +DNP (90 mμmoles) | 2300 |
| +antimycin A+ATP (9 μmoles) | 4306 |
| +antimycin A + ATP + oligomycin (2.8μg/ml) | 2243 |

inhibited by DNP and antimycin A. But even in the presence of antimycin A in the medium, the Pi accumulation was brought about by ATP and was inhibited by oligomycin and TBTC.

## DISCUSSION

The mitochondrial swelling induced by Pi as described in this paper is of a small degree and it is tightly coupled with the oxidation of mitochondria. This mitochondrial swelling required the respiratory substrate and was inhibited by respiratory inhibitor or uncoupler of oxidative phosphorylation. Moreover, the swollen mitochondria induced by Pi and respiratory substrate were shrunken by Na-oleate, DNP and ADP. Therefore, such a swelling of mitochondria is different from the swelling measured by the change of absorption at 520 mμ in the medium of KCl-Tris buffer solution[2,16,36]. A small degree of mitochondrial swelling and shrinkage was observed by PACKER[13,14,18] and he explained it by the mechanism as follows: the swelling-shrinkage of mitochondria is controlled by the intermediate of oxidative phosphorylation, then increase of intermediate induces the swelling and decrease of intermediate induces the shrinkage. But he did not describe what is the intermediate concerned with the regulation of swelling. The present experiment showed that the Pi induced swelling was not inhibited by oligomycin which is the inhibitor of ATP-Pi exchange reaction.

Therefore, the intermediate must be related to the initial step of phosphorylation. Furthermore, the Pi induced swelling of mitochondria is similar to the accumulation of Pi in mitochondria as far as its mechanism is concerned, and it is possible that the intermediate is a compound of phosphate such as phosphate carrier[37,38]. Then it can be deduced that the Pi-induced swelling is induced by the accumulation of Pi in mitochondria. The accumulation of a large amount of Pi in mitochondria required ATP, $Mg^{++}$, respiratory substrate and $Ca^{++}$ and was inhibited by respiratory inhibitors or uncouplers as described by many investigators[11,15,31]. In this case oligomycin does not inhibit the Pi accumulation but accelerates about 25 per cent for 3 minutes at 25°C. The swelling of mitochondria by Pi also required the substrate and was inhibited by uncoupler or respiratory inhibitor. For the accumulation of Pi in a large amount in mitochondria it required $Ca^{++}$ (1 mM), $Mg^{++}$ (10 mM) and ATP (3 mM), but the accumulation of a small amount of $P^{32}$ in mitochondria proceeded in the absence of $Ca^{++}$ and ATP and the amonut of transported Pi was in maximum for 3 minutes as indicated in this paper. This Pi accumulation in the medium containing a low concentration of $Mg^{++}$ (1 mM) was inhibited by uncoupler and respiratory inhibitor, but was accelerated by the inhibior of phosphorylating respiration. The identical phenomenon was observed on the $Ca^{++}$ uptake into mitochondria[39].

In the medium containing no $Mg^{++}$ or with a low concentration of $Mg^{++}$, ATP inhibited slightly the Pi-induced swelling and the Pi accumulation in mitochondria. On the other hand, the mitochondrial swelling induced by Pi and accumulation of Pi in mitochondria were also induced by ATP in the medium containing antimycin A and $Ca^{++}$ as shown in this paper. This Pi-induced swelling and Pi accumulation were inhibited by oligomycin. Namely, the modes of accumulation of Pi and Pi-induced swelling are very similar. Therefore, it is considered that the accumulation of Pi or the Pi-induced swelling is correlated to some intermediate in the initial step of oxidative phosphorylation. Then the inhibition of Pi accumulation by ATP (ATP is a potential source of ADP) would be due to the discharge of intermediate during ATP synthesis, and the Pi-accumulation induced by ATP in the presence of antimycin A would be due to the energy of ATP by a reversal process of phosphorylation to form the intermediateas described by BRIERLEY *et al* [9,21].

Thus both the Pi-induced swelling and the Pi-accumulation in mitochondria require the high energy suggesting that both of them are tightly coupled to form phosphorous compound which is closely correlat an intermediate of phosphorylation in the initial step.

SUMMARY

1. Rat liver mitochondria are swollen by inorganic phosphate in the medium of slightly hypotonic sucrose solution containing respiratory substrate and the mitochondrial swelling is inhibited or turned to shrink by ADP, respiratory inhibitor, anaerobiosis and uncoupler of oxidative phosphorylation. This mitochondrial swelling is not inhibited by the inhibitor of phosphorylating respiration such as oligomycin and tributyltin chloride.

2. Rat liver mitochondria are swollen by ATP in the presence of antimycin A, inorganic phosphate and 0.1 mM of $CaCl_2$ and such a swelling is inhibited by oligomycin.

3. Accumulation of a small amont of $P^{32}$ in acid soluble Pi fraction of rat liver mitochondria proceeds even in the medium containing neither ATP nor $Ca^{++}$ but is inhibited by respiratory inhibitor, ATP, ADP and uncoupler of oxidative phosphorylation. The accumulation of $P^{32}$ in mitochondria, however, is not inhibited by oligomycin.

4. The accumulation of $P^{32}$ is induced by ATP in the presence of antimycin A and $Ca^{++}$(0.1 mM) and such an accumulation of $P^{32}$ is inhibited by oligomycin.

5. It is suggested that the Pi-induced swelling of mitochondria is correlated to the accumulation of inorganic phosphate and both of them are tightly coupled to the initial step in the process of oxidative phosphoryaltion.

ACKNOWLEDGEMENTS

The author wishes to thank professor M, Yamamoto and Professor S. Seno for their interest shown in this work and for many helpful discussions. Thanks are also due to Professor B. Hagihara for the gift of tributyltin chloride and to Dr. S. Minakami for the gift of oligomycin.

REFERENCES

1. LEHNINGER, A. L.: Oxidative phosphorylation in submitochondrial system. *Fedratiou Proceedings* 19, 952, 1960
2. LEHNINGER, A. L.; Reversal of various types of mitochondrial swelling by adenosine triphosphate. *J. Biol. Chem.* 234, 2465, 1959
3. LEHNINGER, A. L.: Ionic environment and the contraction of isolated rat liver mitochondria by adenosine triphosphate. *Biochim. Biophys. Acta* 48, 324, 1961
4. JUDAH, J. D.: The action of antihistamine drugs *in vitro* I. Mitochondrial swelling. *Biochim. Biophys. Acta*, 53, 375, 1961
5. RICHARDSON, T. and TAPPEL, A. L.: Swelling of fish mitochondria. *J. Cell Biol.* 13, 43, 1962
6. KAUFMAN, B. T. and KAPLAN, N, O.: Effect of substrates on the swelling and loss of pyridine nucleotides from rat liver mitochondria. *Biochim. Biophys. Acta* 32, 576, 1959
7. ARCOS, J. C., GRIFFITH, G. W. and CUNNINGHAM, R. C.: Fine structural alterations in

cell particles during chemical carcinogenesis. II. Further evidence for their their involvement in the mechanism of carcinogenesis. The swelling of rat liver mitochondria during a aminoazo dyes. *J. Biophys. Biochem. Cytol.* 7, 49, 1960

8. NEUBERT, D., WOJTCZAK, G. W. and LEHNINGER, A. L.: Purification and enzymatic identity of mitochondrial contraction factor. *Proc. Natl. Acad. Aci.* 48, 1651, 1962
9. VIGNAIS, P. V., VIGNAIS, P. M., ROSSI, C. S. and LEHNINGER, A. L.: Restoration of ATP induced contraction of pre-treated mitochondria by contractile protein. *Biochem. Biophys. Res. Comm.* 11, 307, 1963
10. VIGNAIS, P. M., VIGNAIS, P. V. and LEHNINGER, A. L.: Restoration of ATP induced contraction of pre-treated mitochondria by phosphatidyl inocitide. *Biochem. Biophys. Res. Comm.* 11, 313, 1963
11. EMMELOT, P. E.: The effect of succinate on the glutathion and cystein inducing swelling of liver and hepatoma mitochondria. *Exptl. Res.* 24, 280, 1961
12. CORWIN, L. M. and LIPSETT, M. N.: Studies on stability of rat liver mitochondria II. Relation of the electron transport system to swelling. *J. Biol. Chem.* 234, 2453, 1959
13. PACKER, L.: Metabolic and structural states of mitochondria. I. Regulation by adenosine diphosphate. *J. Biol. Chem.* 235, 242, 1960
14. PACKER, L.: Metabolic and structural states of mitochondria. II. Regulation by phosphate. *J. Biol. Chem.* 236, 214, 1961
15. DI SABATO, G. and FONNESU, A.: Metabolic mechanisms involved in the swelling of isolated mitochondria and in its prevention. *Biochim. Biophys. Acta.* 35, 358, 1959
16. UTSUMI, K., Ohara, S., YAMAMOTO, G., INABA, K., URAKAMI, H. and YAMAMOTO, M.: Mitochondrial swelling and uncoupling activity of long-chain fatty acids. *Acta Med. Okayama* 16, 317, 1962
17. PACKER, T. and GOLDER, R. H.: Structural factors involved in the control of metabolism in ascites tumor cells. *Biochim. Biophys. Acta* 32, 281, 1959
18. PACKER, L.: Metabolic and stractural states of mitochondria. III. Reversal of electron transport and mitochondrial swelling. *J. Biol. Chem.* 237, 1327, 1962
19. PRICE, C. A., FONNESU, A. and DAVIES, R.: Movements of water and ions in mitochondria. *Biochem. J.* 64, 754, 1956
20. AMOORE, J. E. and BARTLEY, W.: The permeability of isolated rat-liver mitochondria to sucrose, sodium chloride and potassium chloride at 0°. *Biochem. J.* 69, 223, 1958
21. BRIERLEY, G. P., MURER, E. and GREEN, D. E.: Participation of an intermediate of oxidative phosphorylation in ion accumulation by mitochondria. *Science* 140, 60, 1963
22. BRIERLEY, G. P., BACHMANN, E. and GREEN, D. E.: Active transport of inorganic phosphate and magnesium ions by beef heart mitochondria. *Proc. Natl. Acad. Sci.* 48, 1928, 1962
23. VASINGTON, F. D. and MURPHY, J. V.: $Ca^{++}$ uptake by rat kidney mitochondria and its dependence on respiration and phosphorylation. *J. Biol. Chem.* 237, 2670, 1962
24. VASINGTON, F. D.: $Ca^{++}$ uptake by fragments of rat liver mitochondria and its dependence on electron transport. *J. Biol. Chem.* 238, 1841, 1963
25. LEHNINGER, A. L., ROSSI, C. S. and GREENAWALT, J. W.: Respiration-dependent accumulation of inorganic phosphate and $Ca^{++}$ by rat liver mitochondria. *Biochem. Biophys. Res. Comm.* 10, 444, 1663
26. ROSSI, C. S and LEHNINGER, A. L.: Stoichiometric relationships between mitochondrial ion accumulation and oxidative phosphorylation. *Biochem. Biphys. Res. Comm.* 11, 441, 1963
27. HOGEBOOM, G. H.: in "*Method in Enzymology*" Vol. 1, Academic Press, New York, 1955, p. 16.
28. HAGIHARA, B.: Teckniques for the application of polarography to mitochondrial respiration. *Biochim. Biophys. Acta* 46, 134, 1961

29. AZZONE, G. F. and ERNSTER, L.: Respiratory control and compartmentation of substrate level phosphorylation in liver mitochondria. *J. Biol. Chem.* 236, 1501, 1961
30. TAKAHASHI, T.: The experimental methods for phosphorous metabolism. Hirokawa Publish, Co., Tokyo, 1958
31. CHANCE, B. and WILLIAMS, C. R.: The respiratory chain and oxidative phosphorylaton. *Advances in Enzymol.* 17, 65, 1956
32. PRESSMAN, B. C. amd LARDY, H. A.: Effect of surface active agents on the latent ATPase of mitochondria. *Biochim. Biophys. Acta* 21, 458. 1956
33. UTSUMI, K., INABA, K., YAMAMOTO M, YAMAMOTO, G., URAKAMI, H. and SENO, S.: The effects of high fatty acid on the glucose metabolism of Ehrlich ascites tumor cells. *Acta Med. Okayama* 16, 177, 1962
34. LOOMIS, W. F. and LIPMAN, F.: Reversible inhibition of the coupling between phosphorylation and oxidation *J. Biol. Chem.* 173, 807, 1948
35. SONE, H. and HAGIHARA, B.: unpublished
36. WOJTCZAK, L. and LEHNINGER, A. L.: Formation and disappearance of an endogenous uncoupling factor during swelling and contraction of mitochondria. *Biochim. Biophys. Acta* 51, 442, 1961
37. HOKIN, L. E. and HOKIN, M. R.: Phosphatidic acid metabolism and active transport of sodium. *Fedration Proceedings* 22, 8, 1963
38. GARBUS, J., DELUCA, H. F., LOOMANS, M. E. aud STRONG, F. M.: The rapid incorporation of phosphate into mitochondrial lipids. *J. Biol. Chem.* 238, 59, 1993
39. UTSUMI, K.: Unpublished
40. UTSUMI, K., YAMAMOTO, G., INABA, K., OHARA, S. and YAMAMOTO, M.: Swelling-shrinkage and oxidative phosphorylation of mitochondria. *Sympo. Cell. Chem.* 13, 153, 1963
41. CLARK, E. P.: "Semi-micro quantitative organic analysis". p. 42, Academic Press, New York, 1943