BIOCHEMICAL AND NUTRITIONAL STUDIES ON POTASSIUM VI. NATURE OF OXYGEN UPTAKE IN THE CELL PARTICULATES AND POTASSIUM ROLE

BIOCHEMICAL AND NUTRITIONAL STUDIES ON POTASSIUM
VI. NATURE OF OXYGEN UPTAKE IN THE CELL PARTICULATES AND POTASSIUM ROLE

By

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I. Introduction

We have already investigated on the separation method of cell particulates and the yield of the chloroplast isolated from the leaves of several plant species. Potassium distribution or autonomous oxygen uptake in the isolated particulates was also examined to explore the effect of potassium at the unit of the cell or cell particulates. The results obtained were that the nature of the isolated particulates differed by the isolation methods and conditions. Then the chloroplast was separated at relatively high degree of purity but mitochondria was obtained at the impure state contaminating 15 per cent chloroplast fragments of the total intact leaves(1). Continuing from the preceding report, the nature of the oxygen uptake of isolated particulates was examined, and the role of potassium in concern with the oxygen uptake was also investigated. In this experiment the isolated barley chloroplast and mitochondria was mainly used as materials, and the experimental materials and methods were similar to the previous one. In addition the following points were emphasized.

1. Influence on the oxygen uptake of the particulates by recentrifugation and the addition of condensed cell sap in contrast to the perfect system similar to the cell. 2. Requirement of some components such as activator, substrate, enzyme and coenzyme. 3. Sensitivity to the inhibitors. 4. Output of carbon dioxide from the particulates. 5. Influence on the oxygen uptake of isolated chloroplast by the addition of potassium to the surrounding medium.

As the investigations concerning the respiratory enzymes have been limitted to the mitochondria in the plant(2-7), the respiratory mechanism in the chloroplast in comparison with the mitochondria and the role of potassium are treated in this report.

II. Experiments and Results

1. *Influence on the oxygen uptake of the isolated particulate by the recentrifugation and the addition of condensed cell sap.*

The purification of isolated particulates were mainly performed by recentrifugation, in which case it had been already reported that the activity of the photosynthesis of the isolated chloroplast decreased due to denaturation or leakage of the particulate components(9). From the result of the previous

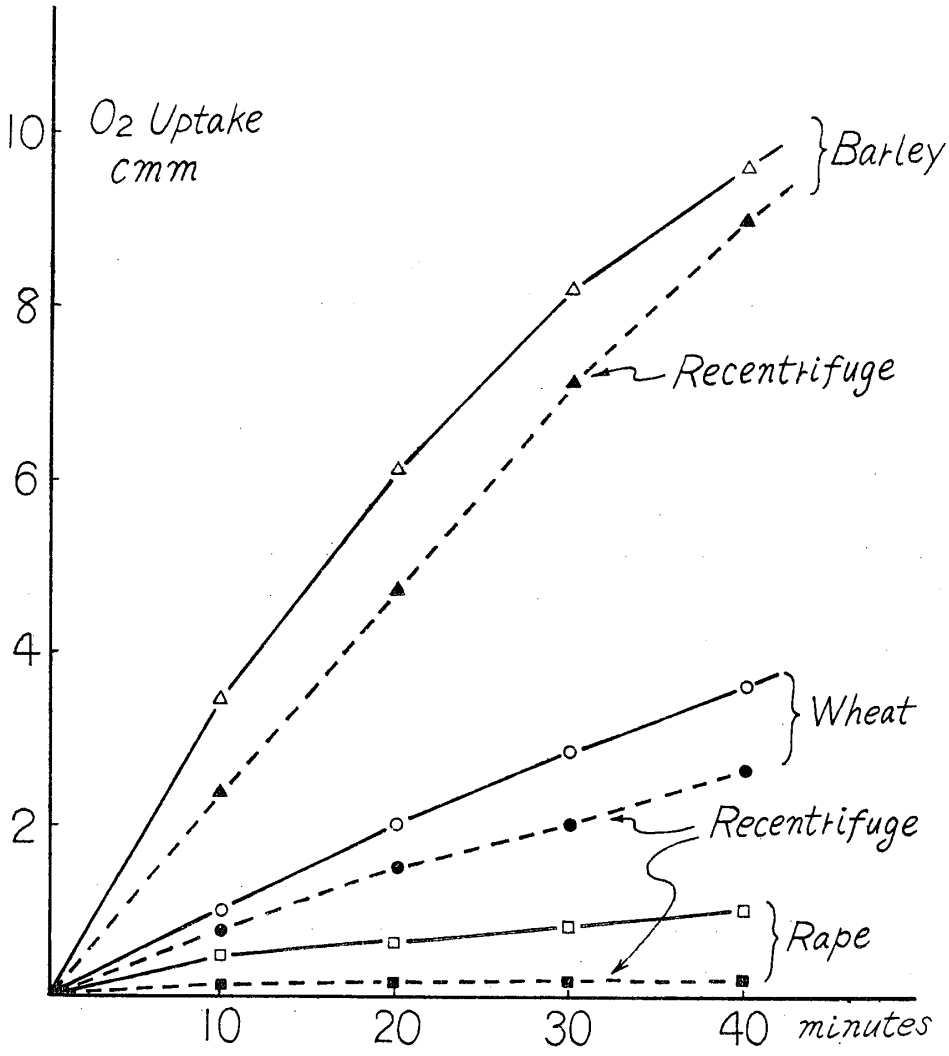

Fig. 1 Inactivation of oxygen uptake of isolated chloroplast by recentrifugation.

report it was stated that the contents of some minerals in the isolated chloroplast altered easily through the process of isolation(1), the same aspect will be naturally supported in the case of organic compounds. In this part concerning the drop of the oxygen consumption in the isolated chloroplast the cause was by recentrifugation. The activity of the oxygen consumption was considerably reduced with once centrifugation relating to the case of photosynthesis. (Fig. 1)

Further problems are in that the connecting structure of the respiratory system will be destroyed when the particulates are separated from the perfect system of the cell, so the isolated particulates were suspended in the condensed cell sap at the place of sucrose medium.

The condensed cell sap was prepared through condensation by the lyophilizer. The content of the intact cell was separated into the following parts, and added to the particulates, that is, the sap retaining the mitochondria and its free sap for the suspension of the isolated chloroplast, for the mitochondrial suspension both cell sap with or without chloroplast. For the control with oxygen uptake influenced by the condensed cell sap, a plot for distilled water as the medium was set. Potato, barley and radish chloroplasts were always activated by suspending in both condensed cell saps, but there was an exception in corn chloroplast, which recreated originally almost no trace of oxyen uptake, and was hardly activated by both condensed cell saps. As high autonomous oxygen uptake in the potato mitochondria was found exceptionally in the previous investigation, also in this case, the potato chloroplast was more influenced by the cell sap containing mitochondria, and less affected by the mitochondria free cell sap. In tobacco chloroplast both condensed cell sap increased the oxygen uptake about two times, radish chloroplast was activated to some extent. (Fig. 2)

From the data, attention must be paid that the autonomous oxygen uptake of the isolated chloroplasts were influenced highly by both condensed cell sap with or without mitochondria. This effect, depending on the species, was observed in the potato, and radish chloroplast. As to the oxygen uptake of the isolated mitochondria, (Fig. 3) however, the influence by the addition of the condensed cell sap was less than that of the isolated chloroplast, and in all cases, the treatment of the condensed cell sap including the chloroplast was more effective than that of chloroplast free cell sap. From these results it became clear that autonomous oxygen uptake of isolated mitochondria was generally low compared with the isolated chloroplast and activated to a small degree by the addition of the condensed cell sap. As we would attempt to survey the respiration in the mitochondria relating to the role of potassium considering from the previous literature, we arrived at the conclusion that isolated chloroplast in almost all species, remained the oxygen uptake system to a high extent. Therefore the investigation will be emphasized concerning the reaction

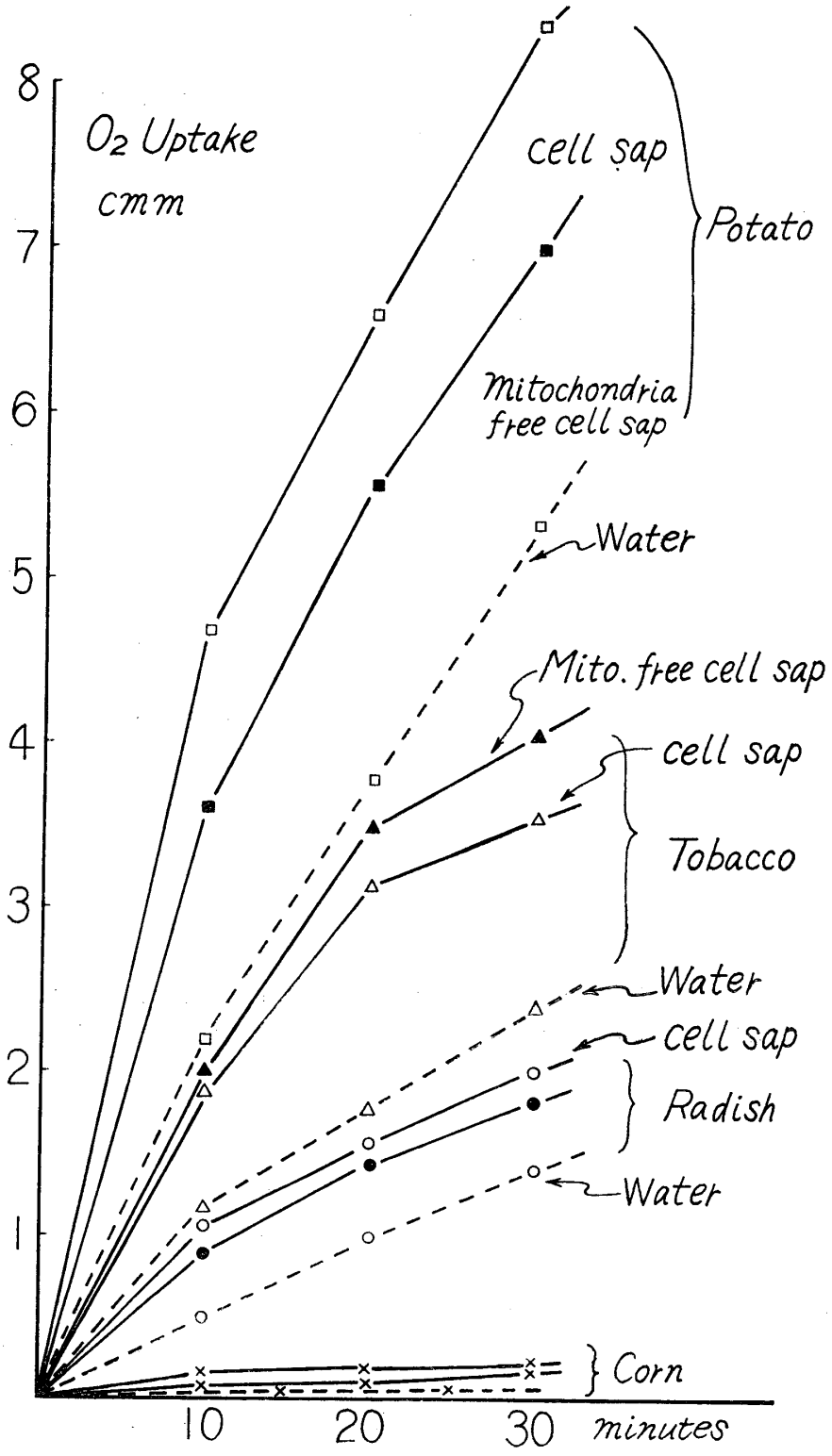

Fig. 2. Effect of the addition of cell sap with or without mitochondria on the oxygen uptake of the isolated chloroplast fraction.

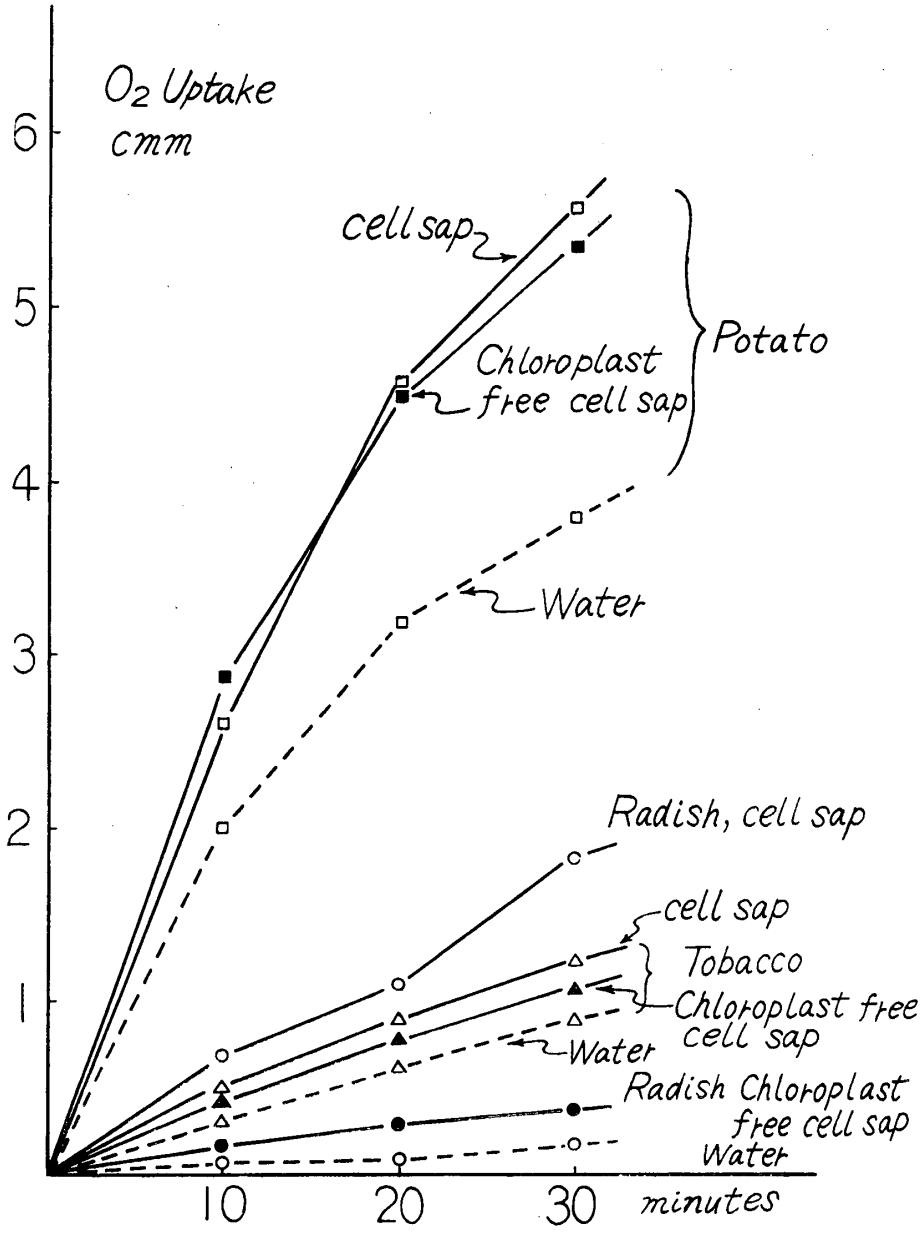

Fig. 3. Effect of the addition of cell sap with or without chloroplast on the oxygen uptake of the isolated mitochondrial fraction.

of chloroplast and carbon dioxide output related with the oxygen uptake of isolated chloroplast.

2. ***Requirement of some components for the oxygen uptake of isolated chloroplast.***

(1) *Effect of Activators,*

According to French(8) the activators of the Hill reaction and photosynthesis of chloroplast have been fully investigated and it was reported that isolated chloroplast retained at 60 per cent level of initial activity for ten days suspended in 15 per cent methanol solution at -5°C. As 20 per cent methanol and 10 per cent, 20 per cent ethanol were used in this experiment, 20 per cent methanol

or ethanol gave a good result at the lapse of one hour, namely, the same effect was observed in the case of the oxygen uptake of the chloroplast. (Fig. 4)

Warburg and Lütgens(9) found that a great amount of hydroquinone, polyphenol, pyrocatechol could maintain the activity of chloroplast. Five mg hydroquinone was added to each vessel which contained the chloroplast immediately

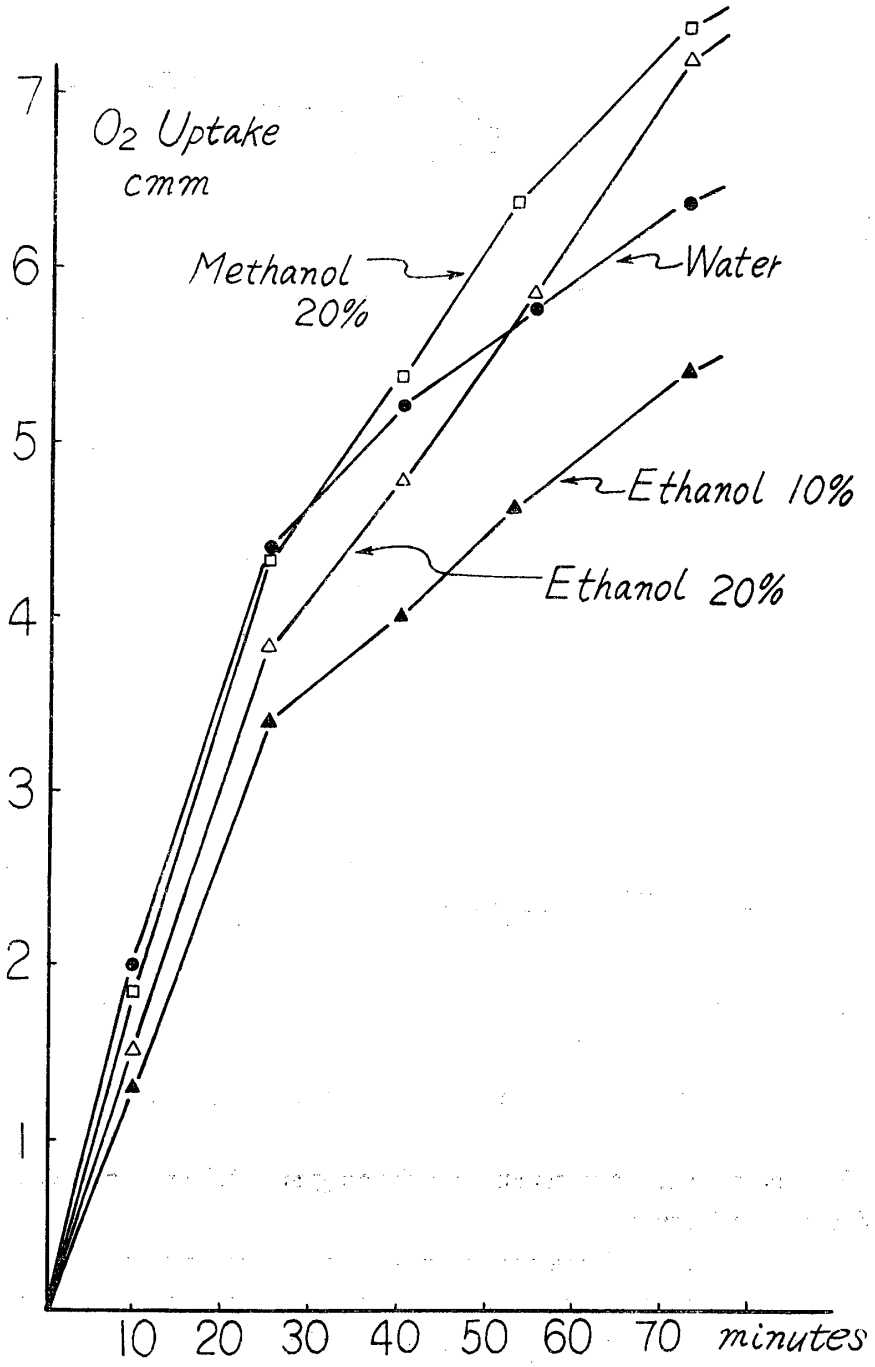

Fig. 4. Influence of addition of ethanol and methanol solution on the oxygen uptake of the isolated chloroplast.

after the isolation and left to stand for 36 hours after separation. The result was that the effect was hardly detected in the former case, but in the latter it was as shown in Fig. 5, the oxygen uptake was highly activated.

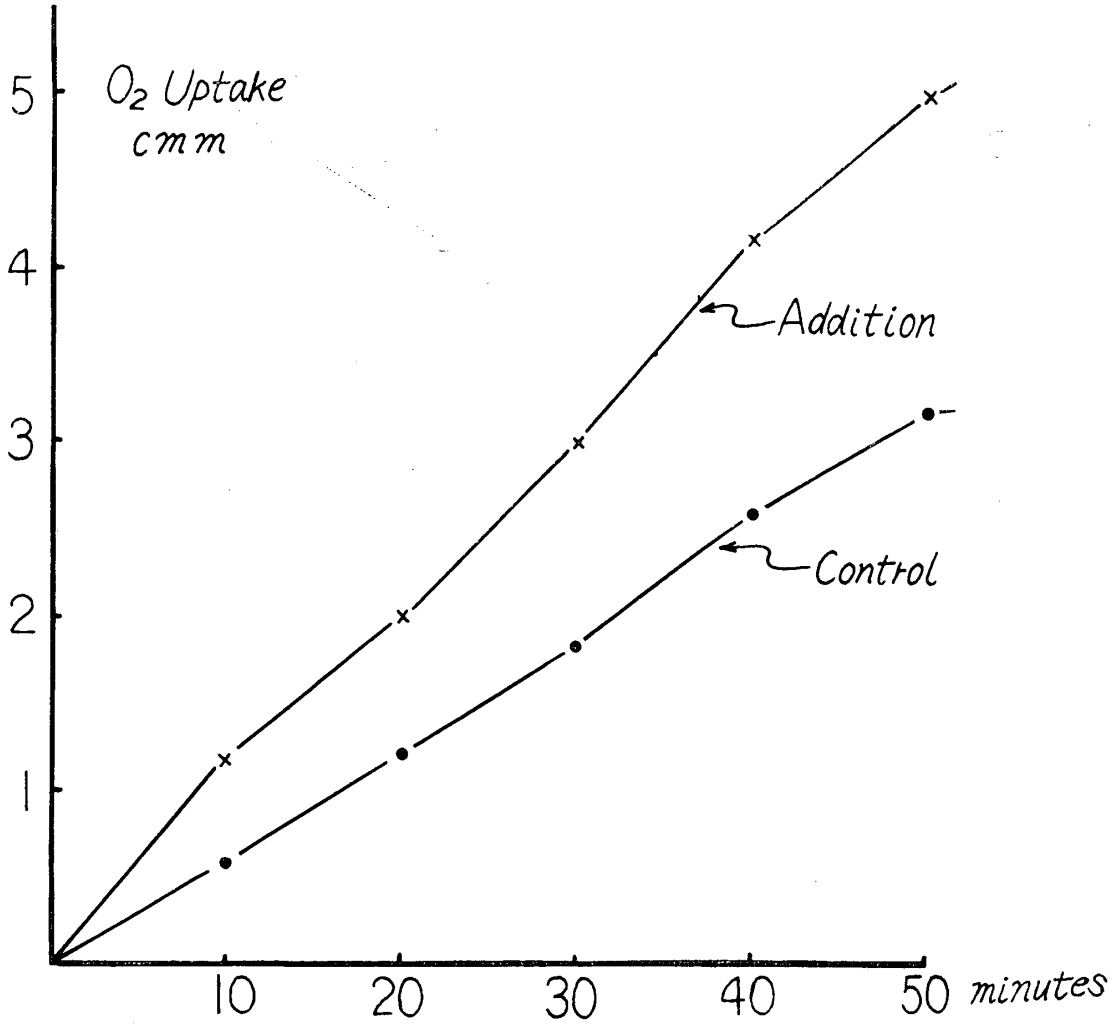

Fig. 5. Effect of the hydroquinon addition (5 mg. per vessel) on the oxygen uptake of 36 hours aged chloroplast.

(2) *Effect of the Substrates*

In the reaction of the mitochondrial system, the selected reaction mixture was often used to increase the activity of the respiration process(10—13) so the reaction mixture was adopted as suitable for the oxygen uptake of isolated chloroplast imitating the case of mitochondria. Namely 0.03 M of succinic acid and 0.015 M of citric acid were added as the substrate. Thus oxygen uptake was increased by 0.03 M succinic acid at about twofold of the control, but not increased by citric acid. From this result it may not yet be concluded whether succinic acid was taken in the krebs cycle or coupled with the other oxidation and reduction process (Fig. 6)

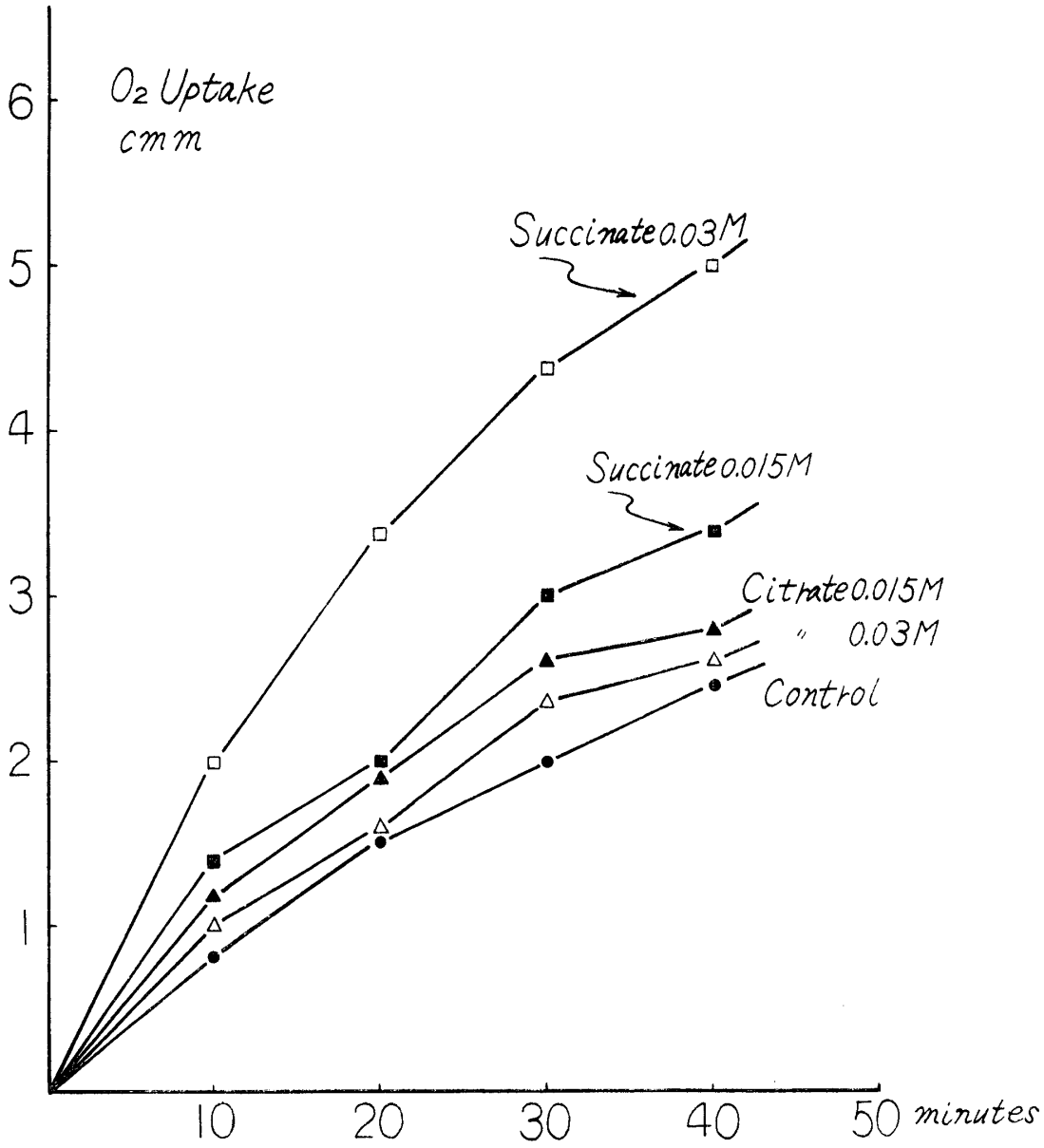

Fig. 6. Effect of the addition of organic acids on the oxygen uptake of the isolated chloroplast

(3) *Influence of the Enzyme and Coenzyme*

The loss of diphosphopyridine from the isolated chloroplast was already found in the investigation of photosynthesis. It may be also considered that other components which have close relation to the process of oxidation mechanism, will be left during the isolation. Following to the above experiment, enzyme and coenzyme were added to the reaction mixture. Adopting 0.03 M succinic acid as the substrate, the following the reaction mixture of mitochondria (10—13), 0.5 μM diphosphopyridine nucleotide (DPN), 1 μM triphosphopyridine nucleotide (TPN), 0.05 μM cytochrome C (cyt-c), 1 μM adenosine monophosphate

(AMP), 1 μM, adenosine triphosphate (ATP) were used, even AMP, which had already been known to influence the mitochondrial respiratory system was hardly effective to the chloroplast isolated from barley and cabbage. Rather little effect was observed by ATP, and the effect was not clear with the addition of the other components in the range of these concentrations. (Fig. 7, 8)

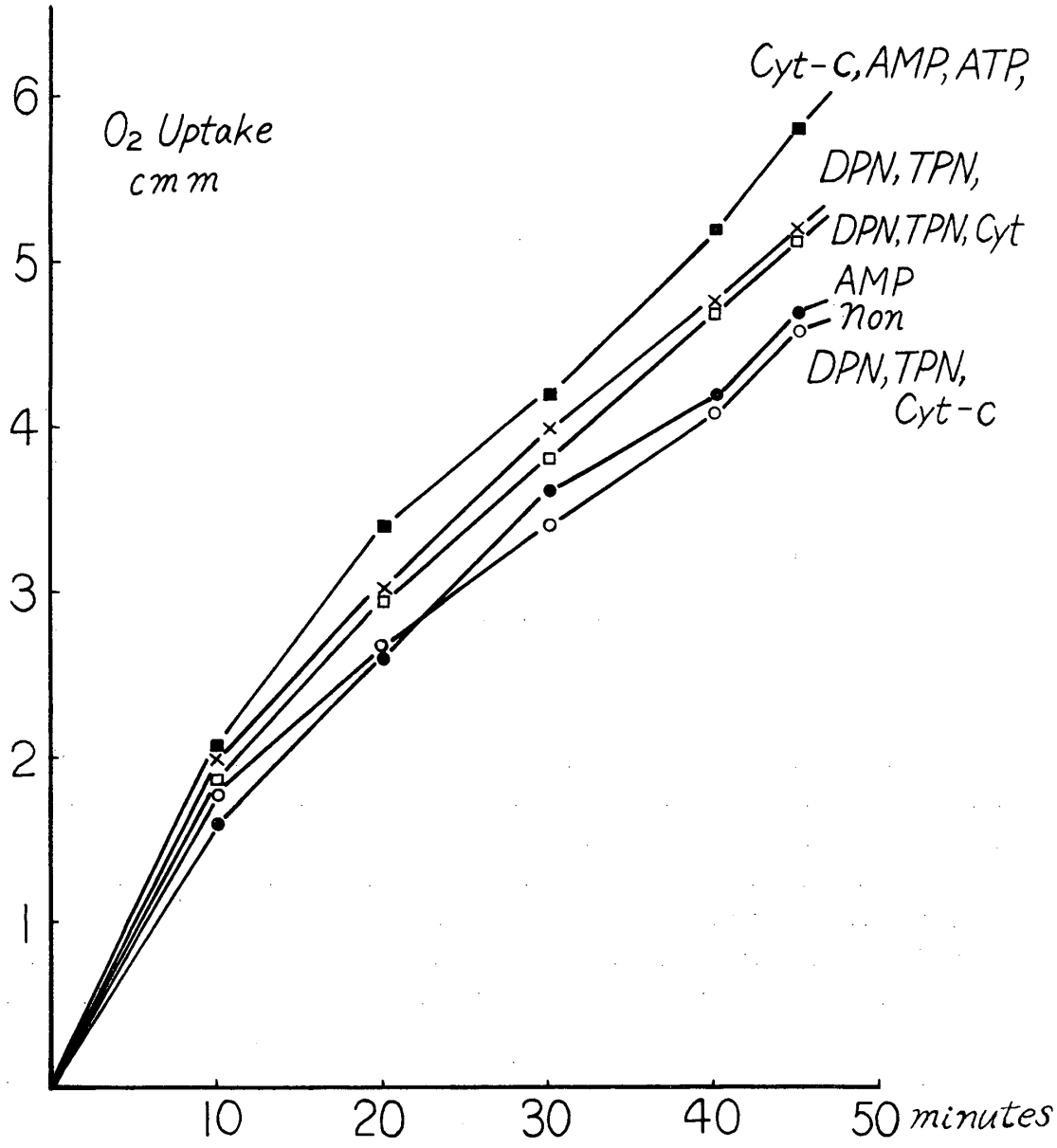

Fig. 7. Influence of the addition of enzyme and cenzyme on the oxygen uptake of the cabbage chloroplast.

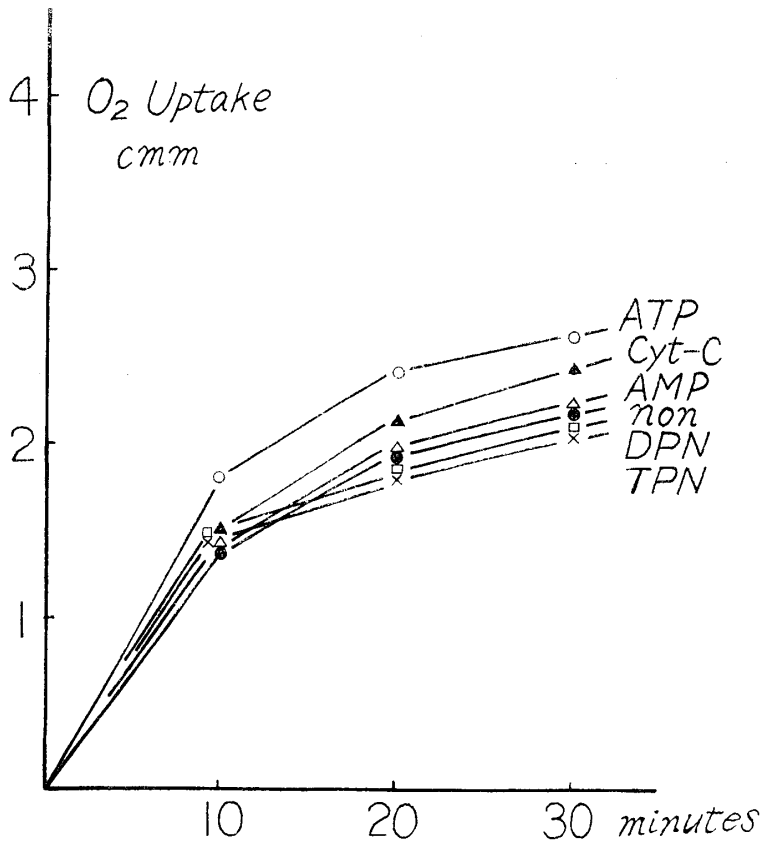

Fig. 8. Influence of the addition of emzyme and coenzyme of the oxygen uptake of the cabbage chloroplast.

III. Sensitivity to the Inhibitors

As the inhibitors of chloroplast reaction, cyanide, azide, and iodoacetate were mainly used in concern with the carbon dioxide fixation and the Hill reaction. For the inhibition of the respiratory chain, Warburg and Lüttgens(9) examined the cyanide inhibition of the chloroplast isolated from the spinach and sugar beet, Gerretsen(14) applied cyanide to the avena chloroplast.

In this experiment, relatively high concentratration of inhibitors, namely 10^{-2} M azide, 7×10^{-3} M malonate, 10^{-1} M cyanide, 7×10^{-3} M salicylaldoxime, were adopted for the inhibition of the oxygen uptake of both barley chloroplast immediately after isolation and aged for 36 hours, to know the sensitivity to the terminal oxidase or Krebs cycle. The data are shown in Fig. 9. The rate of inhibition in fresh chloroplast was strikingly elevated by the treatment of salicylaldoxime and cyanide, which affect the polyphenol and cytochrome oxidase. Also considerable sensitivity was observed by the malonate and azide inhibitors. In the chloroplast which was aged for 36 hours after separation, however, the

inhibition tendency was somewhat different from the fresh one. The aged material was more sensitive to the malonate treatment. It may be concluded that there would be an active oxidation systems of respiratory chain in the barley chloroplast, but the presence of Krebs cycle should not yet be decided merely from inhibition of malonate. From the former results that succinic acid may taken in the oxidation and reduction systems coupling with the dehydrogenase but citric acid of the Krebs cycle was hardly assimilated.

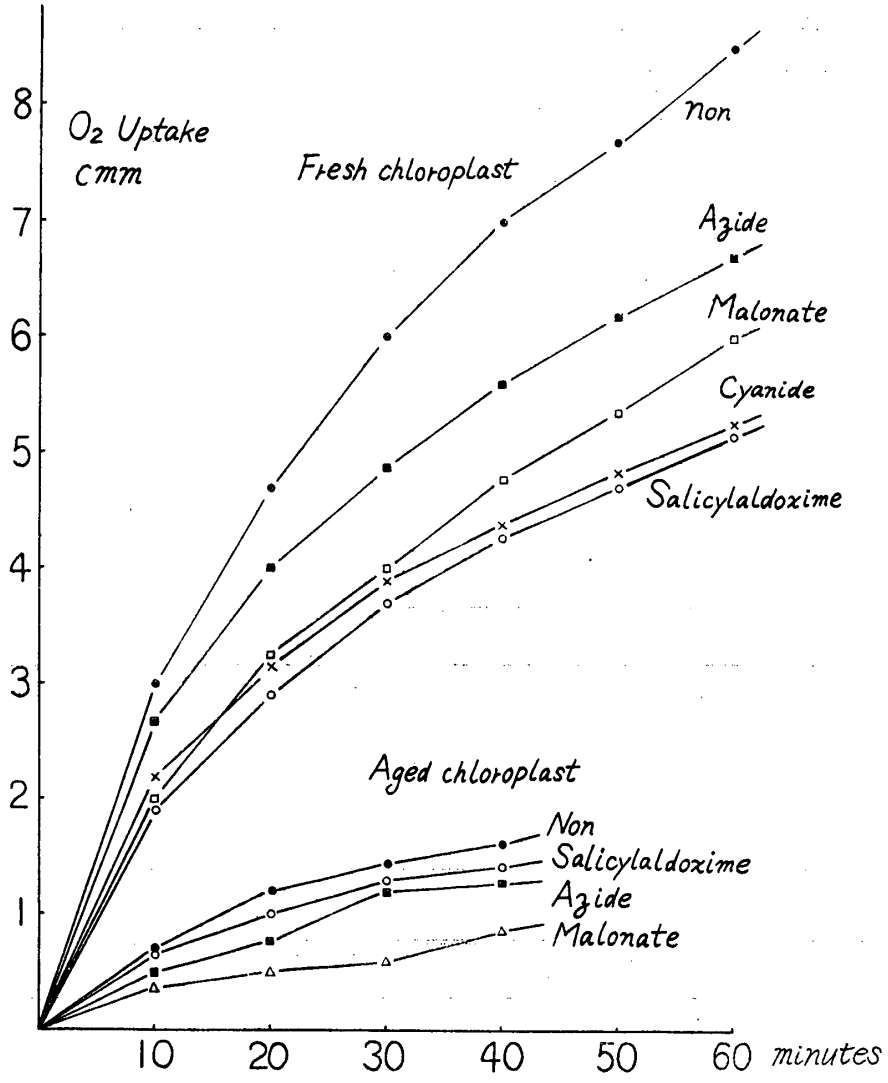

Fig. 9. Inhibition of the addition of respiratory inhibitors on the oxygen uptake of the isolated chloroplast.

IV. The Output of Carbon Dioxide from the Isolated Chloroplast

In general the oxygen uptake and output of carbon dioxide was included in the respiration phenomena of higher plants. As to the oxygen uptake system in the plant cell, it may be concluded that high autonomous oxygen uptake was found in the chloroplast of many species, barley, tobacco, radish, taisai especially. The output of carbon dixide, however, has not yet been clear, so using the potassium sufficient and deficient plants as samples which were labelled with the isotopic carbon, the output of isotopic carbon dioxide from the isolated chloroplast and mitochonodria were determined. Isotopic carbon was inserted into the young barley and spinach plant under the following conditions. Total isotopic carbon 500—250μ c/pot, day light of 25000—20000 lux,

Table. 1 Growth of spinach and barley when isotopic carbon were assimilated

Plot	Spinach (II/12)	Barley (III/10)
Potassium Normal	16.0	17.0
Potassium Deficient	4.5	11.0

g. fresh weight/pot

Table 2. Distribution of assimilated isotopic carbon and output of isotopic carbon dioxide from isolated particulates.

Spinach (sampling date, II/9)

Plot	Fraction	Oxygen Uptake	Output of $C^{14}O_2$	Distribution of assimilated C^{14}
Potassium normal	Intact leaves	46.8	1638	413077
	Isolated chloroplast	1.5	139	64474
	Isolated mitochondria	0.6	73	61369
Potassium deficient	Intact leaves	64.8	1180	278661
	Isolated chloroplast	1.9	177	35644
	Isolated mitochondria	0.5	56	31290

Barley (sampling date, III/7)

Plot	Fraction	Oxygen Uptake	Output of $C^{14}O_2$	Distribution of assimilated C^{14}
Potassium normal	Intact leaves	54.0	4320	1925344
	Isolated chloroplast	3.4	209	2630000
	Isolated mitochondria	2.3	103	166120
Potassium deficient	Intact leaves	66.0	10276	1808000
	Isolated chloroplast	3.3	249	251440
	Isolated mitochondria	2.3	81	176280

Oxygen uptake ; μl/30 min./10 g fresh weight.
Output of $C^{14}O_2$; Counts/min/10 g fresh weight.
Distribution of assimilated C^{14} ; counts/min./10 g fresh weight.

at temperature 18—20°C, for 3 hours, using the apparatus of about 20 *l* photosynthetic bottle. Materials were sampled after seven days. Assimilated carbon was digested by the liquid oxidant and measured with the thin window type G.M. counter, classifying it into chloroplast and mitochondria. Because of the low efficiency of counting, the data are expressed as the result of qualitative analysis (Tables 1, 2).

From the result, isotopic carbon dioxide was broken out from the isolated chloroplast at high degree in comparison with that of isolated mitochondria, that is, it became clear that the chloroplast has the output system of carbon dioxide besides the oxygen uptake system. Assimilation and output of isotopic carbon was surely influenced by the level of potassium nutrient. As isotopic carbon was highly assimilated in the potassium sufficient spinach or barley, so the trend of the out put was the reverse in the slightly potassium deficient plants. These were almost in accord with the phenomena of the intact plants supplied with different potassium levels(15).

V. Effect of Potassium and Other Salts

From the former reports, magnesium and calcium were contained at the condensed state in the chloroplast and potassium, on the other hand, they were mainly found in the cell sap(1). The conclusion was made that the potassium

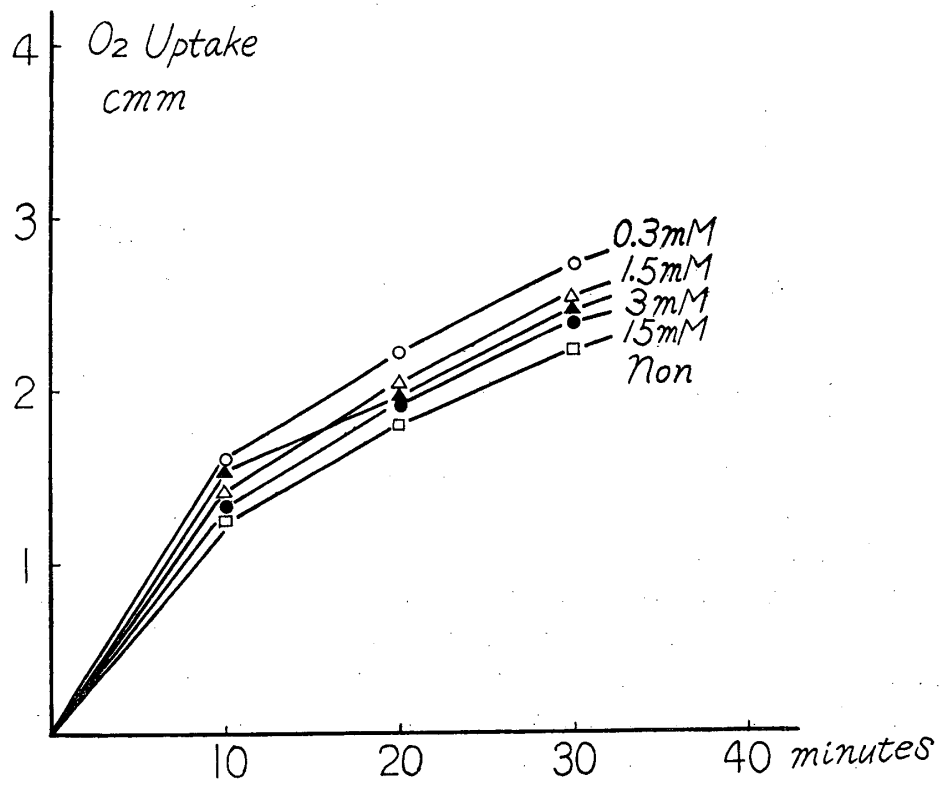

Fig. 10. Influence of the addition of sodium chloride on the oxygen uptake of isolated chloroplast.

content in the particulates or organs was easily altered with the change of physiological conditions such as osmotic pressure, hydrogen ion concentration and the activity of respiration. The levels of the content of these mineral elements may be also varied by the amount of application. In this experiment the isolated chloroplast was suspended in the different levels of mineral concentration, to examine the shift of oxygen uptake rate.

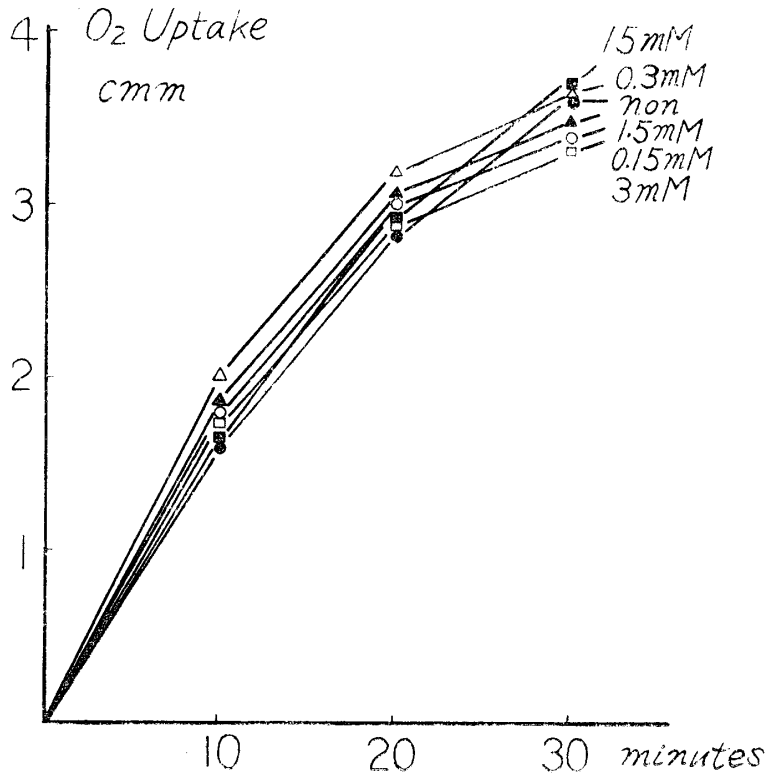

Fig. 11. Influence of the addtiton of phosphate salts on the Oxpgen uptake of isolated chloroplast.

Sodium chloride and sodium phosphate were less effective at the range of 0.3mM-15mM as shown in Figs. 10, 11, and no influenece appeared by the addition of calcium and magnesium salt. The most effective phenomena was confirmed by both chloride or sulfate salts of potassium, when suspended in the 3mM solution of lower concentration of these salts, the oxygen uptake of the isolated chloroplast rose at the 20-40 per cent level, and in 10mM solution of higher concentration of these salts it was contrary depressed. (Fig. 12)

Recently Miller and Evans(16) reported that terminal oxidase in the plant mitochondria system was influenced by the mineral elements. The same role may be considered in this experiment that potassium is required in the process of oxygen uptake in the isolated chloroplast relating to the terminal oxidase or dehydrogenase. To say conclusively potassium will be needed in the surrounding

medium at some concentration and may work with the enzyme or coenzyme to move easily to the center of reaction place in the form of an ion.

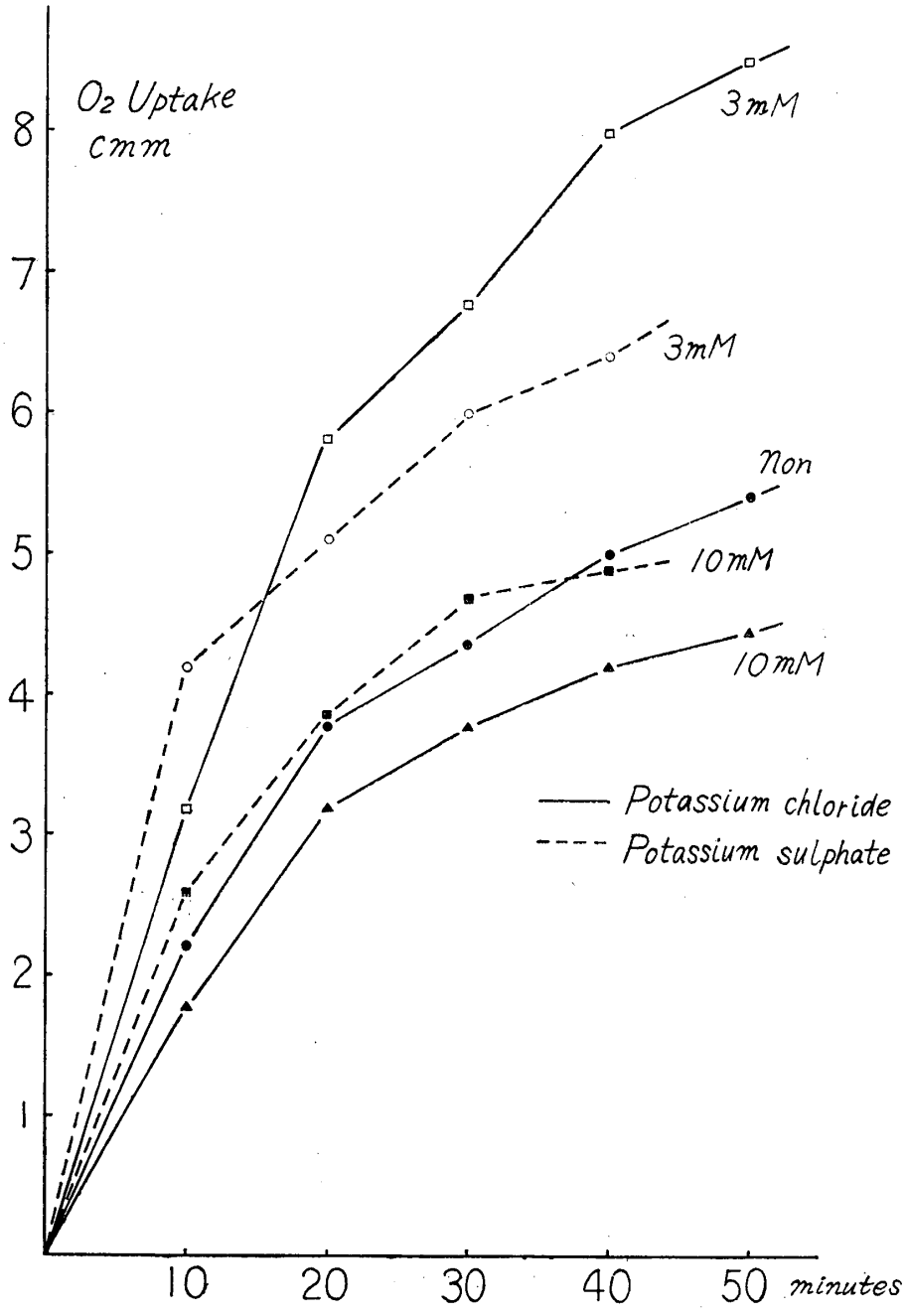

Fig. 12. Effect of the addition of potassium salts on the oxygen uptake of the isolated chloroplast.

VI. Discussion

It has been concluded that mitochondria in the animal is a place for respiratory enzyme(17), but as to the respiratory system of higher plants, the same conception was conducted, although the constitution of the cell particulates in the plant differed strictly from the case of the animal. Contrary to the above conception, in this experiment high oxygen uptake was found in the chloroplast or leucoplast isolated from most plants species, except the potato mitochondria. Presuming the presence of the respiration system in the chloroplast, at first its nature was investigated from the standpoint of reconstructing the perfect system by the addition of condensed cell sap to the isolated particulates, and also judged from the requirement of some components. Oxygen uptake of the chloroplast was activated higly by the addition of the condensed cell sap in comparison with that of mitochondria. Oxygen uptake was also activated by the addition of hydroquinone and methanol as similar to the photosynthetic activity, and to some extent by adenosine triphosphate.

From the sensitivity to the inhibitors, it became clear that terminal oxidase such as cytochrome or polyphenol oxidase might be present in the chloroplast, as mentioned before and it was recognized that it is mainly confirmed in the sugar beet chloroplast. The presence of polyphenol oxidase was already investigated by Arnon(18). However, it has been considered that cytochrome oxidase is mainly present in mitochondria(17)

The output of carbon dioxide from the barley and spinach chloroplast was ascertained by the tracer method of isotopic carbon. It appears that the

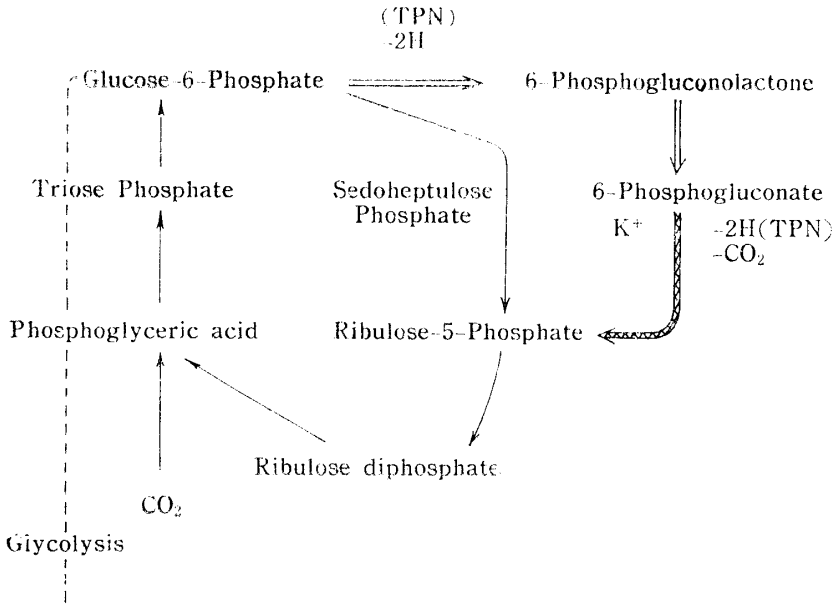

Fig. 13. Presumption figure of O_2 uptake and CO_2 output chain linked with photosynthesis cycle in the chloroplast

chloroplast has a perfect system of respiration, which undergoes, the uptake of oxygen and the output of carbon dioxide, not merely the oxidation of some components or the reverse reaction. The following schema was presumed as the system of reaction. (Fig. 13)

As Bassham and Calvin(19) indicated the connection of photosynthetic and pentose cycle when 6-phosphogluconate was turned to the ribulose-5-phosphate, then hydrogen and carbon dioxide will be outbroken, and hydrogen will be further connected to the triphosphopyridine nucleotide and terminal oxidase. The same case would happen in the process of glucose-6-phosphate, which also liberates hydrogen.

In turn, potassium may be required with the enzyme or coenzyme which has close relation to the above mentioned process, taking in the fact that oxygen uptake of chloroplast was influenced strikingly by the potassium present in the surrounding medium. Furthermore an enzymatic study will be needed to decide the working place and mechanism of potassium.

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