RADIOBIOCHEMICAL STUDIES ON RIPENING PROCESS OF RICE PLANT

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CONTENTS

Chapter I INTRODUCTION

In the ripening process of rice plant, on behaviors of the nutrients, we can see the two conspicious features characterizing the reproductive growth. In the first place, translocation and accumulation of the mineral and organic nutrients, which have absorbed or assimilated before and during the ripening stage, in the grains are observed. In the second place, biosynthesis of the components of the grains from the nutrients thus accumulated takes place. These processes have been known to be decisive factors which govern the crop yield and quality, then it is required that the cultivation of the rice plant had to ground upon the knowledge on the ripening process.

For this reason, the studies on the ripening process of rice plant have been performed by many workers, and the results of these studies have given the theoretical background on the cultivation of rice plant. Before the application of radioisotopes in these studies, the main way of the investigation of translocation and accumulation of the nutrients in the grains from the other parts was based on the observation of the variation of contents of the nutrients in each part of the plant, during its growth.

Ishizuka and Tanaka's work (1) is a representative one, in this connection. In the case of carbon (photosynthetic products), beside the analysis of the organic components of rice plant during its growth, the shading experiment (2) was also carried out. However, the results obtained by these methods gives only the "indirect" evidences on this problem.

As is well known, the introducing of radioisotopes in biochemical investigation have created new techniques, which hitherto could not be supposed, and have given the simple and accurate ways for the tracing of many biologically important substances in biological material, in micro quantity. Further, in the case of the study on the ripening process of rice plant, the application of radioisotopes supplies the "direct" information about the contribution of the nutrients absorbed at a definite growth stage to the grains.

Under these circumstances, in the present article, the author wishes to show the results of the studies on the ripening process of rice plant, using the radio-biochemical techniques.

In Chapter II, the results on the translocation of the photosynthetic products assimilated by the top leaf of rice

plant at the ripening stage is presented, with the results in the case of wheat plant. By application of $^{14}CO_2$ to the top leaf, we are able to determine the contribution of the photosynthetic products assimilated by this leaf to the grain's carbon. The accumulation of phosphorus absorbed at each stage of growth in the grains was studied (Chapter III). From this study, it is confirmed that phosphorus absorbed before the ripening stage is the main source for the grains. In Chapter IV, the metabolic behaviors of phosphorus thus accumulated in the grains are described, that is, the phosphorus compounds of the grains were estimated during the ripening process, and by the feeding experiments of the labeled phosphate, metabolic significance of phytic acid in the ripening rice grains is established. Because at the end of maturing, over 80% of phosphorus of the grains was found as phytic acid and it is supposed that inositol play a role in the accumulation of phosphorus as an acceptor of phosphorus, in Chapter V, the studies about the formation and function of inositol in the ripening rice grains are presented. Phytic acid occurs as a calcium or magnesium salt in the grains, then the accumulation of calcium and strontium in the rice grains was studied (Chapter VI). The results obtained in this chapter also give the fundamental

data on the contamination of rice grains by the fission products.

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Chapter II. TRANSLOCATION OF PHOTOSYNTHETIC PRODUCTS ASSIMILATED BY THE TOP LEAF TO THE EAR OF RICE AND WHEAT PLANTS.*

INTRODUCTION

It has been generally supposed that the carbon contained in the grains of rice plant, mostly in the form of starch, is mainly derived from the photosynthetic products of the leaves after the flowering stage, from the study on the changes of the organic components in each part of the plant at each stage of its growth or from the shading experiments (1,2,3). Therefrom it has been assumed that the carbon dioxide assimilation after the flowering directly affects the crop yield, and consequently, the effects of the nutritional and other environmental factors on the crop yield are attributed to the capacity of photosynthesis of the plant after the flowering.

In the case of rice plant, the carbon assimilated by

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* The contents of Chapter II were announced at the regular meeting of Kansai and Chubu branch of the Society of Soil and Manure, Nagoya, on 16th July, 1960 and appeared in Mem.Res.Inst.Food Sci., Kyoto Univ., No.22, 1 — 11(1960)

the leaves is the main source of the carbon in the grain (4), unlike barley in which sometimes considerable part of the carbon in the grains are attributed to the carbon assimilated by the ear (5). Of all leaves of a rice plant, the top leaf contributes mostly to the formation of the grains by its high percentage of the translocation of the photosynthetic products to the ear, as recognized by Fujiwara and Suzuki (6) and Tanaka (4).

The results presented here is concerned with the fundamental problems on the translocation of the photosynthetic products assimilated by the top leaf to the grains of rice and wheat plants, such as 1) the rate of translocation, 2) effect of light on the top leaf, 3) distribution of the assimilation products in the route of translocation, 4) chemical form of translocation and 5) disturbance of translocation by the relation between phyllotaxis and inflorescence, from the tracing of $^{14}CO_2$ assimilated by the top leaf.

EXPERIMENT

Culture of Plants: Rice and wheat plants grown by the sand and gravel culture method were used (7). On the 10th day after flowering, i.e., at the milk stage, $^{14}CO_2$ fixation

was performed.

$^{14}CO_2$ Fixation and Sampling: The top leaf was sealed in a glass tube (Fig. 1, B, 40 cm in length and 2.5 cm in diameter). After the pressure in "A" and "B" was reduced, lactic acid in "C" was dropped into "A" to produce $^{14}CO_2$ from $Na_2{}^{14}CO_3$. Then the carbon dioxide free air was introduced to "A" and "B", and during a definite time $^{14}CO_2$ assimilation was performed, under natural light. The concentration of carbon dioxide in the photosynthetic tube was 1%. The experiments of $^{14}CO_2$ assimilation was carried out between a.m. 10 — 12. After the fixation, $^{14}CO_2$ in the tube was replaced with air. To avoid the reassimilation of $^{14}CO_2$ produced by the respiration (8) of the top leaf,

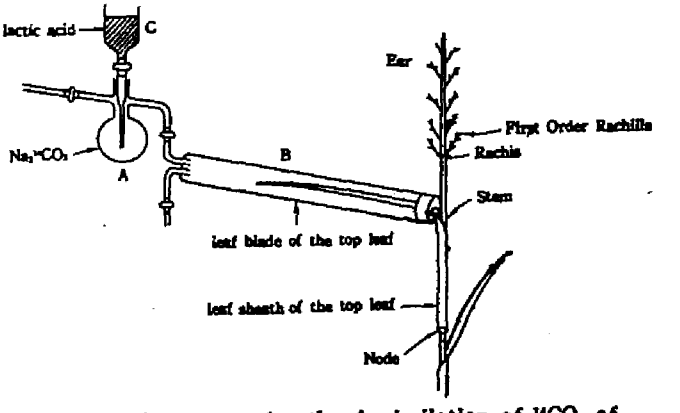

Fig. 1. Apparatus for the Assimilation of $^{14}CO_2$ of the Top Leaf.

the air in the tube B was replaced by fresh air several times before sampling. After a definite time, the plants were divided into leaf blade and leaf sheath of the top leaf, stem, grains and others, and each material was killed in boiling 80% ethanol for 1 min.

Fractionation and Determination: The materials were homogenized with 80% ethanol and sand, and were fractionated into alcohol soluble (supernatant) and insoluble fractions (residue), by centrifugation. In the case of wheat, amino acids, organic acids and sugar phosphate esters among the alcohol soluble fraction were removed by Amberlite IR-120 (H^+) and IRA-410 (OH^-) and the fractions adsorbed by these resins were eluted with 1 N NH_4OH and 1 N HCl, respectively. The parts that passed through the columns of these resins were concentrated under reduced pressure and were separated by paperchromatography (Buthanol-Acetic acid-Water: 4:1:5 (v/v), upper layer, three multiple developments). In the case of rice, separation by paperchromatography was performed with the alcohol soluble fraction. Distribution of the radioactivity of ^{14}C on the paperchromatograms was determined by a G.M. counter through 5 mm slit width. Radioactivity of the alcohol insoluble fraction was deter-

mined as $BaCO_3$ after the wet combustion (9) and the self absorption was corrected. Determination of the radioactivity of the other material was made, as usual, by a G.M. counter.

RESULTS

The translocation of the photosynthetic products from the top leaf of rice and of wheat is shown in Figs. 2 and 3 and Table 1. In Figs. 2 and 3, most part of the ^{14}C in the route of the translocation, i.e., leaf sheath of the top leaf, node and stem, was recovered in the alcohol soluble fraction, therefore, the total ^{14}C and the concentration of ^{14}C in each 3 cm section in the route of the translocation to the ear were determined by measuring the radioactivity of the alcohol extracts and are shown in Figs. 4 and 5. The distribution of ^{14}C on the paperchromatograms of the alcohol extracts are shown in Figs. 7 and 8, and the percentage of ^{14}C in each compound calculated from these chromatograms are shown in Table 2. The results in Table 1 were also calculated by the same method. The contents of ^{14}C in each first order rachilla (cf. Figs 1 and 11(a)) and the autoradiograms showing the distribution of ^{14}C in each part of rice plant are shown in Figs. 9 and 11.

DISCUSSION

Rate of Translocation

The rate of the translocation of the photosynthetic products to the grains from the top leaf was about 5 and 15% of the total $^{14}CO_2$ assimilated for an hour and determined at the 3rd and 5th hour after the assimilation, respectively (Figs. 2 and 3). Though the distribution of ^{14}C in the lower parts of the stem was observed (Figs. 3 and 11), its contents were low. From these results it is supposed that the assimilation products of the top leaf mainly translocated to the grains. This agrees with the study on the translocation of the photosynthetic products of each leaf at the ripening stage of rice plant (4,5). The ratio of ^{14}C found in the grains was not so large, and the most parts of the ^{14}C was retained in the top leaf, during these periods. However compared with the experiment, in which $^{14}CO_2$ fixation was performed by the whole rice plant, and the ^{14}C found in the grains was 17% of total ^{14}C at the 24th hour after the assimilation, we can suppose that the translocation from the top leaf to the grains was relatively active.

Effects of Light in the Top Leaf

From Table 1, it is suggested that the interruption

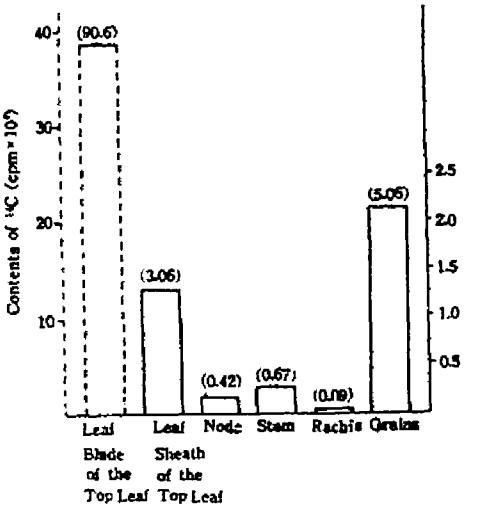

Fig. 2 Rate of Translocation of Photosynthetic Products Assimilated by Top Leaf in Each Part of Rice Plant.
Assimilation of top leaf was carried out for an hour in $^{14}CO_2$ and at 3rd hour after that contents of ^{14}C was determined. Values in parentheses are percentage of ^{14}C in each part of the plant.

of the photosynthesis caused by the dark treatment of the top leaf after the $^{14}CO_2$ assimilation did not affect the translocation of ^{14}C to the grains. Then the increase of the concentration of sugars due to the hydrolysis of starch formed during photosynthesis in the dark treated leaf, as generally supposed, does not result the active translocation from the top leaf. The most parts of the photosynthetic products are sugar, as it was the case in this experiment (Table 1), the active translocation of the photosynthetic

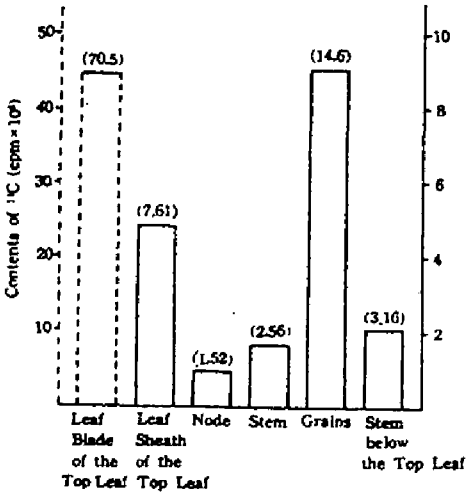

Fig. 3 Rate of Translocation of Photosynthetic Products Assimilated by Top Leaf in Each Part of Rice Plant.
Assimilation of top leaf was carried out for an hour in $^{14}CO_2$ and at 5th hour after that contents of ^{14}C was determined. Values in parentheses are percentages of ^{14}C in each part of the plant.

Table 1 Translocation of Photosynthetic Products Assimilated by Top Leaf in Each Part of Wheat Plant. (cpm x 10^4)
Assimilation of top leaf was carried out for 2 hours in $^{14}CO_2$ and at 6th hour after that contents of ^{14}C was determined. Dark treatment of top leaf was performed by covering photosynthetic tube with black paper, after the assimilation of $^{14}CO_2$.

Parts	Leaf blade of the top leaf		Leaf sheath of the top leaf		Stem		Grains	
Treatments	Control	Dark	Control	Dark	Control	Dark	Control	Dark
Sucrose	19.4	17.1	2.31	3.36	3.95	3.94	2.23	2.12
Glucose	0.435	5.40	0.098	0.168	0	0	0	0
Fructose	0.621	2.25	0.012	0.066	0	0	0	0
Maltose (?)	—	—	—	—	—	—	0.105	0.093
Fraction adsorbed to resins*	11.7	10.1	1.14	1.43	0.190	0.663	0	0.201
Total	32.2	34.9	3.56	5.02	4.14	4.60	2.34	2.41

* Amberlite IR-120 (H^+) and IRA-410 (OH^-)

products assimilated in the day time is not expected in the night.

Distribution of ^{14}C in the Route of Translocation

From ^{14}C contents in each 3 cm section in the route of translocation to the ear, it was observed that there existed the concentration gradients in the leaf sheath, stem and rachis at 3rd hour after the assimilation of $^{14}CO_2$ (Fig.4), but at 5th hour after the assimilation no gradients was observed (Fig. 5). Increase of the total ^{14}C in the lower part of the leaf sheath of the top leaf may be due to the increase of the leaf sheath's area per 3 cm section. Between the distance of the migration and log of the concentration of isotopes in stem or leaves, recently, reverse proporational relation was observed with respect to ^{14}C, ^{32}P and ^{3}H (11,12), and from this relation, the calculation of the translocation velocity of nutrients was attempted. The concentration gradients at 3rd hour, observed in this experiment, was not so sharp. It may be due to the lack of the supply of $^{14}CO_2$ after the assimilation of $^{14}CO_2$, since the reverse proportional relation in the leaf sheath of the top leaf and stem was observed in the experiment that the ^{32}P-phosphate solution was supplied to the top leaf of the rice plant continuously before sampling (Fig.6). Therefrom,

it is supposed that the low concentration gradients of ^{14}C result from the fact that the amounts of ^{14}C transported to the route of translocation from the top leaf was the same as that received by the ears or from the fact that the transport of ^{14}C to the route of the translocation from the top leaf was depressed. From Figs. 2 and 3, during this interval, the contents of ^{14}C in the grains increased, so it is supposed that the former was the case.

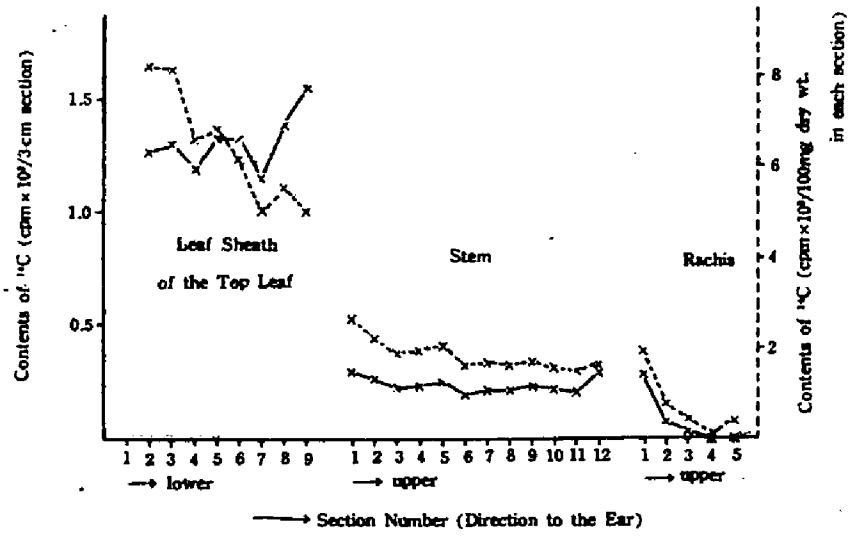

Fig. 4 Distribution of Photosynthetic Products Assimilated by Top Leaf of Rice Plant in Route of Translocation to Ear (3 hours).

Experimental conditions were same as in Fig. 2. Solid and dotted lines represent total ^{14}C and concentration of ^{14}C in each 3 cm section, respectively.

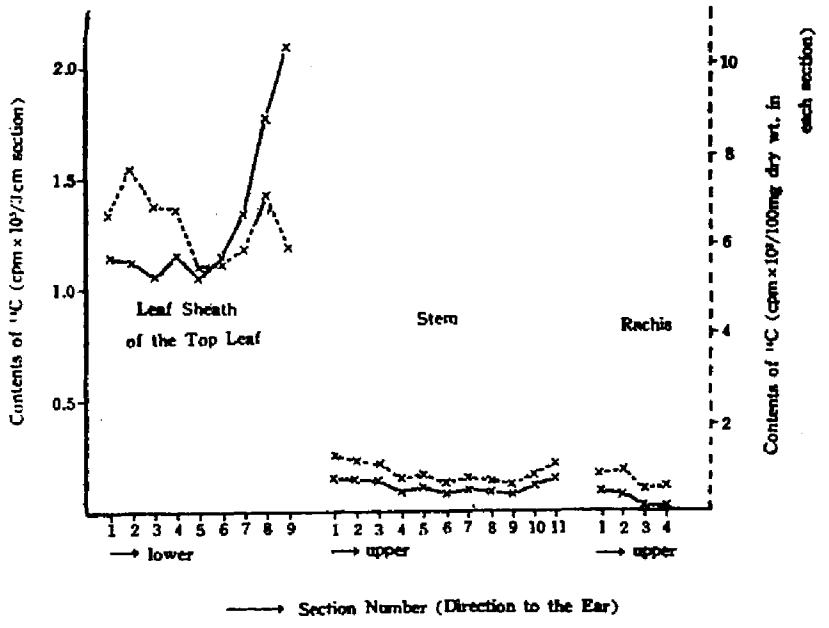

Fig. 5 Distribution of Photosynthetic Products Assimilated by Top Leaf of Rice Plant in Route of Translocation to Ears (5 hours).

Experimental conditions were same as in Fig. 3. Solid and dotted lines represent total ^{14}C in each 3 cm section, respectively.

Chemical Form of Translocation

Since the ^{14}C labeled compound in the route, i.e., leaf sheath, node and stem, was mainly sucrose, in both rice and wheat (Table 1 and Figs. 7 and 8, Table 2), it is supposed that the chemical form of the translocation of the photosynthetic products from the top leaf to the ear is sucrose. From Table 2, it is observed that, to the stem

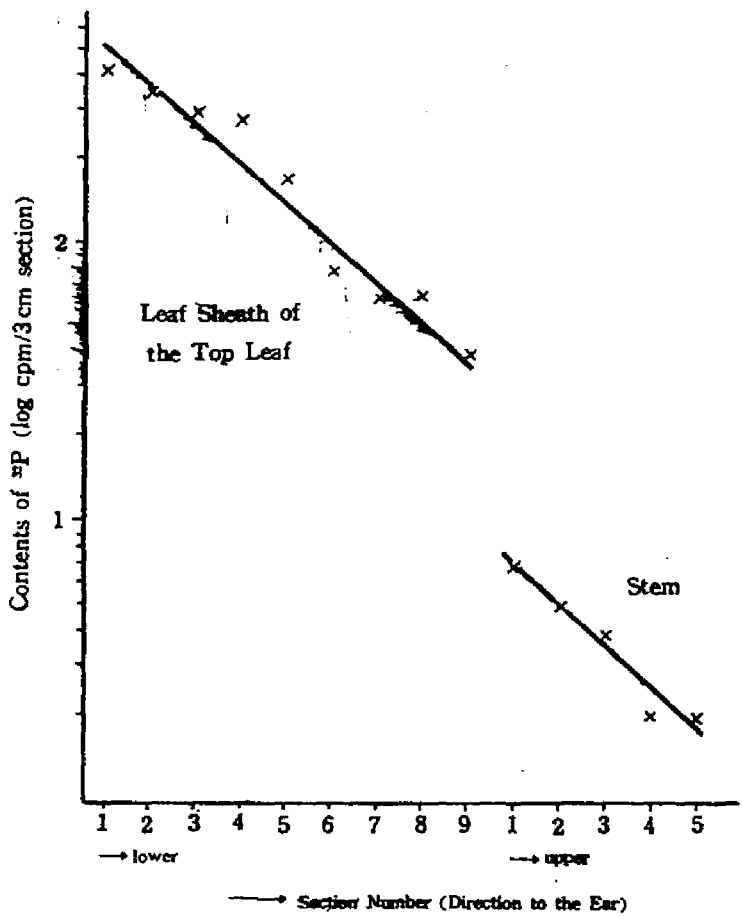

Fig. 6. Distribution of ^{32}P Absorbed by Top Leaf of Rice Plant in Route of Translocation to Ear.

Top leaf, of which end part was cut off to leave the base 15 cm, was immersed in ^{32}P-phosphate solution and after 8 hours, ^{32}P in each 3 cm section was determined.

below the top leaf, the photosynthetic products was translocated also in the form of sucrose. Translocation of the photosynthetic products in the form of sucrose was also observed by Tanaka with rice (4), and was shown with the other plants (12, 13, 14). Because in the $^{14}CO_2$-feeding experiment, sucrose is labeled faster than hexoses and starch, it is suggested that the primary free sugar produced by photosynthesis is sucrose (14, 15). As shown in Table 1, the main photosynthetic products in the top leaf was sucrose even after several hours after the assimilation of $^{14}CO_2$, then it is supposed that the sucrose translocated to the grains is the direct product of the photosynthesis and is not the secondary products, for example, the hydrolysis products from starch. From the viewpoint of the preservation of the chemical energy obtained by photosynthesis of the plant, the translocation of sugar in the form of sucrose prevents the loss of energy, due to the preservation of the glycosidic bond of sucrose molecule, and its utilization on the synthesis of starch in the grains is expected (16, 17, 18).

Distribution of ^{14}C in Each First Order Rachilla and the Relation between Phyllotaxis and Inflorescence

From Fig. 9, it is observed that the amounts of ^{14}C

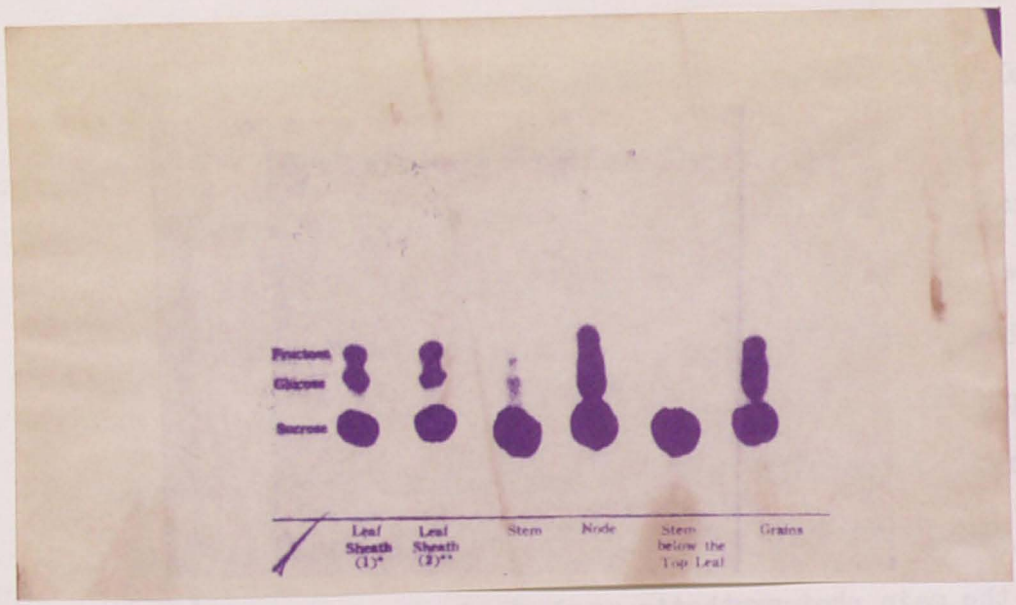

Fig. 7 Form of ^{14}C Labeled Compounds in the Route of Translocation.
Autoradiograms showing distribution of ^{14}C on paperchromatograms of ethanol extracts in each part of rice plant.
Experimental conditions of $^{14}CO_2$ assimilation and sampling were same as in Fig. 3.
* upper half, ** lower half

Table 2. Percentages of ^{14}C in Each Compound Found in the Route of Translocation.

Parts	Leaf Sheath of the Top Leaf (1)*	Leaf Sheath of the Top Leaf (2)**	Node	Stem	Grains	Stem below the Top Leaf
Sucrose	95.3	93.2	85.6	89.6	88.2	95.7
Glucose & Fructose	3.9	4.3	9.3	5.6	8.2	2.2
Others	0.8	2.5	5.1	4.8	3.6	2.1

* upper half, ** lower half

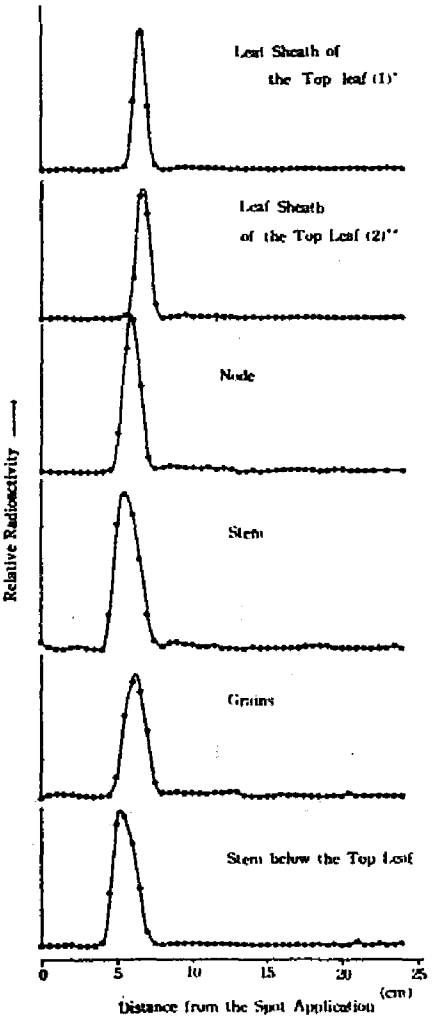

Fig. 8. Distribution of ^{14}C on the Paperchromatograms of Fig. 7.
* upper half, ** lower half

in each first order rachilla (cf. Figs. 1 and 11(a)) was irregular. Previously, Inosaka (19) explained the distribution of ^{32}P in each first order rachilla of the ear absorbed from each leaf of rice plant, from the relation between the phyllotaxis and inflorescence. The relation between phyllotaxix having 1/2 and the inflorescence of the ear of the rice plant having 2/5 is illustrated schematically in Fig. 10. In this figure, the first order rachilla having number 1, 3, 4 and 6, which contained relatively large

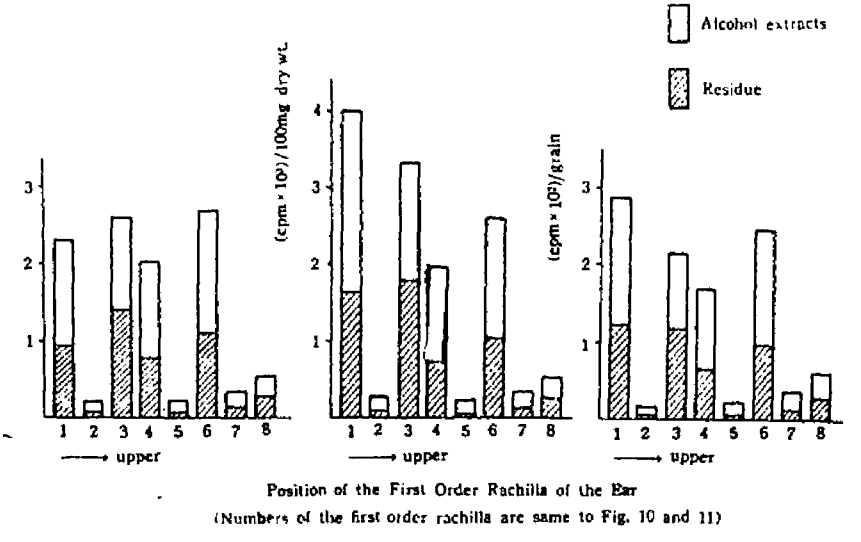

Fig. 9 Distribution of Photosynthetic Products Assimilated by Top Leaf of Rice Plant in Each First Order Rachilla of Ear.
Experimental conditions of $^{14}CO_2$ assimilation and sampling were same as in Fig. 3.

amounts of ^{14}C (Fig. 9), is located near the top leaf. On the other hand, the first order rachilla of the numbers 2 and 5 is located far from the top leaf and the contents of ^{14}C in these first order racilla was small (Fig. 9). From these relations, it is supposed that the photosynthetic products assimilated by the top leaf flowed in the nearest parts of the stem attached to the top leaf, and each first order rachilla of the ear uptook the photosynthetic products from its nearest part of the cross section of the stem.

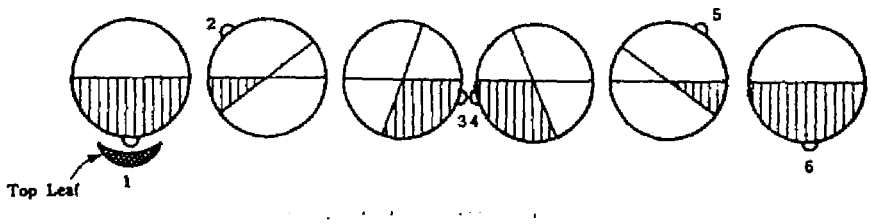

Fig. 10 Relation of Situation between Phyllotaxis and Inflorescence of First Order Racilla of Ear of Rice Plant.
The numbers are those of first order rachilla from the lower to upper and are same as in Figs. 9 and 11.

Then the contents of ^{14}C in each first order rachilla is determined by the area overlapped by the two parts of the nearest regions of the top leaf and the first order rachilla of the ear (parts marked strips in Fig. 10). These areas

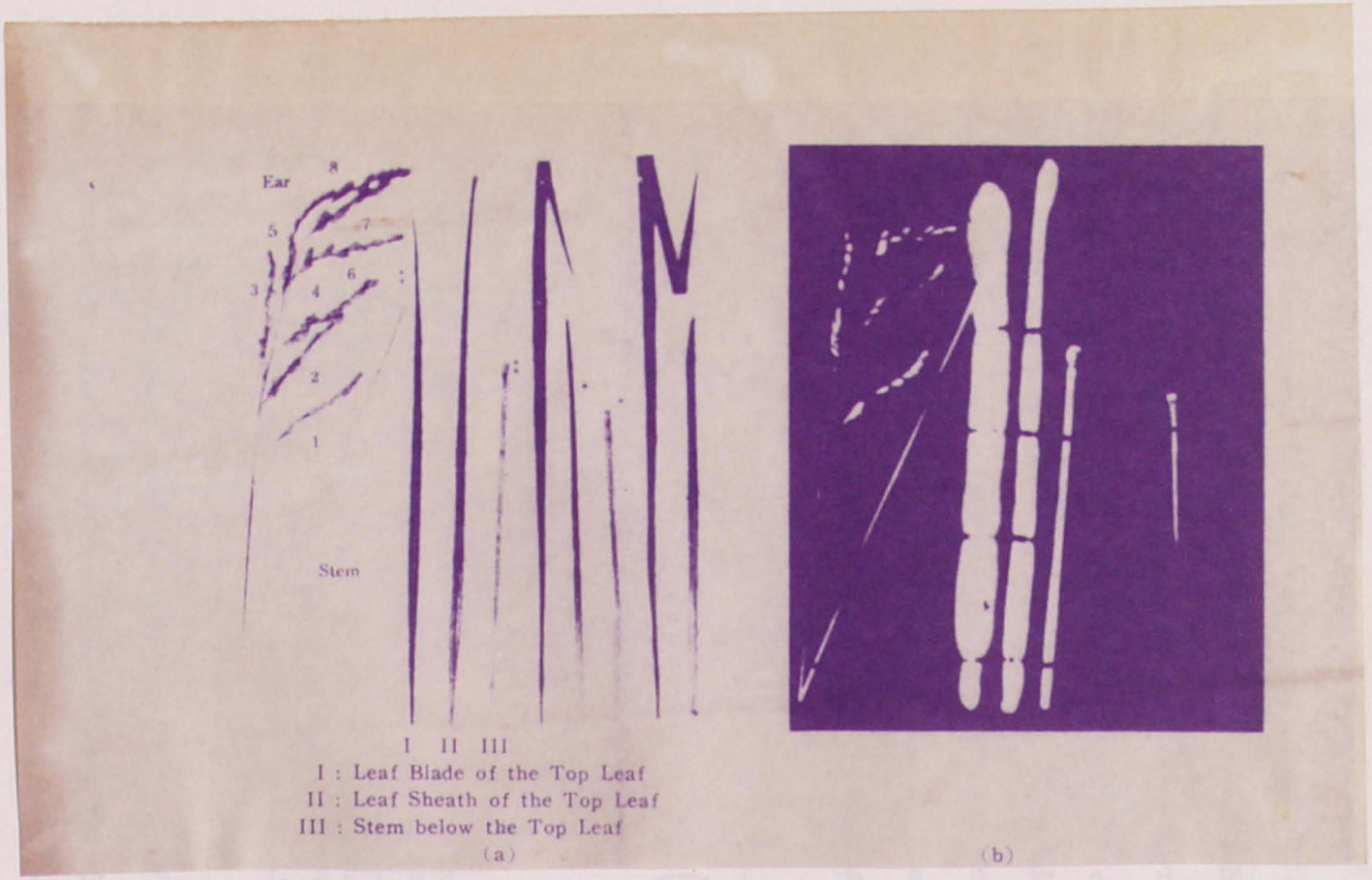

Fig. 11 Autoradiogram Showing Distribution of ^{14}C Assimilated by Top Leaf in Each Part of Rice Plant, Especially in Each First Order Rachilla of Ear.

Experimental conditions were same as in Fig. 3. (a) is plant material for autoradiography and (b) is autoradiogram. Numbers of first order rachilla of ear are same as in Fig. 10.

in each first order rachilla from the numbers 1 to 6 are succesively 1, 1/5, 3/5, 3/5, 1/5 and 1. The results obtained in this experiment, as shown in Fig.9 and in autoradiogram of Fig. 11, show the same tendency of the decrease and increase as the hypothesis described above. Recently, the results showing the fact that the distribution of the nutrients affected by the morphological factor have been obtained (19, 20, 21, 22, 23, 24), also more directly from the observation of the isotopes in stem microautoradiographycally (20, 22, 23). The results presented here show that the distribution of the photosynthetic products from the top leaf to the ear of rice plant is disturbed morphologically, though this disturbance is gradually removed by the lateral movements of the photosynthetic product in the stem during a long time.

SUMMARY

The translocation of the photosynthetic products from the top leaf of rice and wheat at the development was investigated by tracing the $^{14}CO_2$ assimilated by the top leaf. The results obtained are as follows;

1) At 3rd and 5th hour after the assimilation of $^{14}CO_2$, about

5 and 15% of ^{14}C was found in the grains.

2) Translocation to the grains was not affected by the interruption of the photosynthesis of the top leaf.

3) The concentration gradients of the ^{14}C in the route of the translocation was very low, may be due to the lack of $^{14}CO_2$ after the assimilation and the balance between the transport from the top leaf and the uptake by the grains.

4) Photosynthetic products translocated in the form of sucrose.

5) Distribution of ^{14}C in each first order rachilla of the ear of rice plant was affected by the relation of situation between the phyllotaxis and inflorescence of the first order rachilla.

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Chapter III. BEHAVIOR OF PHOSPHORUS ABSORBED AT EACH STAGE OF GROWTH IN RIPENING PROCESS OF RICE PLANT*

INTRODUCTION

The role of mineral nutrients absorbed at each stage of growth of crops has been investigated by many workers, in numerous ways and from various viewpoints. By the application of isotope techniques, the behavior of the nutrients absorbed at a definite period of growth stage of plant can clearly trace. Kasai et al studied the role of mineral nutrients such as ^{32}P, ^{45}Ca and ^{35}S, by applying them to plants at each stage of growth and by determination of the distribution of the respective nutrient in each part of plants upon harvest. (1) This chapter deals with the results of the investigation on the differences of roles

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* The contents of Chapter III were announced at Isotope Conference of Japan, on 4th Feb., 1958 and at the regular meeting of Kansai and Chubu branch of the Society of Soil and Manure, Kyoto, on 14th Dec., 1958 and appeared in Mem. Res. Ins. Food Sci., Kyoto Univ., No.18, 22 — 31 (1959) and in Dojohiryogaku zasshi, 30, 479 — 482 (1960) (in Japanese).

of phosphorus absorbed at each stage of growth of rice plants cultured at low and high phosphorus levels, in their ripening process, by ascertaining the following points. 1) Distribution of ^{32}P absorbed at each stage of growth in each part of the crops and 2) in acid soluble and insoluble fractions of phosphorus of the grains, at the harvest.

EXPERIMENT

Culture of Crops : Seedlings of rice plant (Kyoto Asahi) having no tiller were chosen and were transplanted on 24th June, 1957, three seedilings in each pot. The plants were cultured by the customary sand and gravel culture mathod, with 1.5 l of the culture solution (Table 1). The plants were cultured at two phosphorus levels, viz., a low phosphorus (P: 5 pp m) and high phosphorus levels (P: 10 ppm). The culture solution was not circulated, but it was renewed every five days. The decrease of the culture solution due to evaporation and absorption was supplemented by tap water every day. Process of growth, i.e., the stages of growth, the length of grasses and the number of tillers are shown in Table 2. The growth of the plants were normal, except at the stage of tillering a slight chlorosis due to iron deficiency was observed. This chlorosis was cured

by a foliar application of EDTA-Fe solution. Any distinct difference in the growthes of the plants due to the different application of phosphorus, i.e., low and high phosphorus treatments, was not observed. After 13th Sept., the culture solution of one half concentration other than phosphorus, and after 4th Oct., only water were supplied, respectively.

Table 1 Composition of Culture Solution

Elements	Form of Fertilizer	Concentration (ppm)
N	NH_4NO_3 and $Ca(NO_3)_2$	70
K	KCl	16
Ca	$Ca(NO_3)_2$	12
Mg	$MgSO_4 \cdot 7H_2O$	10
S	$MgSO_4 \cdot 7H_2O$	12.8
Fe	EDTA-Fe	1.0
B	$Na_2B_4O_7 \cdot 10H_2O$	0.2
Mn	$MnSO_4 \cdot 7H_2O$	0.5
Zn	$ZnSO_4 \cdot 7H_2O$	0.05
Cu	$CuSO_4 \cdot 5H_2O$	0.02
P	$Na_2HPO_4 \cdot 12H_2O$	High phosphorus treatment—10 Low phosphorus treatment— 5

Treatment with ^{32}P: During five days shown in Table 2 (Experimental No. 1 -- 9), phosphorus labeled with ^{32}P was supplied. In this experiment, the radioactivity of ^{32}P corresponding to the quantity of phosphorus in the culture solution was measured and the quantity of phosphorus labeled

with ^{32}P (P*) was illustrated by the absolute value of phosphorus, in spite of radioactivity of ^{32}P.

Table 2 Stage of Growth of Rice Plant and the Period Treated with ^{32}P

Experiment No.	Period treated with ^{32}P	Length of grasses (cm) Low-P	High-P	Number of tiller (per plant) Low-P	High-P	Stage of Growth
	24/VII	48.8	44.3	3.0	3.0	
	4/VIII	61.9	55.3	5.4	5.5	
1	8/VIII	67.1	63.4	7.6	7.6	Tillering
	14/VIII	70.2	69.0	8.6	7.7	
2	18/VIII					Ear-forming
	24/VIII	75.4	74.9	8.8	8.0	
3	28/VIII					
	29/VIII					
4	2/IX	82.7	79.2			Booting
	8/IX					
5	12/IX	100.3	93.4			Flowering
6	13-17/IX					
7	18-23/IX					
8	24-28/IX					Milk stage
9	29/IX-3/X					
	at harvest (21/X)	102.2	98.2	6.9**	7.1**	Maturing

* Only this period was six days.
** Number of ears.

Sampling and Determination: Rice plants were harvested on 21st Oct., that was the maturing stage, and were divided into grains, leaves and stems and roots. ^{32}P and phosphorus in each part of the plants were determined, as usual. The first five ears of each plant were checked at the flowering stage and the grains obtained from these ears were used in the following experiment.

Fractionation of Phosphorus Compounds of the Grains: With 10% cold trichloroacetic acid 7.5 g of the grains, that had been taken the hulls off, was groumded and was fractionated into acid soluble- (supernatant) and insoluble-phosphorus (residue). The residue was re-extracted twice with 5% cold trichloroacetic acid and the extracts were combined. Radioactivity of ^{32}P and phosphorus in the acid soluble and insoluble fractions were measured, by the usual method.

RESULTS

The quantities of ^{32}P absorbed at each stage of growth and its distribution in each part of the rice plant are shown in Figs. 1, 2 and 3. The ratio of ^{32}P absorbed at each stage of growth in each part of the plants are shown in Fig. 4. The ratios of phosphorus absorbed at each stage

of growth in each part of the plants are shown in Fig. 4. The ratios of phosphorus absorbed at each stage of growth to total phosphorus in each part of the plants, i.e., μ g P*/mg P, are illustrated in Figs. 5 and 6. The average value of the dry weight and phosphorus contents (A) of the plants at the harvest are shown in Table 3. The phosphorus absorbed during the treatment with ^{32}P (after 4/VIII) (B) and the phosphorus absorbed before the treatment with ^{32}P (before 4/VIII) (A - B = C) were calculated and are shown in Table 3. In the calculation of (B), as the amounts of phosphorus absorbed during the period without the treatment with ^{32}P, for example 3/IX - 7/IX, average value of the phosphorus absorbed before and after that period were taken. The results above illustrated are based on the value per pot (three plants). Distribution of ^{32}P absorbed at each stage of growth and μg P*/mg P in each fraction of the grains are shown in Fig. 7 and Table 4, respectively. Phosphorus contents (A'), phosphorus absorbed during the treatment with ^{32}P (B') and the phosphorus absorbed before the treatment with ^{32}P (A' - B' = C) per gram of the grains are shown in Table 5. The value of (B') was calculated in the same manner as in the case of (B).

DISCUSSION

Absorption of Phosphorus at Each Stage of Grwoth and Its Distribution at Harvest

The amount of phosphorus absorbed at each stage of growth by the high phosphorus treatment plant was larger than that by the low phosphorus treatment plant, up to the flowering stage, but after that stage, the situation reversed (Fig. 1). Since no difference was observed in the growth of the plants between the two phosphorus treatments, it may be assumed that the difference in the amount of phosphorus absorbed was not due to the difference in the stage of growth. ^{32}P in the grains of both treatments showes the tendencies similar to the total amounts of phosphorus absorbed at each stage of growth (Fig. 2). From Fig. 2, it is seen that the amounts of translocation to the grains of ^{32}P absorbed after the flowering stage show a decrease in the high phosphorus treatment, as compared with the low phosphorus treatment. Translocation of phosphorus absorbed after the flowering stage to the leaves and stems also showes a decrease, and an accumulation of ^{32}P is particularly seen in the roots. This is observed conspiciously in the plants cultured with high phosphorus (Fig. 3). These observation agree with the results previously obtained with wheat, sesame and rape plants (1).

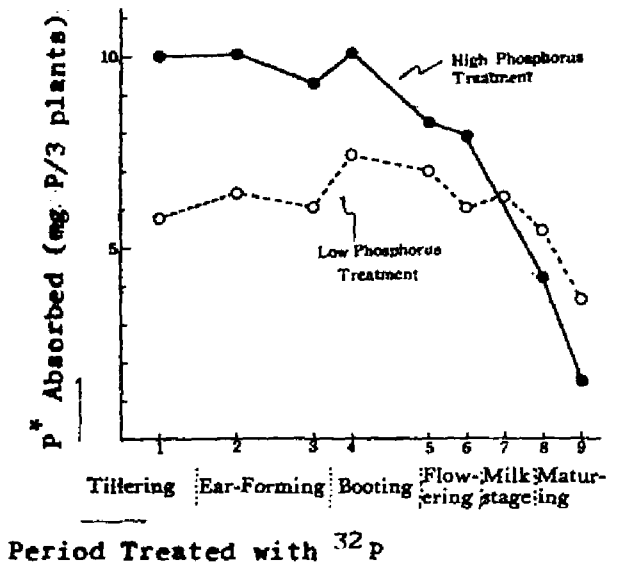

Fig. 1 Absorption of ^{32}P at Each Stage of Growth of Rice Plant

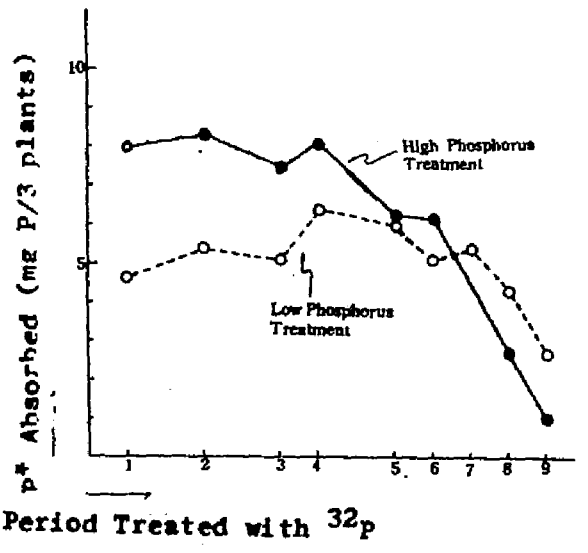

Fig. 2 Distribution of ^{32}P Absorbed at Each Stage of Growth in Grains of Rice Plant

The results described above are clearly seen in the ratio of phosphorus absorbed at each stage of growth in every parts of the plants, as shown in Fig. 4. From this figure, it is seen that the translocation of phosphorus absorbed at each stage of growth to the grains in the ripening process was enhanced to such an extent that over 60% of phosphorus absorbed, even at milk stage, viz., last stage of growth, was translocated to the grains, and this phenomenon is more conspicious in the low phosphorus treatment. As may easily be supposed from these results, at the later stage of growth, the values of μg P*/mg P of the leaves and stems, and of grains decreased and that of the roots increased (Figs. 5 and 6). These trends are more marked in the high phosphorus treatment. This figure also shows that the value of μg P*/mg P of the leaves and stems throughout all the period of application of ^{32}P was lower than the other parts. This is because of the facts that the phosphorus in the leaves and stems, which had been absorbed before the treatment with ^{32}P (C), was larger than the phosphorus absorbed during the treatment with ^{32}P (B), as compared with the other parts of the plant (Table 3).

From these observations, it is suggested that the phosphorus absorbed before the treatment with ^{32}P (before

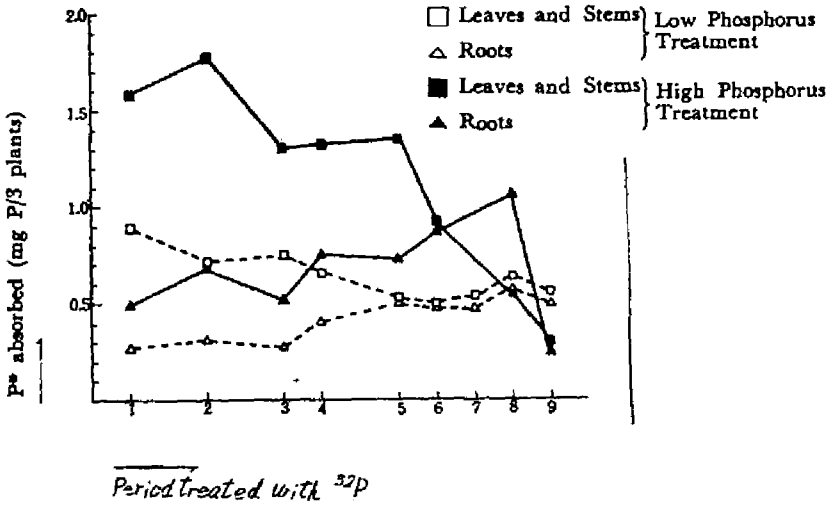

Fig. 3 Distribution of ^{32}P Absorbed at Each Stage of Growth in Leaves and Stems and Roots of Rice Plant.

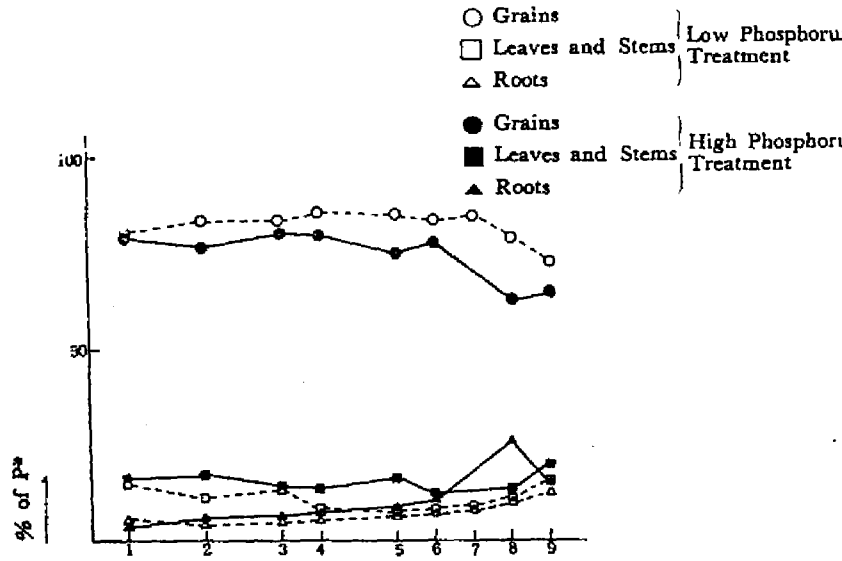

Fig. 4 Distribution of ^{32}P Absorbed at Each Stage of Growth in Each Part of Rice Plant. (% of P*)

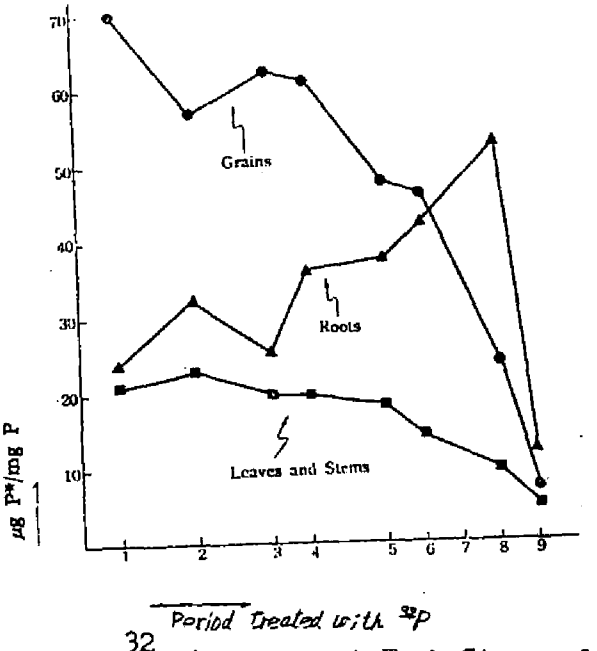

Fig. 5 Ratio of ^{32}P Absorbed at Each Stage of Growth to Total Phosphorus in Each Part of Rice Plant Cultured with High Phosphorus.

the later period of tillering) did not turn over with the phosphorus absorbed after that period, and that it was not translocated to the other part, especially to the grains, but remained in the leaves and stems. As one of the reason of this fact, it is supposed that the phosphorus absorbed at an early stage of growth of the plant precipitated as Fe-phosphate in the cell walls by EDTA-Fe foliar applied during the experiment and went outside of metabolism (3). But in this respect, further experiments have to be carried

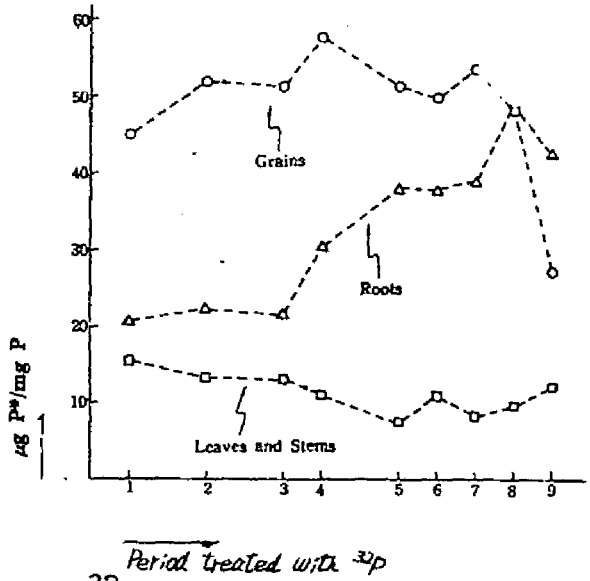

Fig. 6 Ratio of ^{32}P Absorbed at Each Stage of Growth to Total Phosphorus in Each Part of Rice Plant Cultured with Low Phosphorus.

out, in order to ascertain whether this is also observed with rice plant of normal growth.

The changes of the amounts of ^{32}P absorbed at each stage of growth and the accumulation of ^{32}P absorbed at the later stage of growth in the roots agree with the results of Kasai et al (2), in which a similar ^{32}P treatment was applied to rice plant and the harvest was made at milk stage. It should be mentioned in this connection, however, that in the previous experiment (2), the phosphorus absorbed at each stage of growth was chiefly detected in the leaves

and stems, since the harvest was made at the milk stage. Though it may be inadequate to compare directly the results of these two sets of experiment, we can learn the movement of phosphorus absorbed at each stage of growth during the milk stage and maturing. From the fact that the distribution ratio of ^{32}P in the roots remained equal in the two experiments, as compared with that in leaves and stems, it is deduced that the phosphorus translocated to the grains from milk stage to maturing almost originates from the leaves and stems.

Distribution of Phosphorus Absorbed at Each Stage of Growth in Acid Soluble and Insoluble Fractions of Grains.

With regard to the phosphorus contents in the acid soluble and insoluble fractions of the grains (Table 5), it is observed that in the plants of the high phosphorus treatment, the amounts of acid soluble phosphorus is rich, but the acid insoluble phosphorus shows no difference between the high and low phosphorus treatments. Over 90% of acid soluble phosphorus is phytin phosphorus (4), and these results agree with the findings by Fujiwara and Nihashi (5).

Table 3. Dry Weight, Phosphorus Contents and Phosphorus Absorbed before and during Treatment with ^{32}P in Each Part of Rice Plant

(The Values are based on 3 plants)

Treatment	Parts of plant	Dry Wt. (g)	Phosphorus contents(A) (mg P)	Phosphorus % (dry Wt.base)	Phosphorus absorbed during the treatment with ^{32}P (B) (mg P)	Phosphorus absorbed before the treatment with ^{32}P (A-B = C) (mg P)
Low phosphorus treatment	Grains	46.0	102.2	0.22	61.0	41.2
	Leaves and stems	42.6	58.8	0.14	7.9	50.9
	Roots	9.0	12.8	0.14	4.9	7.9
	Total	97.6	173.8		73.8	100.0
High phosphorus treatment	Grains	44.7	127.7	0.29	75.1	52.6
	Leaves and stems	41.3	68.5	0.17	14.3	54.2
	Roots	8.0	20.5	0.26	8.2	12.3
	Total	94.0	216.7		97.6	119.1

Table 4. The Ratio of ^{32}P Absorbed at Each Stage of Growth to Total Phosphorus in Each Fraction of Grains of Rice Plant (g P*/mg P)

Period treated with ^{32}P	Low phosphorus treatment		High phosphorus treatment	
	Acid insoluble-P	Acid soluble-P	Acid insoluble-P	Acid soluble-P
1	35.1	37.6	63.0	62.1
2	42.8	44.4	54.9	52.8
3	32.8	42.8	49.4	47.1
4	56.4	57.5	64.6	63.8
5	39.5	38.7	36.6	53.2
6	44.9	42.9	36.2	40.7
7	41.3	49.5	—	—
8	35.5	55.8	25.5	30.4
9	21.5	26.8	5.8	7.4

The ratio of ^{32}P absorbed at each stage of growth in each fraction of the grains was the same with total phosphorus, i.e., ^{32}P and phosphorus were distributed at the same ratio in each fraction of the grains. As may be supposed from Fig.7, the values of μg P*/mg P in the two fractions are found closely resembling. These observations naturally suggest that phosphorus absorbed at each stage of growth turned over each other in the grains. However, as shown in Table 4, after the flowering stage, the value of μg P*/mg P of acid insoluble phosphorus is lower than that of acid soluble phosphorus and this is remarkably observed in the low phosphorus treatment. From these results, it is considered that phosphorus absorbed after the flowering stage was easily incorporated to the acid soluble phosphorus and that the acid insoluble phosphorus formed at an early period of ripening did not turn over with the phosphorus translocated after the formation of acid insoluble phosphorus in the grains.

The conclusion above described, however, is applicable only to the mutual relations between the acid soluble and insoluble fractions of the grains, so that there may be certain inconsistencies with respect to the incorporation of ^{32}P absorbed at each stage of growth in the individual

Table 5. Phosphorus Contents and Phosphorus Absorbed before and during the treatment with ^{32}P in Each Fraction of Grains of Rice Plant.

(The values are based on gram of grains)

Treatment	Fractions	Phosphorus contents(A') (mg P)	Phosphorus absorbed during the treatment with ^{32}P (B') (mg P)	Phosphorus abosrbed before the treatment with ^{32}P (A'-B' = C') (mg P)
Low phosphorus treatment	Acid insoluble-P	0.732	0.347	0.387
	Acid soluble-P	1.641	0.870	0.771
High phosphorus treatment	Acid insoluble-P	0.778	0.418	0.360
	Acid soluble-P	2.457	1.382	1.075

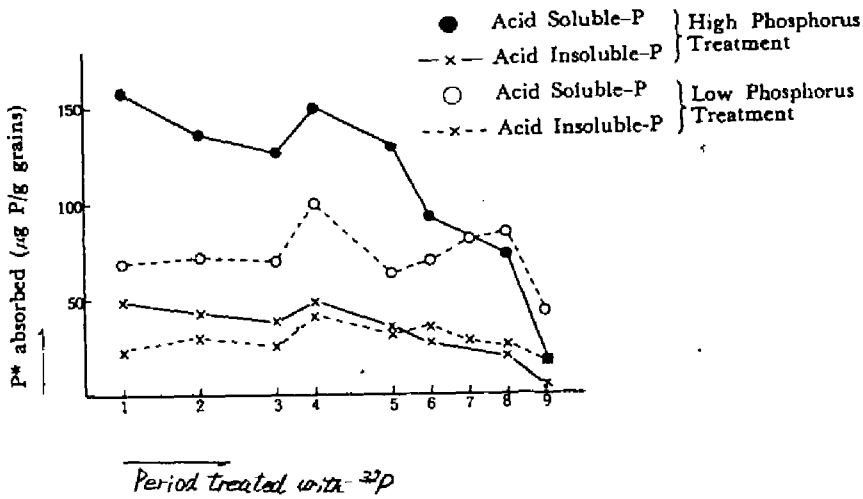

Fig. 7 Distribution of ^{32}P Absorbed at Each Stage of Growth in Each Fraction of Grains of Rice Plant

phosphorus compounds and histological distribution of ^{32}P. As a means to clarify the histological and chemical uniformity of ^{32}P in the grains, the germination of the grains obtained in the above experiments was studied. The grains were germinated in the culture solution that did not contain phosphorus and the seedlings were sampled at the 5th and 7th day after sowing. The seedlings were divided into the synthetic organ (leaves and roots) and the decomposing organ (endosperm) and the comparison of μg P*/mg P between the two organs was made. However, any distinct difference in any sample could not be found. In

other words, no histological and chemical ununiformity on the distribution of ^{32}P was observable in the grains, through germination.

SUMMARY

1. To study the role of phosphorus absorbed at each stage of growth in ripening of rice plants cultured at low and high phosphorus levels, distribution of ^{32}P at the harvest, that was supplied as a tracer at each stage of growth, in each part of the plant and in acid soluble and insoluble fractions of the grains were investigated.

2. Absorption of phosphorus decreased after the development of ears in the case of rice plants cultured with high phosphorus, but the plants cultured with low phosphorus continued the absorption. From 60 to 80% of phosphorus absorbed at each stage of growth was translocated to the grains. The translocation of phosphorus absorbed after flowering to the grains decreased and its accumulation in the roots increased. These tendencies were conspicious in the case of the plants cultured with high phosphorus.

3. Distribution of phosphorus absorbed at each stage of growth in acid soluble and insoluble fractions of the grains was uniform, and it is suggested that phosphorus absorbed at each stage of grwoth in the grains turned over each

other. But the incorporation of phosphorus absorbed after the flowering to acid soluble fraction increased slightly.

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Chapter IV. PHOSPHORUS METABOLISM IN RIPENING RICE GRAINS

Section I. ACID-SOLUBLE NUCLEOTIDES IN RIPENING RICE GRAINS*

INTRODUCTION

Accumulation of starch, protein and other components in the grains of rice plant during the ripening process is one of the prominent features characterizing the change to the reproductive growth from the vegetative growth. Despite the fact that it has been assumed that in these processes nucleotides play a role, especially in the energy transformation and as a co-factor of enzymic action, no survey on the nucleotides in the ripening rice grains was performed. But several studies are found on the other phosphorus containing compounds. Fractionation of phosphorus

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* The contents of Chapter IV, Section I were announced at the annual meeting of the Society of Soil and Manure, Tokyo, on 2nd April, 1962 and appeared in Soil Sci. & Plant Nutrition, 9, 13 — 17 (1963)

compounds in the ripening rice grains was studied by Aimi and Konno (1) and by author (2). Kurasawa et al (3) identified glucose-1-phosphate from the ripening rice grains.

On the nucleotides in plant materials, our information is poor compared with that in the animal and microorganism, partially due to the troubles in the separation and identification of these substances from plant materials. Recent findings that nucleotides are adsorbed on charcoal with comparative specificity, however, gives a means of the separation of nucleotides from the other miscellaneous components, and by the application of this principle Bergkvist (4) analysed the total acid-soluble nucleotides of wheat plant.

In this section, the separation and identification of the acid-soluble nucleotides of the ripening rice grains are presented. The separation was carried out according to the following procedures: extraction by perchloric acid, adsorption on charcoal, elution from charcoal and separation by ion exchange chromatography and identification of the peaks on the chromatogram by paperchromatography.

EXPERIMENT

Plant Material : The rice plant (Kyoto Asahi) grown in the farm of Kyoto University was used. The ears were sampled at about 10 days after flowering (at milk stage) and the grains from these ears were used immediately.

Extraction : One thousand grams of the grains (about 40,000 grains) was ground thoroughly with 3 l of 0.5 M perchloric acid and together with silica sand, in a cold room (5°C). All operations described below were carried out below 5°C, except chromatography. After the homogenate was allowed to stand an hour, the perchloric acid extract was obtained by centrifugation at 1,500 g for 10 min. The residue was re-extracted with 2 l of 0.2 M perchloric acid and the supernatant obtained by centrifugation was combined with that of the first extraction. Then the perchloric acid extract was filtered with super cell in Büchner funnel to remove fine precipitate. Total E_{260}* of the perchloric acid extract was 39,400.

Adsorption on Charcoal: To 4.3 l of the perchloric acid extract 50 g of charcoal (Norit SX-30) was added and the

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* Total E_{260} = Optical density at 260 m μ in 1 cm light path x Volume (ml)

adsorption of nucleotides was performed for 60 min. with stirring. Then the charcoal was filtered and was washed with water, until no acidic reaction was observed. Total E_{260} of the filtrate was 3,280, so the total E_{260} of 36,120 was adsorbed on charcoal.

Elution from Charcoal : The procedures described below were typical. A part of charcoal, corresponding to total E_{260} of 8,210, was eluted with the mixture of aqueous 10% pyridine and 60% ethanol (5), three times. After the evaporation of the solvent in vacuo, the nucleotides obtained were dissolved with water. Based on E_{260}, in this step complete elution from charcoal was observed. The nucleotides solution was made acidic (pH 1.5) with 0.04 N HCl and was extracted with ether for 9 hours by the apparatus of Kutscher and Steul, with an ice cold sample flask, to remove the pigments and the other disturbing substances (4). After this treatment, the ether contained in the solution was removed by the aeration, and pH of the solution was adjusted to 6 with 0.1 N NH_4OH. Total E_{260} of the solutionmthus obtained was 4,750.

Ion Exchange Chromatography : Twenty five milliliters of the anion exchange resin, Dowex-1 (X-10, 200 - 400 mesh), which was made free from fine and coarse particles by sedimentation

and decantation, was poured into column (22 x 1.2 cm) as an aqueous slurry. The resin was converted to formate form by applying 200 ml of 6 M sodium formate. After this treatment, the column was washed with 70 ml of water and then with 70 ml of 85% formic acid. Then the column was washed with water until pH of the effluent was about 5. The nucleotides solution obtained above was applied to the column at the flow rate of 0.6 ml/min. The column was washed with about 400 ml of water, until E_{260} of the washings was below 0.1. Total E_{260} found in the washings was 2,387. A gradient elution of "formic acid" system, according to Hurbert et al (6), was carried out starting with 790 ml of 4 M formic acid as the reservior and 450 ml of water in the mixing chamber, at the flow rate of 0.4 ml/min. The elution was continued with the following solvents in the reservior without changing the contents of the mixing chamber: 450 ml of 4 M formic acid and 0.2 M ammonium formate, 675 ml of 4 M formic acid and 0.4 M ammonium formate, and 450 ml of 4 M formic acid and 0.8 M ammonium formate. Total E_{260} recovered in the effluents was 1,633, when E_{260} of the blank is 0.12. Total E_{260} in each step are summarized in Table 1.

Ultraviolet Absorption : Optical density of each effluent

fraction (5.7 ml) at 260 and 275 mμ was measured in 1 cm light path. E_{275}/E_{260} was used as characterization of the kinds of the bases. Absorption spectrum in ultraviolet region of the concentrated solution of each peak, obtained by the procedures described below, was measured.

Table 1. Extraction of Acid-soluble Nucleotides from Ripening Rice Grains.

step	total E_{260}/1,000 g grains in fresh weight
Perchloric acid extract	39,400
A part adsorbed on charcoal	36,120
Charcoal eluate, after ether extraction	20,900
A part adsorbed on Dowex-1	10,400
Recovered in effluents of chromatography	7,190

Paperchromatography : The fractions in each peak on the column chromatogram were collected, and to this solution charcoal was added. After two hours the charcoal was collected by centrifugation and was washed three times with 40 ml of water. The nucleotide adsorbed on charcoal was eluted overnight by the addition of 5 ml of 60% aqueous

ethanol containing 1% NH_4OH. The concentrated nucleotide solution thus obtained was subjected to paperchromatography, using the following solvent systems (7): a) Ammonium sulfate-0.1 M Phosphate buffer (pH 6.8)-Propanol, (150 g: 250 ml: 10 ml), b) iso-Propanol-conc.HCl-Water (v/v) (65: 16.7: 18.3) and c) iso-Butyric acid-conc. NH_4OH-Water, (v/v) (33: 0.5: 16).

RESULTS AND DISCUSSION

The ion exchange chromatogram, employing the gradient elution of "formic acid" system is shown in Fig.1. Elution by "ammonium formate" system of Hurbert et al (6) was also carried out with the same sample, which is not reproduced here. The nucleotides in each peak were identified, based on the following observations: comparison of the position of the peaks on the ion exchange chromatogram of the two solvent systems with that of Hurbert et al (6), the ratio of E_{275}/E_{260}, absorption spectrum (230 - 340 mμ) and paper-chromatography. Approximate quantities of nucleotides calculated from E_{260}, using the molar absorbancy of Bergkvist (4), are shown in Table 2. As a molar absorbancy of DPN and TPN*, that of oxidized form (16.5×10^3) was used.

* In regard to the abbreviation used in this section, see the note of Fig. 1.

It is found that some peaks on the column chromatogram have several components, as described in the note of Fig.1. For this cause, the values presented in Table 2 are approximate one.

From these results presented here, the occurrence of the nucleotides is confirmed, having different bases, at the different levels of phosphorylation, in the ripening rice grains. The absence of CMP and CDP may be due to their low contents because of the overlapping with the other nucleotides on the chromatogram. Identification was performed only for 5'-monophospho nucleotides, hence the presence of 2'- or 3'- monophosphonuclectides in the ripening rice grains was not determined. These 2'- or 3'-monophospho nucleotides may be found in the unidentified peaks on the column chromatogram or in the unidentified spot on the paperchromatogram.

As is found in Table 2, about 80% of the total nucleotides of the ripening rice grains, in moles, is existent as the adenosine and uridine derivatives, and the contents of the cytidine and guanosine derivatives are very low. In wheat plant, the predominance of adenosine and uridine derivatives was also observed (4). The presence of uridine derivatives in the ripening rice grains, with high contents, is of interest for their special role in the metabolism of sugar.

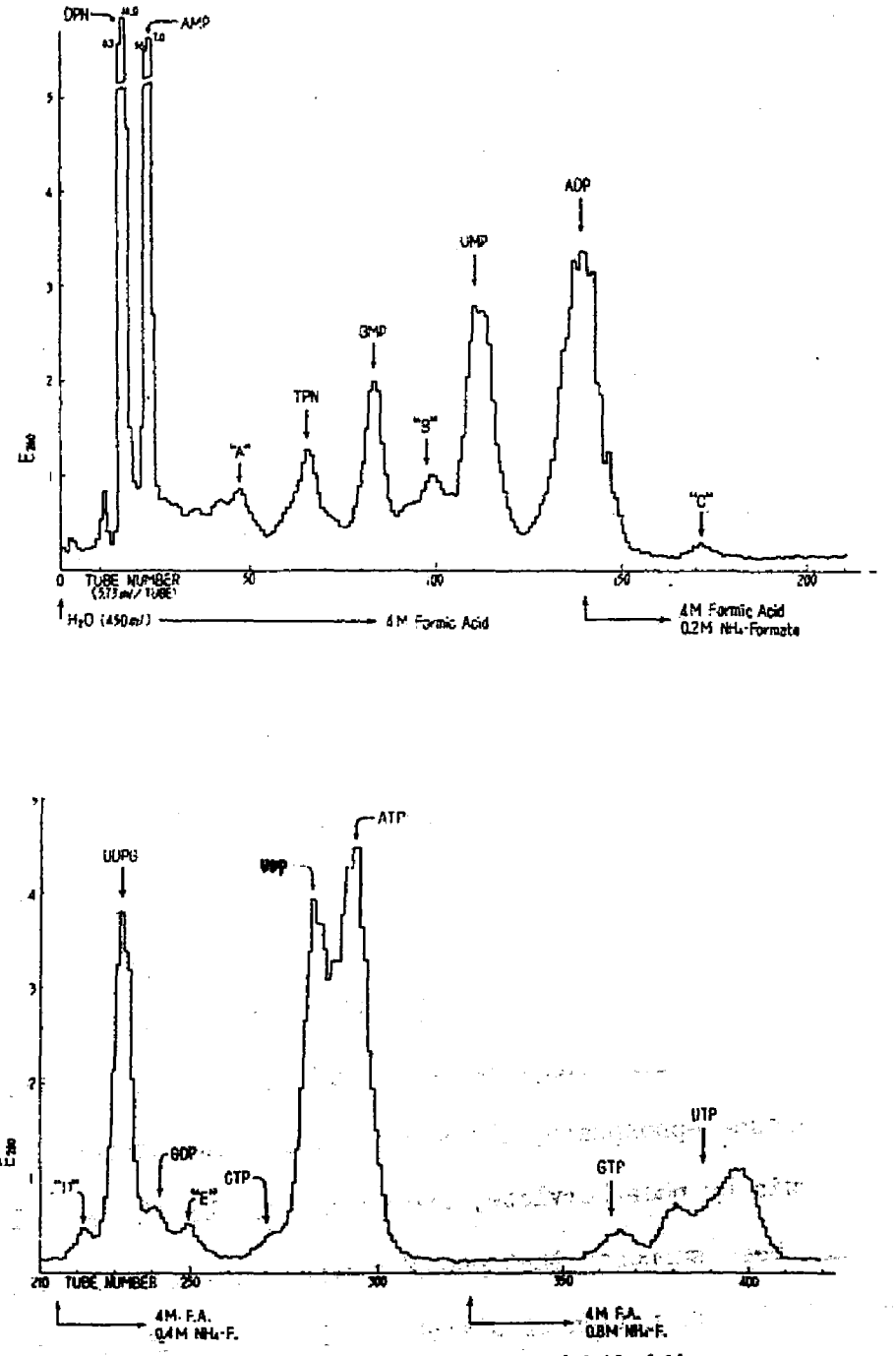

Fig. 1. *Ion Exchange Chromatogram of Acid-soluble Nucleotides from Ripening Rice Grains*

Fraction	Note (PC.: paperchromatography)
DPN: diphosphopyridine nucleotide	
AMP: adenosine-5′-monophosphate	unidentified, E_{max} was not found in UV region.
"A"	other one unidentified spot was observed by PC.
TPN: triphosphopyridine nucleotide	other two unidentified spotes were observed by PC.
GMP: guanosine-5′-monophosphate	GMP and other two unidentified spotes were observed by PC.
"B"	other one unidentified spot was observed by PC.
UMP: uridine-5′-monophosphate	
ADP: adenosine diphosphate	unidentified
"C"	unidentified
"D"	glucose was identified by PC. after the material was hydrolysed in 0.1 N HCl, at 100℃, for 5 min.
UDPG: uridine diphospho glucose	
GDP: guanosine diphosphate	unidentified
"E"	E_{275}/E_{260} was 1.4.
CTP: cytidine triphosphate	spot of ATP was also observed by PC.
UDP: uridine diphosphate	
ATP: adenosine triphosphate	
GTP: guanosine triphosphate	other one unidentified spot was observed by PC.
UTP: uridine triphosphate	

To elucidate the route of biosynthesis of starch from sucrose, the dominant form of carbon source for the starch syntehsis in the grains that is translocated from leaves (8), the presence of UDPG, with high contents, is of special interest. Turner et al (9, 10) in the study of the ripening pea plant supposed that UDPG is the intermediate to glucose-1-phosphate, the substrate for the starch synthesis by phosphorylase, from sucrose. Until recently it has been supposed that phosphorylase play a predominant role in the formation of starch or glycogen. It is now known, however, that the second pathway of glycogen or

starch synthesis in animal or in plant (11 - 13) is also functioned, that is, UDPG is the direct substrate for the glycogen or starch synthesis. Though the presence of phosphorylase in the ripening rice grains was confirmed (14, 15), no survey is found on the second pathway of starch synthesis in ripening rice grains.

Table 2. Contents of Acid-soluble Nucleotides in Ripening Rice Grains.

nucleotide	*μ moles*/1,000 g in fresh weight
DPN	42
AMP	30
TPN	(18)
GMP	(31)
UMP	(67)
ADP	72
UDPG	56
GDP	7
UDP	67
ATP	84
GTP	8
UTP	50
Adenosine derivatives	246
Uridine derivatives	240
Guanosine derivatives	46
Cytidine derivatives	trace

Value in bracket is an approximate one, for the presence of unidentified nucleotides. See the note in Fig. 1.

SUMMARY

Analysis of the total acid-soluble nucleotides in the ripening rice grains (at milk stage) were performed according to the following procedures: extraction by perchloric acid, separation by charcoal treatment and separation and identification by ion exchange chromatography. The occurrence of 5' -mono-, di- and tri-phosphates of adenosine, uridine and guanosine, beside DPN, TPN, UDPG and CTP, was confirmed and their contents were determined.

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Section II FORMATION OF PHYTIN AND ITS ROLE IN THE RIPENING PROCESS OF RICE PLANT*

INTRODUCTION

It has been generally supposed that phytin** which is sometimes composed of over 80% of the total phosphorus contained in the cereal grain, is a storage form of phosphorus and that it is utilized in the process of germination (1). Fujiwara and Nihashi (2) observed that in the grains of rice plant cultured with high phosphorus fertilizers alone phytin showed an increase, while other phosphorus compounds remained unchanged. They suggested therefrom that phytin was a storage form of phosphorus that was translocated into the grains excessively for the process of ripening. On the other hand, from investigations

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* The contents of Chapter IV, Section II were announced at the regular meeting of Kansai and Chubu branch of the Society of Soil and Manure, Kyoto, on 14th Dec., 1958 and appeared in Mem.Res.Ins.Food Sci., Kyoto Univ., No.18, 32—40(1959) and in Dojohiryogaku zasshi, 30, 540—544(1960)(in Japanese).

** In this section, the terms of phytin and phytin derivatives are used to imply myo-inositol hexaphosphate and myo-inositol phosphate at lower levels of phosphorylation, respectively.

on phosphorylase in the grains of rice plant Aimi (3). suggested that, for the formation of starch in the grains by phosphorylase, it is required to exclude inorganic phosphorus from the site of action of phosphorylase and that myo-inositol is an acceptor of inorganic phosphorus. The work contained in this section is an attempt to study the role of phytin in the ripening process of rice plant cultured at low and high phosphorus levels by observing the following phenomena.

1) Changes of phosphorus compounds in the grains during the ripening process. 2) Incorporation of ^{32}P-phosphate absorbed through ear stalks at the milk stage in each fraction of the phosphorus compounds of the grains. 3) Incorporation of ^{32}P-phosphate absorbed through roots at the yellow ripening stage in each fraction of phosphorus compounds of the grains.

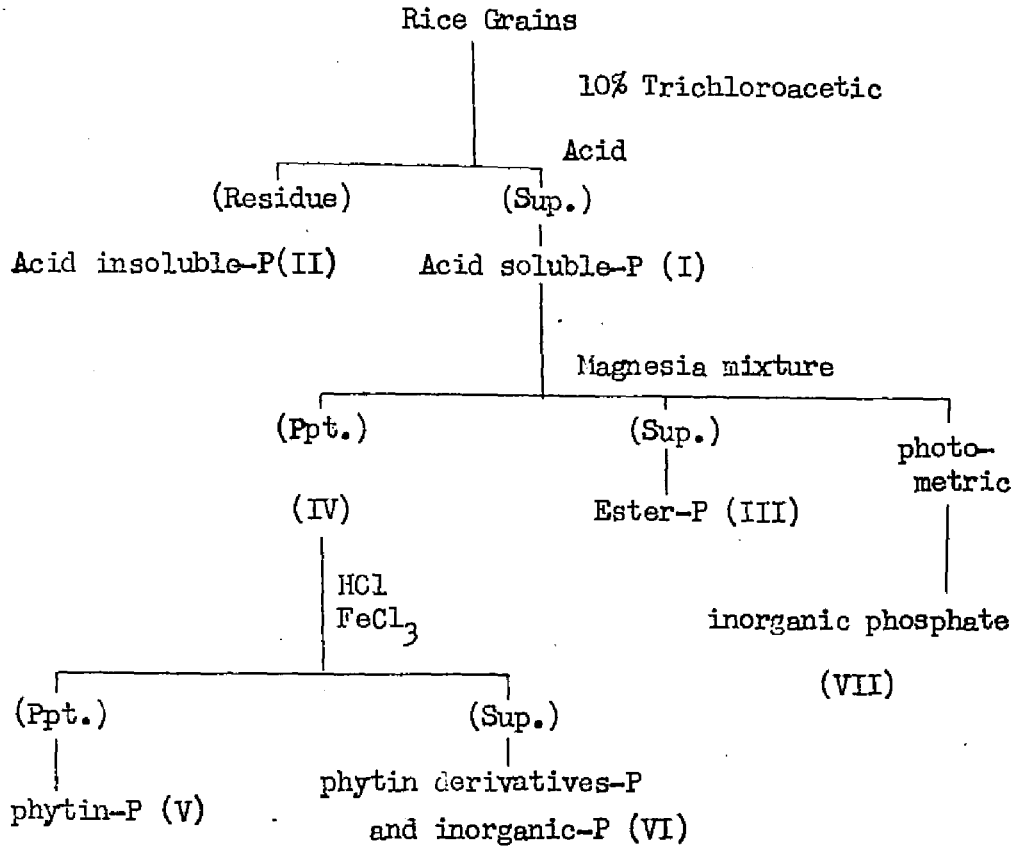
EXPERIMENT

Culture of Crops : Seedlings of rice plant (Kyoto Asahi) having no tiller were transplanted on 4th June, 1958 and were cultured by means of water culture. The composition of the culture solution was the same to that of Chapter III, but the concentration of Fe was 2 ppm. The plants were

cultured at the two phosphorus levels, viz., high phosphorus (P: 10 ppm) and low phosphorus treatments (P:5 ppm). The culture solution was renewed every seven days. After 2nd Sept., the culture solution of one half concentration except phosphorus was supplied. Process of growth was the same to that of Chapter III. The first five ears of each plant were checked at the flowering stage and were used in the following experiments.

Fractionation of Phosphorus Compounds: After flowering, three to five ears were sampled with the intervals of certain days during the ripening process. The ears sampled were homogenized with cold 10% trichloroacetic acid and were fractionated into acid soluble- (I) and insoluble-phosphorus (II), by centrifugation. Acid soluble-phosphorus was further fractionated into ester-phosphorus (III) (supernatant) and phytin- and phytin derivatives-phosphorus and inorganic phosphorus (IV) (precipitate), by adding magnesia mixture. Fraction IV was dissolved with 0.1 N HCl and the pH was adjusted about 2 by thymol blue. The solution of 1% of $FeCl_3$ was added to this solution (over 5 mole equvalents of Fe-phytate) and phytin (V) was made precipitate as Fe-phytate (5,6). The supernatant phosphorus was phytin derivatives- and inorganic phosphorus

Fig. 1 Fractionation of Phosphorus Compounds of Rice Grains



(VI). Inorganic phosphorus (VII) was determined photometrically by fraction I. Phytin derivatives-phosphorus (VIII) was determined by subtracting inorganic phosphorus from fraction VI. The schema of the fractionation are shown in Fig. 1. This experiment had been also performed in 1957 and the same results had been obtained.

Absorption of ^{32}P-phosphate through Ear Stalks : On the 12th day after flowering, the ears were taken and were cut off at the ear stalk. Then the ear stalk was steeped in the solution of 10 ppm ortho phosphate labeled with ^{32}P, at room temperature. In order to avoid contamination of ^{32}P having any other chemical forms than ortho phosphate, the ^{32}P-phosphate solution was heated in the boiling water bath for 2 hours before the experiment (7). At 2 hours after the steeping of the ear stalks, ^{32}P absorbed through ear stalks was detected in the grains. Then the grains were taken off and were placed in the test tubes having the cheesecloth moistured with water and were kept at 25°C. Grains were sampled at the intervals of two hours and were killed by homogenation with 10% trichloroacetic acid. Acid soluble-phosphorus (I) was neutralized with KOH and, after the addition of phosphate carrier (0.5 mg P) and the formation of phosphomolybdate by adding sulfuric acid and ammonium molybdate, ^{32}P in the form of inorganic phosphate was extracted by isobuthanol-benzene (1:1, v/v) mixture, three times (8). After the extraction of inorganic phosphate, by adding of magnesia mixture, ester-(III) and phytin- and phytin derivatives-phosphorus (IX) were separated. Radioactivity of ^{32}P and phosphorus contents in each fraction

were measured by the usual methods. Inorganic phosphate was determined photometrically by fraction I.

Absorption of ^{32}P-phosphate through Roots: From 9th to 16th Oct., the culture solution labeled with ^{32}P was supplied to the rice plants, through roots. The plants were harvested on 24th Oct. and the phosphorus compounds in the grains were fractionated in the same way as described above, and the radioactivity of ^{32}P and and phosphorus contents in each fraction were determined. In this case, fraction IX was further fractionated into phytin- and phytin derivatives-phosphorus by the same method, described above.

RESULTS

Changes in the fresh weight and in the phosphorus compounds of the grains are illustrated in Figs. 2, 3, 4 and 5. Distribution of ^{32}P absorbed through ear stalks in each fraction of the grains is shown in Fig. 6 and the specific activity in each fraction is shown by ^{32}P %/P % in Fig.6. Distribution of ^{32}P absorbed through roots and the specific activity in each fraction of the grains are shown in Fig. 8.

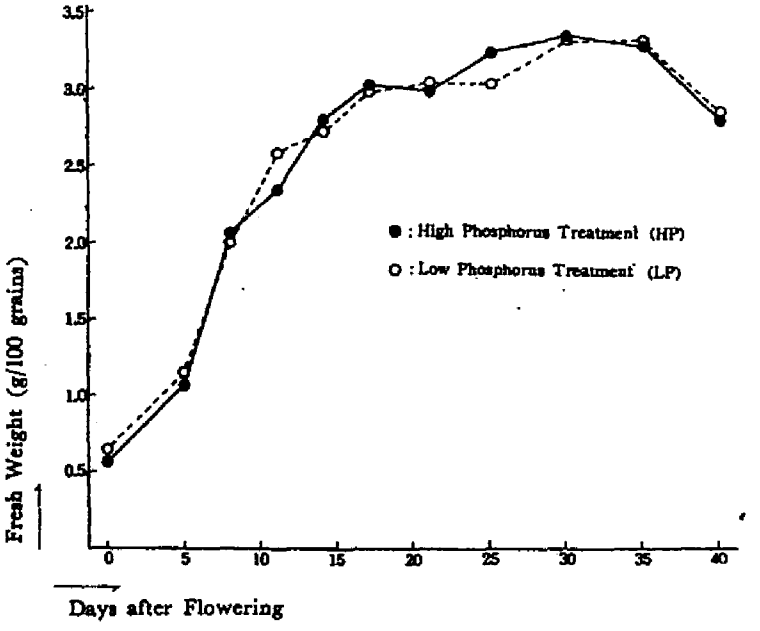


Fig. 2 Changes in Fresh Weight of Ripening Rice Grains

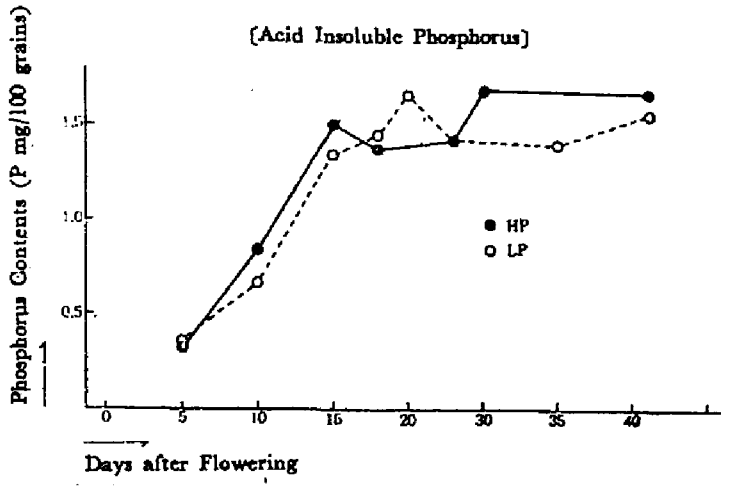


Fig. 3 Changes in Acid Insoluble-Phosphorus of Grains during the Ripening Process of Rice Plant Cultured at Two Phosphorus Levels.

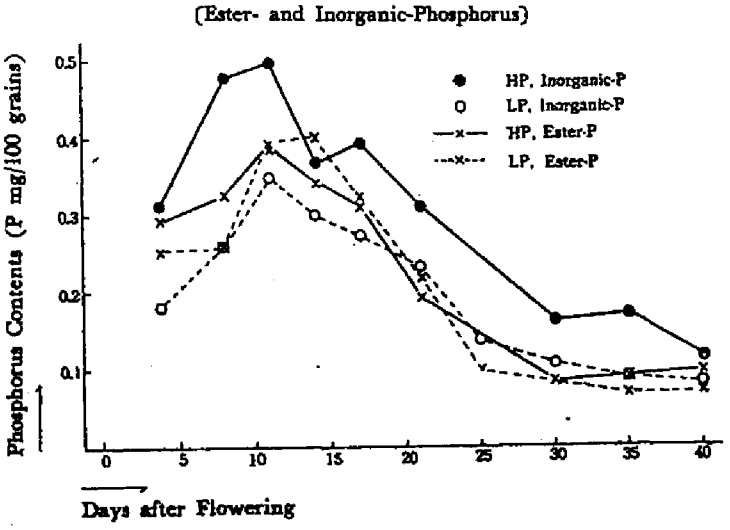


Fig. 4 Changes in Ester- and Inorganic-Phosphorus of Grains during Ripening Process of Rice Plant Cultured at Two Phosphorus Levels.

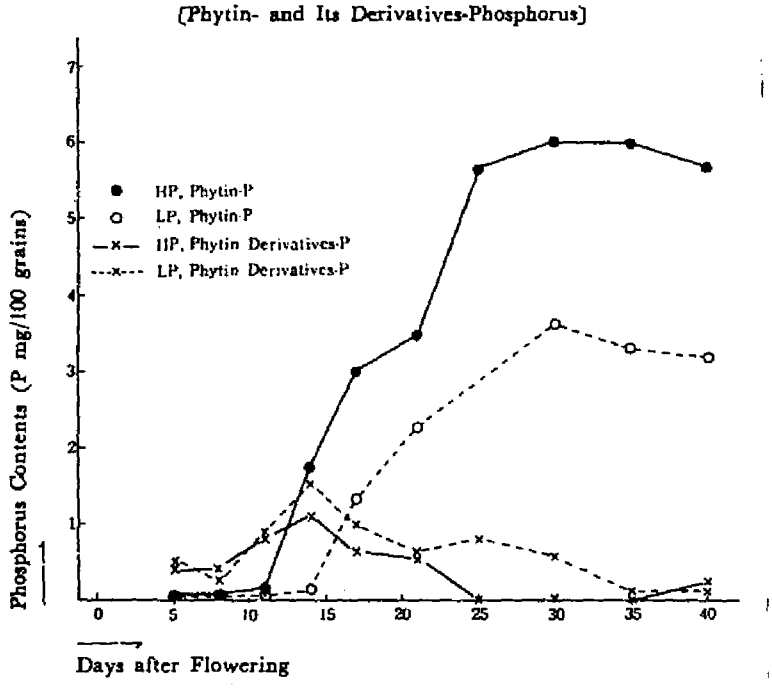


Fig. 5 Changes in Phytin- and Its Derivatives-Phosphorus of Grains during Ripening Process of Rice Plant Cultured at Two phosphorus Levels.

DISCUSSION

Changes of Phosphorus Compounds of Rice Grain

As shown in Fig. 3, it is observed that, from the changes of the phosphorus compounds in the ripening process, acid insoluble-phosphorus formed itself at an early period of ripening and reached its maximum on the 15th day after flowering. This observation appears to agree with the results that RNA was formed at an early stage of the ripening

process of rice plant (9), since it is supposed that one of the components of acid insoluble fraction is nucleic acid. Ester- and inorganic-phosphorus gradually increased up to the 10th day after flowering and after 25 days they showed a decrease (Fig. 4). During 10 - 25 days after flowering, as is supposed from the changes in fresh weight of the grains, starch accumulated in the grains, and this period corresponds to that having high contents of ester-phosphorus. Turner et al (12) likewise obtained results showing the facts that in the ripening process of peas the period observed high contents of glucose-6-phosphate corresponded to the period of starch accumulation. Phytin derivatives-phosphorus increased at an earlier period of the ripening process and decreased gradually as phytin-phosphorus increased. Phytin reached to maximum level at the 25th day after flowering (Fig.5). Though the separation of phytin- and phytin derivatives-phosphorus by Fe salt precipitation method was not accomplished very satisfactory, yet it may well be presumed that phytin is formed through phytin derivatives. It is widely known that, when phytin is decomposed by phytase, phytin derivatives are formed as an intermediate products and, in fact, these phytin derivatives have been separated by means of various methods

(13 - 18). However, the survey on phytin derivatives in the phosphorylation of myo-inositol is not found yet.

The results obtained above also agree with the observation by Aimi et al who investigated about the histological distribution of phosphorus compounds in the grains of rice during the ripening process (10).

The most striking difference which is noticeable with regard to phosphorus nutrition is the high contents of phytin-phosphorus, in the case of the high phosphorus treatment (Fig. 5). Phosphorus compounds other than phytin were not affected by the phosphorus levels of the culture solution, except the fact that inorganic-phosphorus showed slightly high value in the high phosphorus treatment.

Incorporation of ^{32}P-phosphate Absorbed through Ear Stalks in Each Fraction of Rice Grains

At the 12th day after flowering, when the experiment was performed, as shown in Fig. 4, it is observed that ester- and inorganic-phosphorus were at their maximum levels and that phytin- and acid insoluble-phosphorus showed a gradual increase. This period exactly corresponds to the early period of milk stage where an accumulation of starch was observed. At 2 hours after the absorption of ^{32}P-phosphate, it was found that ^{32}P had been already incorporated

into ester-phosphorus. The incorporation of ^{32}P into phytin gradually increased and then a decrease of ester- and inorganic-phosphorus followed (Fig. 6). The effect of the phosphorus nutrition upon the incorporation of ^{32}P was observed in the velocity of the formation of phytin- ^{32}P as well as in the ratio of ester- and inorganic phosphorus. The specific activities of phytin- and acid insoluble-phosphorus were far from that of ester- and inorganic phosphorus, as shown in Fig. 7, therefore, it is considered that phytin- and acid insoluble-phosphorus did not turn over with ester- and inorganic-phosphorus.

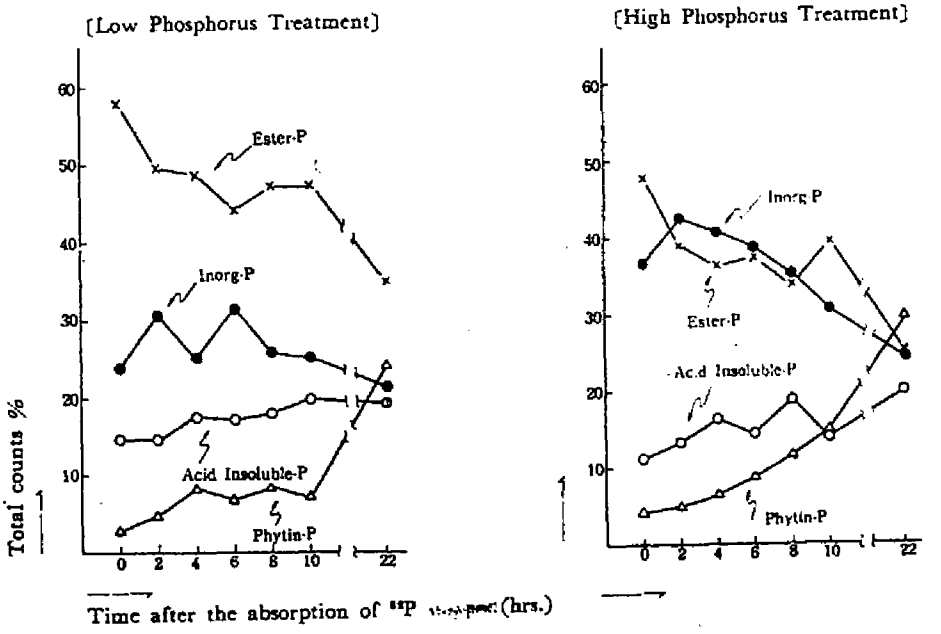


Fig. 6 Incorporation of ^{32}P-phosphate Absorbed through Ear Stalks in Each Fraction of Rice Grains (% of Total ^{32}P)

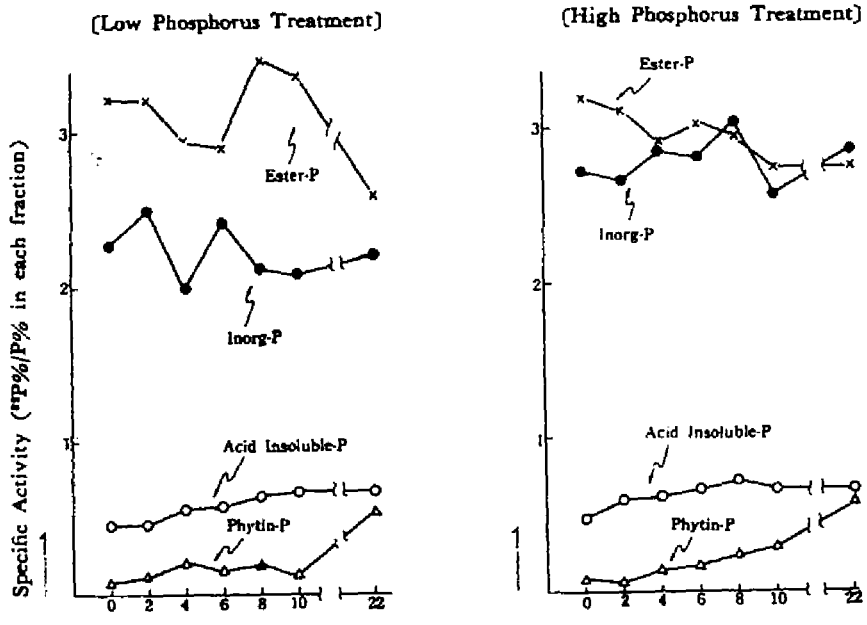


Fig. 7 Incorporation of ^{32}P-phosphate Absorbed thorough Ear Stalks in Each Fraction of Rice Grains (specific activity)

Incorporation of ^{32}P-phosphate Absorbed through Roots in Each Fraction of Rice Grains.

In the case of ^{32}P-phosphate absorbed through the roots at the yellow ripening stage (25 - 32 days after flowering), the absence of ^{32}P at the top of the ears was observed by autoradiography. At the time of harvest, it was found that over 70% of ^{32}P absorbed had incorporated

into phytin- and phytin derivatives-phosphorus (Fig.8). In agreement with the results of Chapter III, the value of the specific activity of acid insoluble-phosphorus was lower than the other fractions (Fig. 8). The lower specific activity of phytin appears to show that a large amounts of phytin had been already formed before the application of ^{32}P (Fig. 5) and that phytin did not turned over. From these observations we can deduced that phytin was the final product in phosphorus metabolism of the grains in the ripening process of rice plant. The high specific activity of phytin derivatives is due to the fact that this is

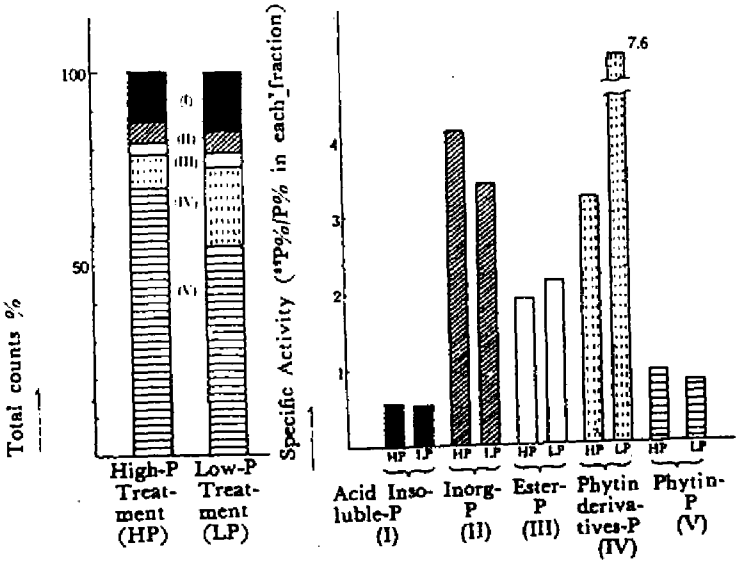
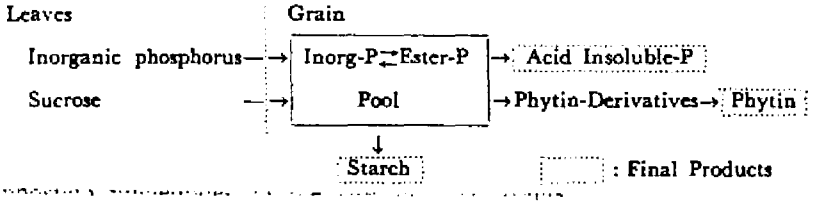


Fig. 8 Incorporation of ^{32}P-phosphate Absorbed through Roots at the Yellow Ripening Stage in Each Fraction of Rice Grains.

the intermediate product in the formation of phytin. At the same time, it was observed that ester- and inorganic-phosphorus turned over, from their high specific activities as compared with that of acid insoluble- and phytin-phosphorus.

From the results thus obtained, we suppose the following schema of phosphorus metabolism in the grains of rice plant in its ripening process (Fig. 9). The form of sugar

Fig.9. Metabolic Pattern of Phosphorus in Rice Grain



that is translocated from leaves to the grains is sucrose (4). From the sucrose, ester-phosphorus is formed with inorganic-phosphorus through the chain of the reactions, as supposed by Turner et al (12, 19, 20) and this, in turn, works as a substrate for the biosynthesis of starch by phosphorylase (11, 21). Phosphorus translocated to the grains is incorporated into the pool of inorganic- ⇄ ester-phosphorus, turns over each other and then incorporates

into the acid insoluble- and phytin-phosphorus, that do not turn over. Since it is presumed that, in the formation of phytin, inorganic-phosphorus does not directly combine with myo-inositol but through some intermediates (22), and so the pool mentioned above is related to the synthesis of both phytin and starch. In other words, as the results of the formation of phytin, the decrease of inorganic phosphorus follows thus enhancing the formation of starch by phosphorylase. The properties that Ca, Mg salt of phytic acid are insoluble at the pH observed in the rice grains (11) is suitable for eliminating of inorganic phosphorus from the site of phosphorylase activity. Thus, in the high phosphorus treatment, the increase of inorganic-phosphorus in the pool greatly enhanced the formation of phytin in the process.

SUMMARY

1) To study the role of phytin in the ripening process of rice plant, with this purpose in view, the phosphorus compounds of the ripening grains of rice plants cultured with low and high phosphorus treatments were estimated, and the incorporations of ^{32}P-phosphate, absorbed through ear stalks at the milk stage and of ^{32}P-phosphate, absorbed

through roots at the yellow ripening stage, into each fraction of the grains were studied.

2) In the ripening process, any difference in the contents of phosphorus compounds of the grains was unobservable by the phosphorus nutrition, but the contents of phytin was high by the high phosphorus treatment.

3) Phosphorus transported to the grains was incorporated at first into the pool of inorganic-ester-P and then into acid insoluble-P and phytin-P which did not turn over.

4) From these results, the role of phytin in the accumulation of starch of the grains was discussed.

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Chapter V PHYSIOLOGICAL FUNCTIONS OF MYO-INOSITOL IN RIPENING RICE GRAINS

Section I PREPARATION OF MYO-INOSITOL-^{3}H BY THE RECOIL TRITON AND TRITIUM GAS EXPOSURE METHODS*

INTRODUCTION

The application of tritium labeled compounds in the biochemical study has steadily increased due to the current developments in the determination of soft -ray of tritium (0.018 Mev) by the liquid scintillation counter and the other instruments, and in the method of labeling organic compounds (1,2). Concerning the labeling methods, recent two findings, that is, the tritium labelings with the recoil

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* The contents of Chapter V, Section I were announced at the regular meeting of the Agricultural Chemical Society, Kyoto, on 28th Oct., 1961 and appeared in Mem.Res.Ins.Food Sci., Kyoto Univ., No.24, 13 — 20(1962). The author wish to express sincere thanks to Prof. Nakajima and Dr. Kurihara, Faculty of Agriculture, Kyoto University, for the infrared analysis, and also to Assistant Prof. Yamaguchi of the same faculty, for the advice in the design of the apparatus.

triton by Wolfgang et al (3), and with the tritium gas exposure method by Wilzbach (4) have provided the simple and effective means of the preparation of the tritium labeled organic compounds. By these methods, when a specific labeling is not required**, organic compounds, even that having the complex structure and high molecular weight, can be labeled, without using the techniques or processes of organic synthesis.

Recently the labeled myo-inositol has been applied in the biochemical and physiological studies of inositol. Until now, myo-inositol-^{14}C and myo-inositol-2-^{14}C (6,7) were prepared by the chemical synthesis, and myo-inositol-^{14}C by the biosynthesis in plant, from $^{14}CO_2$ (8) and glucose-^{14}C (9). However their radiochemical yields were low. On the other hand, hydrogen labeled inositol, myo-inositol-2-D (10), myo-inositol-2-^{3}H (11,12) and myo-inositol-^{3}H (12, 13, 14) were prepared by the chemical synthesis and by the tritium gas exposure method.

This section describes the preparations of the randomly labeled myo-inositol-^{3}H by 1) the recoil trition produced in $^{6}Li(n,\alpha)^{3}H$ reaction, and by 2) the tritium gas exposure method, for the application to the study of the physiological

** Recently it was reported that the specific labeling was also possible by the tritium gas exposure method (5)

function of myo-inositol in plants. In the former case, the mixture of lithium carbonate and myo-inositol was irradiated with the thermal neutron and the labeling was performed by the replacement of the bound hydrogen of myo-inositol with the energetic tritium produced in the nuclear reaction. In the latter case, myo-inositol was exposed to tritium gas and it has been supposed that in the mechanism of labeling, the bound hydrogen excited by the β-ray of tritium is replaced with tritium.

EXPERIMENT

Recoil Triton Method

Irradiation; The powders of myo-inositol and Li_2CO_3 were mixed thoroughly in the different ratios (A: 1 g myo-inositol and 9 g Li_2CO_3, B: 5 g myo-inositol and 5 g Li_2CO_3) and the mixtures were irradiated with the thermal neutrons of the atomic pile of the Japan Atomic Energy Research Institute (JRR-1) for 15 hours (neutron flux: $3 - 5 \times 10^{11}$ n/cm^2/sec). After the irradiation, the materials turned brownish in color, especially of sample B, probably due to the decomposition of myo-inositol by the triton produced in the nuclear reaction, and by the γ-ray in the pile.

Separation and Identification; The irradiated materials were

dissolved in hot water and lithium carbonate was filtered off. The brownish solution obtained was passed through both columns of Amberlite IR-120 (H^-) and -IRA-410 (OH^-), to remove Li^+ and the decomposition products of myo-inositol. The fraction passing through both columns and the washings were slightly brownish and, by the treatment with charcoal, the clear solution was obtained. The solution was concentrated in vacuo and the recrystallization from aqueous ethanol was performed three times. The constant specific activity was observed in each step of crystallization. 3.906 g in sample A and 0.766 g in sample B of myo-inositol could be obtained in the first crystallization. From these results, it was indicated that 21.9 % (sample A) and 23.4% (sample B) of the original myo-inositol had been decomposed by the irradiation.

Beside the constant specific activity in each crystallization step, the crystals obtained were identified by means of the following observations; melting point (222-223°C (uncorrected)), infrared spectrum (Fig. 1) and paperchromatography (Fig. 2). Distribution of ^3H on the paperchromatogram was determined by a windowless gas flow counter, with 5 mm section of a paperchromatogram. To study the ratio of ^3H in hydrogens bound to oxygen and carbon of myo-inositol, acetylation of the inositol-^3H was performed in acetic anhydride and pyridine. After the crystallization from ethanol,

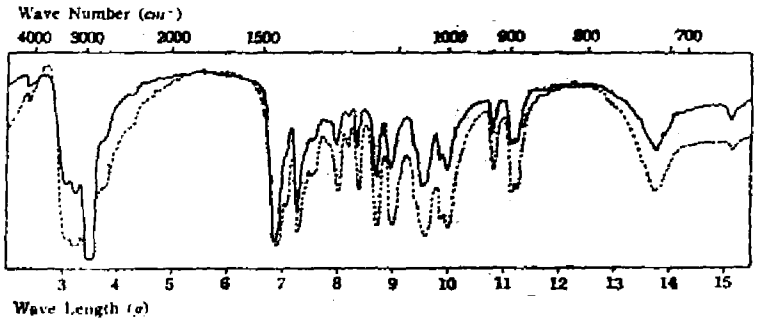

Fig. 1 Infrared Spectrum of *myo*-Inositol and *myo*-Inositol-^{3}H, Labeled by Recoil Triton Method. (Nujol mull)
: *myo*-Inositol —— : *myo*-Inositol-^{3}H

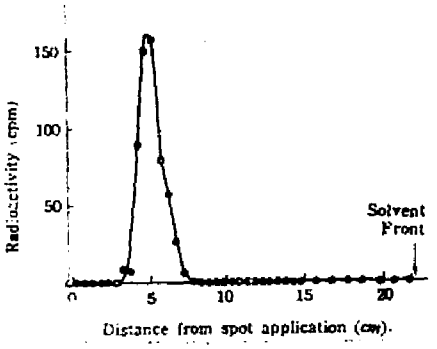

Fig. 2 Distribution of Inositol-^{3}H, Labeled by Recoil Triton Method, on Paperchromatogram. Buthanol-Acetic acid-Water, 4:1:5 (upper layer), three multiple developments.

the decrease of ^{3}H in myo-inositol hexaacetate-^{3}H compared with the myo-inositol-^{3}H, that is ^{3}H bound to oxygen, was determined.

Tritium Gas Exposure Method

Apparatus; The apparatus used for this method is shown in

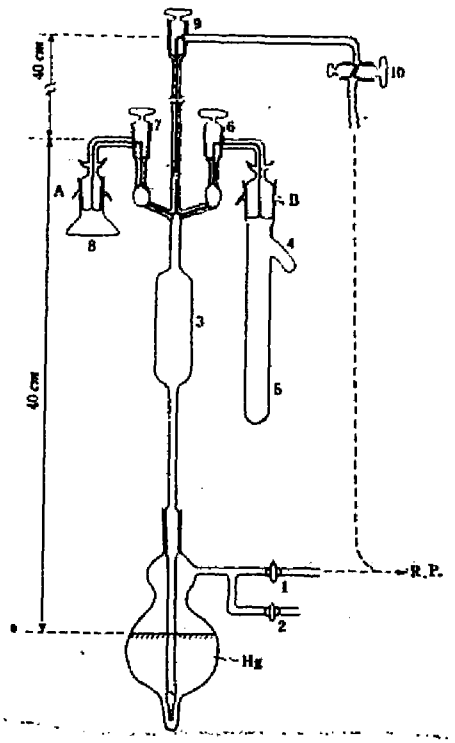

Fig. 3 Apparatus for Labeling by Tritium Gas Exposure Method.

Fig. 3. This was designed for the facility of the safe handling of the small volume of tritium gas, without loss of the gas, for a long time use, as that had been reported (15 -21) At 1 and 10, in Fig. 3, the line is connected to a rotary pump. Tririum gas ampoule and the substance to be exposed are put in 5 and 8, respectively. After the tritium gas was transfered from 5 to 8, the vessel connected to B is

exchanged with the vessel suitable for the exposure or storage of the gas for the further use. The type of vessel connected to A or B is varied according to the case of the exposure, for example, to spread over the surface area of the material, the type shown in Fig. 3 is used and when at the same time many kinds of substances in tubes are exposed, the cylinder type is used. After the system was evacuated, all stopcocks are closed. Then the tritium gas ampoule in 5 is broken by small iron rod in 4. Tritium gas in 5 is transfered to vessel 8, with Toepler pump by the operation of the stopcocks 1 and 2, and of 6 and 7. After the desired time of exposure, tritium gas is transfered from A to B with Toepler pump, for the exposure or storage of the tritium gas.

Exposure; 100 mg of myo-inositol in the tube was exposed to 2 c of tritium gas for 37 days, at room temperature ($30^{\circ}C$). The volume of $^{3}H_{2}$ was 0.8 ml at N.T.P. and the volume of the vessel was 14 ml. (pressure of $^{3}H_{2}$: 43 mm Hg).

Purification and Identification; The myo-inositol exposed to tritium gas was dissolved in water, with the addition of 200 mg of carrier. To eliminate the labile tritium, the drying up and dissolution in water were repeated five times. From the second dissolution, the constant specific activity was observed. And the

recrystallization from aqueous ethanol was performed three times. The crystals obtained was identified by paperchromatography, with two solvents, and autoradiography. (Fig. 4). The distribution of ^{3}H on the paperchromatogram was measured as described above. The autoradiogram was obtained by the exposure of the paperchromatogram with the medical X-ray film in the scintillating solution (scintillator; p-diphenylbenzene 3 g and wave shifter; POPOP (2,2'-p-phenylene-bis-(5-phenyloxazole)) 0.1 g per liter of toluene), according to Wilson (22). Acetylation of the myo-inositol-^{3}H was performed, as mentioned above, to eliminate the ^{3}H bound to oxygen.

Assay of Tritium

The radioactivity of tritium was determined by a windowless gas flow counter. The sample to be counted was pipetted onto a stainless planchet and ethanol was added to the planchet, to obtain the uniform distribution in drying. The self absorption was corrected by the self absorption curve of Fig. 5. When the amount of the sample to be counted could not be determined or was over 0.2 mg/cm^{2}, the paper disk method, recommended by Mizuno et al (23), was used. In this case, the filter paper (diameter 21 mm, 33.5 + 1.0 mg in weight) was attached on a stainless planchet by cemedine, and 0.1 ml of the material to be counted was applied onto the paper

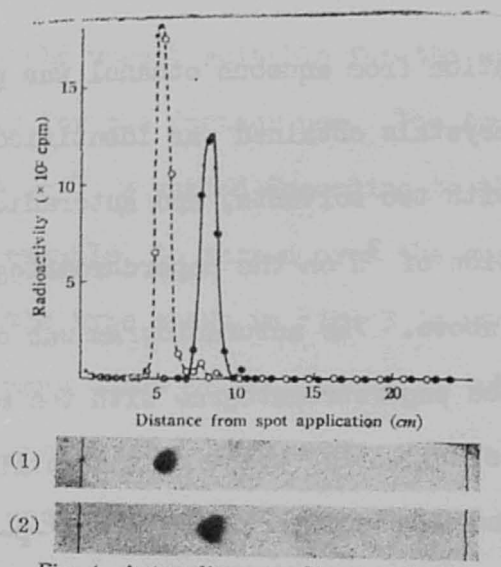

Fig. 4 Autoradiograms Showing Distribution of Inositol-^{3}H, Labeled by Tritium Gas Exposure Method, on Paperchromatograms and Radioactivity along Them.
---○--- (1) : Buthanol-Acetic acid-Water, 4 : 1 : 5 (upper layer)
——●—— (2) : Propanol-Ethylacetate-Water, 7 : 1 : 2
Three multiple developments in both cases.

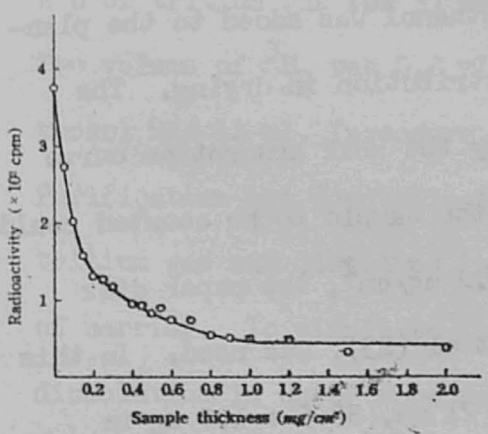

Fig. 5 Self-absorption of Inositol-^{3}H. Inositol-^{3}H containing different amounts of carrier was pipetted onto stainless planchet (diameter 25 *mm*), and after drying radioactivity was determined.

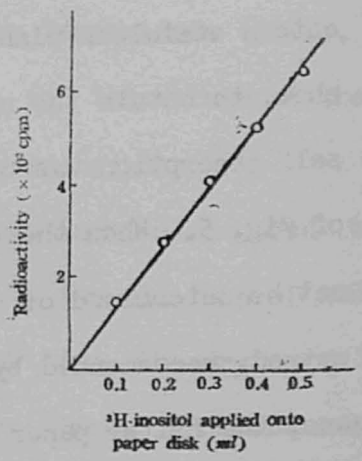

Fig. 6 Radioactivity of Inositol-^{3}H Measured by Paper Disk Method. Different amounts of inositol-^{3}H (45.2 *μg/cm²* per 0.1 *ml*) was applied onto paper disk, and after drying radioactivity was determined.

disk and was dried. By this method, the radioactivity was not affected by the amounts of a carrier between 0 - 1 mg/cm^2, as shown in Figs. 6 and 7. However, the radioactivity was reduced to 1/41.9 of that in infinite thinness. The results are shown by the radioactivity in infinite thinness, in all cases.

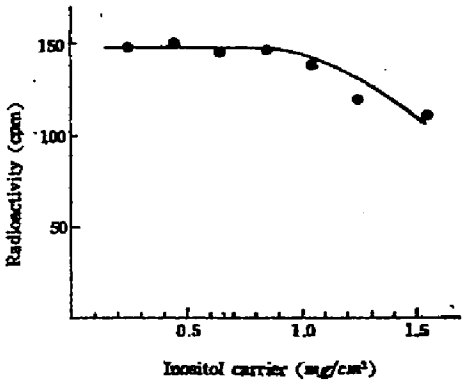

Fig. 7 Radioactivity of Inositol-^{3}H Measured by Paper Disk Method. Inositol-^{3}H containing different amounts of carrier was applied onto paper disk, and after drying radioactivity was determined.

RESULTS AND DISCUSSION

Labeling by the Recoil Triton Method

The tritium found in the irradiated myo-inositol is shown in Table 1. From this table, it is observed that specific activity is not affected by the mixing ratio of myo-inositol and lithium carbonate, so the percentage of tritons

retained in the myo-inositol is significantly varied by the mixing ratio. In the case of glucose and galactose, 10 and 12% retentions were observed, respectively (24).

Table 1 Tritium Labeling of myo-Inositol by Recoil Triton Method

	Irradiated Material		Yield of Inositol-^{3}H	Total ^{3}H in Inositol	Specific Activity (per *mg*)	^{3}H produced in ^{6}Li$(n, \alpha)^3$H Reaction**	Retention of ^{3}H in Inositol (%)
	Li_2CO_3	Inositol					
A	5 *g*	5 *g*	3.906 *g*	2.54×10^4 cpm 0.572 *mc**	6.50×10^1 cpm 0.146 *μc**	2.91 *mc*	19.6
B	9 *g*	1 *g*	0.766 *g*	4.60×10^7 cpm 0.104 *mc**	6.01×10^4 cpm 0.135 *μc**	5.24 *mc*	1.98

* In calculation of these values, as counting efficiency of counter, 20% was used.

** These values were calculated according to following equation;

$$A = Nf\sigma\left(1-e^{-0.693\frac{t}{T}}\right)$$

A ; ^{3}H produced
N ; Number of atom of ^{6}Li
f ; Neutron flux, 4×10^{11} *n/cm²/sec*
σ ; Nuclear cross section of ^{6}Li
t ; Time of irradiation, 15 hrs.
T ; Half life of ^{3}H, in hours.

The range of triton in the mixing powder is 40 - 50μ (1), so in the case of the sample containing high ratio of lithium, the greater part of triton lost its energy in the particles of lithium carbonate and did not attack myo-inositol. From the facts described above, the lithium contents in the irradiated material was in the saturated level, even in sample A, so, to get the myo-inositol-^{3}H having the higher specific

activity, the further irradiation of neutron is required (increase of f and/or t in the equation of Table 1).

Table 2 Distribution of ^{3}H in H-C and H-O of myo-Inositol, Labeled by Recoil Triton Method

	A	B
Inositol-^{3}H	10.0	9.70 ($\times 10^{3}$ cpm/μM)
Inositol-hexaacetate-^{3}H	8.95	7.71 ($\times 10^{3}$ cpm/μM)
^{3}H in H-O	10.5	20.5 (%)
^{3}H in H-C	89.5	79.5 (%)

However, since in this experiment about a fifth part of the original myo-inositol was decomposed by the radiation (Table 1), the further irradiation should result in the more radiation decomposition of the material. From the distribution ratio of ^{3}H in hydrogens bound to oxygen and carbon (Table 2), most part of ^{3}H is found in H-C. This is higher than that of myo-inositol-^{3}H, labeled by the tritium gas exposure method (Table 4), probably due to the exchange reaction in the ion exchange resins treatment.

Labeling by the Tritium Gas Exposure Method

From the results shown in Table 3, by the tritium gas

exposure method, the myo-inositol-^{3}H having about 600 times higher specific activity than the myo-inositol-^{3}H, labeled by the recoil triton method, is obtained. During the exposure process, no radiation decomposition of myo-inositol was observed. As shown in Table 4, about half of ^{3}H is found in H-C.

From the results obtained, in the labeling of the organic substances with tritium, the tritium gas exposure method is superior to the recoil trition method, except the long time required for the exposure, in the following points, a) radiation damage of the material is less, b) so the purification is easy and c) high specific activity is obtained.

Table 3 Tritium Labeling of myo-Inositol by Gas Exposure Method.

Condition of Exposure	Total ^{3}H in Inositol	Specific Activity (per *mg*)
100 *mg* inositol in 2 *c* $^{3}H_2$ for 37 days	1.14×10^{10} cpm 25.7 *mc**	3.80×10^{7} cpm 85.6 *μc**

* In calculation of these values, as counting efficiency of counter, 20% was used.

Table 4 Distribution of ^{3}H in H-C and H-O of myo-Inositol Labeled by Tritium Gas Exposure Method

myo- Inositol-^{3}H	17.5×10^{4}cpm/μM
myo-Inositol-hexaacetate-^{3}H	8.84×10^{4}cpm/μM
^{3}H in H-O	49.5%
^{3}H in H-C	50.5%

SUMMARY

By the recoil triton and tritium gas exposure methods, randomly labeled myo-inositol-^{3}H was prepared and it was identified, based on the constant specific activity in crystallization, melting point, infrared spectrum and paper-chromatogram. The specific activities of the myo-inositol-^{3}H obtained were 0.14 μc/mg by the former method and 85.6 μc/mg by the latter method. By the acetylation of myo-inositol-^{3}H, the tritium bound to oxygen or carbon was determined.

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Section II FORMATION OF MYO-INOSITOL AND PHYTIN IN RIPENING RICE GRAINS*

INTRODUCTION

During the ripening process of rice plant, the accumulation of phytic acid**, myo-inositol phosphoric acid, in the grains is a distinctive feature of the phosphorus metabolism. This accumulation of phytic acid is enhanced on applying phosphorus to the plant, without affecting the other phosphorus compounds in the grains, as shown in Chapter IV (1,2). Although the fractionation of phosphorus compounds in the ripening rice grains has been performed (2,3), the studies on the determination of myo-inositol, and on its formation and function, in connection with the phosphorus metabolism, have not done yet. myo-Inositol is a ubiquitous constituent of plants and it is now known to exist in free

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* The contents of Chapter V, Section II were announced at the annual meeting of the Society of Soil and Manure, Tokyo, on 2nd April, 1962 and appeared in Plant & Cell Physiol., 3, 397--406 (1962).

** In this section, the term phytic acid is used to imply myo-inositol phosphoric acid at different levels of phosphorylation.

and combined states as phosphate ester and phosphoinositide, but its function has remained obscure.

In the present study, using the rice grains in the process of ripening, observations were made on the variation of myo-inositol, in free and combined states, the formation of myo-inositol from ^{14}C-sugars, the formation of phytin from myo-inositol-^{3}H and the incorporation of ^{32}P from glucose-1-^{32}P. The results obtained led us to the conclusion that myo-inositol exists chiefly as phytic acid, biosynthesis of myo-inositol occurs using sugars as substrate and that phytic acid is a final product in the phosphorus metabolism in the ripening rice grains.

EXPERIMENT

Preparation of Labeled Compounds : Uniformly labeled glucose-^{14}C and sucrose-^{14}C were prepared from leaves of rice plant or canna which had been allowed to assimilate in $^{14}CO_2$. Separation of ^{14}C-sugars was performed according to the procedures previously reported (4) followed by purification by paperchromatography which was repeated three times. Randomly labeled myo-inositol-^{3}H was prepared by the method of Wilzbach (5), as described in Section I of this Chapter. ^{32}P labeled glucose-1-phosphate was prepared from starch and

^{32}P-phosphate using partially purified potato phosphorylase (6). The reaction mixture contained: 35 ml of 0.7 M ^{32}P-phosphate (pH 6.8) (129 x 10^4 cpm), 50 ml of 2% starch solution and 10 ml of phosphorylase solution. Incubation lasted for 28 hours at 37°C with the addition of toluene. When the reaction was over, inorganic phosphate was removed as the precipitate of magnesium phosphate by the addition of 4.3 g of magnesium acetate at pH 8.5 and this procedure was repeated three times, with the addition of carrier phosphate. The supernatant thus obtained was passed through the column, the adsorbate was eluted with 1 N KOH. Glucose-1-^{32}P was crystallized by the addition of three volumes of methanol to the effluent, and the recrystallization from aqueous methanol was carried out. Radiochemical yield: 2.23%; specific activity: 6.75 x 10^3 cpm/m mole.

Plant Material : Rice plants were cultured by the gravel culture method, using the culture solution, described in Chapter III (phosphorus; 5 ppm).

Absorption of Labeled Compounds : Administration of labeled compounds to the grains was performed by steeping the ear stalks in the solution during a definite time.

Fractionation of myo-Inositol Containing Compounds : The materials were ground thoroughly with 0.2 M perchloric acid

together with silica sand. After the homogenate was allowed to stand for an hour, the acid extract was obtained by centrifugation. The residue was reextracted two times with the same solvent and the extracts were combined. Sodium ethylenediamine tetraacetate, 20 mM in final concentration, was added to the perchloric acid extract to prevent the coprecipitation of phytic acid (7), and the extracts was neutralized with KOH to pH 7.0. $KClO_4$ was removed by centrifugation and the supernatant was passed through the column of Amberlite IRA-410 (OH^-). The effluent and the washings were combined (free myo-inositol). The adsorbate was eluted with 2N HCl (myo-inositol phosphate). The residue was washed with water to remove perchloric acid, was dried in vacuo, and then extracted with chloroform-methanol (1:1) using a Soxhlet apparatus (phosphoinositide). Myo-Inositol phosphate and phosphoinositide fractions, from which the solvent had been previously removed, were hydrolysed with 2 N HCl, in a sealed tube, at 123° C for 48 hours (8). The hydrolysate was concentrated in vacuo to remove HCl and the residue was dissolved in water and the pH was adjusted to 4.8 with NaOH. These materials were subjected to the bioassay of myo-inositol.

In the experiments reproduced in Fig. 1 and Table 1,

free myo-inositol in 0.04 N HCl extract of the grains was determined (8) and the quantity of myo-inositol phosphate was determined by subtracting the amount of free myo-inositol found in the 2 N HCl hydrolysate of the 1 N HCl extract.

Bioassay of myo-Inositol : Saccharomyces carlsbergensis 4228 was used as the assay organism, with the medium used by McKibbin (9).

Isolation of myo-inositol-^{14}C : According to Eagle et al (10), acid resistant property of myo-inositol was utilized for its separation. The grains which had absorbed ^{14}C-sugars from the ear stalks were homogenized thoroughly with 1 N HCl and the acid extracts obtained by the procedures described above was hydrolysed in 6 N HCl for 30 hours at 140° C with refluxing. The hydrolysate was dried in vacuo, and after the addition of methanol, the mixture was again brought to dryness in order to remove the trace of residual HCl. The residue was dissolved in water and after the neutrlalization to pH 7.0 with KOH, the solution was deionized by passing successively through the columns of Amberlite IR-120 (H^+) and IRA-410 (OH^-). The effluents and the washings were concentrated in vacuo, and myo-inositol-^{14}C was crystallized from aqueous ethanol with the addition of carrier myo-inositol. The recrystallization from aqueous

ethanol was repeated five times until the constant specific activity was observed.

Fractionation of Phosphorus Compounds : The compounds containing ^{32}P were fractionated by the procedures described in Chapter IV, Section II.

RESULTS AND DISCUSSION

Variation of the Quantity of myo-Inositol in Rice Grains During Ripening

A typical result obtained in the fractionation of myo-inositol from the rice grains is shown in Table 1, together with the results obtained for Chlorella and yeast. At the early stage of ripening, a considerable part of myo-inositol was in free state, as shown also in Fig. 1, whereas at the end of ripening period, the most part of myo-inositol was found in the phosphate ester form. The mole percentage of phosphoinositide in the total myo-inositol was small and did not vary during the ripening (Table 1). Darbre and Norris (8) observed low contents of phosphoinositide in the germinating oat, as compared with the other form of myo-inositol. Eagle et al (10) found about 30% of total myo-inositol in the lipid fraction of mammalian cells. These facts seem to indicate that the low content of phosphoinositide is a general

Table 1 Myo-Inositol of Different States in Ripening Rice Grains, in Chlorella and in Yeast

Material	Free *myo*-inositol	*myo*-Inositol phosphate	Phospho-inositide	Total
Rice grains at 10th day after flowering[a]	0.97 (26.4)	2.62 (71.1)	0.091 (2.5)	3.68 (100)
Rice grains at maturing[b]	0.58 (3.5)	15.3 (93.3)	0.52 (3.2)	16.40 (100)
Chlorella[c]	0.40 (2.7)	12.7 (87.2)	1.48 (10.1)	14.58 (100)
Yeast[d]	0.33 (33.3)	0.62 (62.6)	0.041) (4.1)	0.99 (100)

Values in parentheses are percentage of *myo*-inositol in respective states.

[a] μmole *myo*-inositol/100 grains (fresh weight: 2.26 *g*).

[b] μmole *myo*-inositol/100 grains (fresh weight: 3.13 *g*).

[c] *Chlorella ellipsoidea* cells were generously supplied by Dr. S. IDA, Faculty of Agriculture, Kyoto University. μmole *myo*-inositol/*g* dry weight.

[d] Commercial baker's yeast. μmole *myo*-inositol/*g* dry weght.

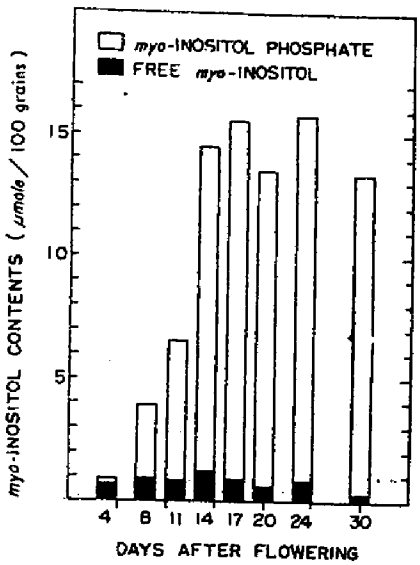

Fig. 1 Change in amounts of free and phosphate ester forms of *myo*-inositol in rice grains during ripening.

Fig. 1 Change in Amounts of Free and Phosphate Ester Forms of myo-Inositol in Rice Grains during Ripening Period

feature of cereal grains. Brockerhoff and Ballou (11), who studied the chemical configuration of phosphoinositide, proposed the hypothesis that phytic acid is formed through phosphoinositide. Although Hoffman-Ostenhof et al (12)

found myo-inositol kinase activity in yeast hexokinase***, direct phosphorylation of myo-inositol has not yet been demonstrated. The results obtained in the present study, together with that of the ^{32}P-phosphate feeding experiment (2), may be taken to point to the occurrence of direct phosphorylation of myo-inositol.

As may be seen from Fig.1, the content of myo-inositol in the grains reached the maximum level at the 14th day after flowering and did not vary appreciably after that. On the other hand, the results of Chapter IV, Section II confirmed that phosphorus in phytic acid of the rice grains increased until the 25th day after flowering. Therefore it is supposed that from about the 14th day to 25th day after flowering, corresponded to the middle stage of ripening of rice plant, there occurred only the phosphorylation of myo-inositol.

Formation of myo-Inositol from ^{14}C-Sugars

myo-Inositol contents in different states of a rice

*** The author tested the myo-inositol kinase activity of yeast hexokinase, using myo-inositol-^{3}H as substrate. The result was, however, negative, which was in agreement with the results reported by Ballou (11) and Sols and Crane (13).

plant in the ripening stage are shown in Table 2.

Table 2 Contents of myo-Inositol in Different Parts of Ripening Rice Plant (μmole/whole plant)

Parts	Fresh weight (g)	Free *myo*-inositol	*myo*-Inositol phosphate	Total
Grains[a]	43	18.2	54.0	72.2
Leaves & stems	159	8.17	33.3	41.47
Roots	132	0.59	4.08	4.67

Material used was rice plant at the 13th day after flowering of the first ear.
[a] about 1,700 grains

The content in the grains amounted to two-thirds of that in the whole plant, even at the early stage of ripening, and from the results presented in Fig.1, it is deduced that the grains in whole plant at the stage of maturation contain 200 μmoles of myo-inositol. Considering the low contents of myo-inositol in the leaves, stems and the roots, they can hardly supply the myo-inositol required for the formation of phytic acid in the grains. It is, therefore, inferred that biosynthesis of myo-inositol is a process occurring in the grains. To confirm this point, ^{14}C labeled sucrose and glucose were fed to the rice grains. In Chapter II, it was

confirmed that the photoshyntheates formed in leaves are translocated to grains in the form of sucrose. As shown in Table 3, a large part of ^{14}C-sugars administered was converted into the acid insoluble fraction, i.e., starch. From the acid

Table 3 Incorporation of ^{14}C-Sugars into myo-Inositol and Starch in Ripening Rice Grains ($\times 10^4$ cpm)

Sugars administered	Sucrose-^{14}C		Glucose-^{14}C	
Expt. No.	1	2	1	2
Sugars-^{14}C absorbed (A)	2181	2046	3133	3531
Acid-soluble fraction (B)	427	341	942	1039
Acid-insoluble fraction (starch) (A−B=C)	1754	1705	2191	2492
myo-Inositol (D)	1.023	0.735	2.72	2.75
D/C (%)	0.0584	0.0431	0.124	0.110

^{14}C-Sugar was administered as a 0.1 *M* solution through ear stalks for 25 *hr* at 30°. Fifteen ears of rice at the 10th day after flowering were used in each test.

soluble fraction, myo-inositol-^{14}C was isolated, whose radioactivity is also shown in Table 3. To establish that ^{14}C-sugar supplied was not contaminated with myo-inositol-^{14}C, the ^{14}C-sugar was treated as described in Experiments, and it was confirmed that the radioactivity found in the form of myo-inositol was below 0.005% of that of sugar. ^{14}C found in myo-inositol was about 0.05 - 0.1% of that found in starch (Table 3). This value is close to the mole ratio of myo-inositol (0.13 μ mole/grain) and starch (83 μ mole/grain, in hexose unti) in the rice grains at the harvest, i.e., 0.16% of starch. These results lead to the conclusion that the biosynthesis of myo-inositol takes place in rice grains in their ripening stages. The formation of myo-inositol from sugars has been shown to occur in yeast (14) and mammalian cells (10, 15, 16), while so far no relevant information has been obtained for plant tissues.

Formation of Phytin from myo-Inositol-^{3}H

To investigate the fate of myo-inositol biosynthesized in the rice grains, myo-inositol-^{3}H, which was free of the labile tritium, was administered to the ripening rice grains. The most part of tritium was recovered in the acid extract, as shown in Table 4, and the separation by the ion exchange resins showed that tritium was present in the neutral and

anionic fractions. In the neutral fraction only the unmetabolized myo-inositol-^{3}H was found by paperchromatography with the two solvent systems. Since the anionic form is considered to be phytic acid, it may be concluded that myo-inositol does not undergo other reactions than phosphorylation in the ripening rice grains. Lowes et al (17) showed in their feeding experiment of myo-inositol-^{14}C and -^{3}H to parsley leaves and strawberry fruits that 30 - 50% of ^{14}C or ^{3}H appeared in the 70% ethanol insoluble fraction, and that the labeled myo-inositol was further converted into many other compounds. The results of our feeding experiments of ^{14}C-sugars and of myo-inositol-^{3}H allow us to draw the conclusion that myo-inositol is one of the final products in the carbon metabolism in the ripening rice grains.

Incorporation of ^{32}P into Phytic Acid from Glucose-1-^{32}P

The experiment of Chapter IV, in which the ripening rice grains were fed ^{32}P-phosphate and the distribution of ^{32}P in different fractions of grain materials was investigated, have suggested that ^{32}P would be incorporated first into the "pool" of inorganic-P $\rightleftarrows$ ester-P and then into acid insoluble-P and phytic acid-P, both of which seemed to perform no turn over. To confirm this point further,

Table 4 Distribution of myo-Inositol-^{3}H Incorporated in Different Fractions of Ripening Rice Grains

	Radioactivity ($\times 10^{i}$ *cpm*)
myo-Inositol-^{3}H absorbed	5.26
^{3}H recovered in 0.2 *M* $HClO_4$ extract	5.10
Neutral fraction (*myo*-inositol)	4.64
Cationic fraction	0
Anionic fraction (phytic acid)	0.165

myo-Inositol-^{3}H was administered as a 0.033 *M* solution through ear stalks for 17 *hr* at 30.

Fifteen ears of rice at the 15th day after flowering were used. *myo*-Inositol containing compounds were fractionated as described in Materials and Methods, except the use of a cation exchange resin.

a feeding experiment of glucose-1-^{32}P was performed. The results presented in Table 5 show that the specific activities of inorganic-P and ester-P were higher than those of acid insoluble-P and phytic acid-P, a situation identical with that observed in the ^{32}P-phosphate feeding experiment.

Table 5 Incorporation of Glucose-1-^{32}P into Phosphorus Compounds of Ripening Rice Grains (per 5 g grains)

(per 5 *g* grains)

Fraction	^{32}P contents (*cpm*)	Phosphorus contents (*mg*)	Specific activity (*cpm/mg* P)
Acid-insoluble-P	162	2.63	62
Inorganic-P	713	1.54	463
Ester-P	300	1.30	230
Phytin-P	100	5.05	20
Total	1275	10.52	

Glucose-1-^{32}P was administered as a 0.07 *M* solution through ear stalks for 24 *hr* at 30°.
Ears of rice at the 15th day after flowering were used.

These facts suggest the existence of the "pool" of inorganic-P ⇄ ester-P and confirm that in the ripening rice grains phytic acid- and acid insoluble-P do not perform turn over with the phosphorus in the "pool". These experiments also show that the phosphate released from glucose-1-phosphate was not removed immediately from the "pool" to form phytic acid.

From the results obtained above, it may be concluded

that myo-inositol and its phosphate ester are both final products in the carbon and phosphorus metabolism in the ripening rice grains. Such an idea seems to be in harmony with the hypothesis proposed by Aimi (18) that in the ripening process of rice grains myo-inositol functions as an acceptor of phosphorus to lower the level of inorganic phosphorus in favor of starch synthesis by phosphorylase. According to this assumption, inorganic phosphorus in the "pool" is removed by the formation of phytic acid, probably through some intermediate, and the "pool" maintains the favorable condition for the starch synthesis. The occurrence of phosphorylase in the ripening rice grains has been confirmed (19, 20); however, the analysis of inorganic phosphorus and glucose-1-phosphate (3) in ripening rice grains showed that ratio of the two constituents present in toto was unfavorable for starch synthesis by phosphorylase, although no one knows the ratio on the actual site of starch synthesis. If the recently discovered second route of starch synthesis, uridine diphosphate glucose starch synthetase (21,22), which is independent of the concentration of inorganic phosphorus, is predominant in rice grains, some other assumption is required for the interpretation of the function of myo-inositol. Clarification of this point as well as elucidation of the mechanism of

phosphorylation of myo-inositol is a matter for further investigation.

SUMMARY

With the view to elucidate the role of myo-inositol in the ripening process of rice grains, its distribution, formation and conversion were studied.

1. myo-Inositol in the ripening rice grains was fractionated into free-, phosphate ester- and phosphoinositide-forms. At the early stage of ripening, a considerable part of myo-inositol was found in free state, and at the end of the ripening, the most part was found in phosphate-ester-state, phytic acid. The content of phosphoinositide in the grains was low during the ripening period.

2. The occurrence of biosynthesis of myo-inositol in the ripening rice grains was confirmed by the observation of incorporation of ^{14}C into myo-inositol from ^{14}C-sugars, and it was found, from the feeding experiment of myo-inositol-^{3}H, that myo-inositol does not undergo reactions further than phosphorylation.

3. The feeding experiment of glucose-1-^{32}P showed that the distribution pattern of ^{32}P in different fractions of grain material was same as that of ^{32}P-phosphate, indicating that

phytic acid is one of the final products of phosphorus metabolism in the ripening rice grains.

4. These results led to the assumption that myo-inositol might act as an acceptor of phosphorus to remove inorganic phosphorus in favor of starch synthesis by phosphorylase.

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Chapter VI DISCRIMINATION BETWEEN STRONTIUM AND CALCIUM ON THE ABSORPTION AND TRANSLOCATION IN RICE PLANT*

INTRODUCTION

In the estimation of the contamination of radioactive "fall out" products resulting from the atomic- and hydrogen-bombs experiments in the food chain, the ratio of Sr and Ca have been emphasized, from the long half life of ^{90}Sr, in physical and biological, and its similarity of the chemical and biological behaviors with calcium in human body. Recently the discrimination between Sr and Ca or Cs and K between two systems, for example, soil and plant, and feed and milk, was illustrated by the discrimination factor, D.F., (or distribution factor or observed ratio) that was expressed by the following equation (1):

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* The contents of Chapter VI were announced at the regular meeting of Kansai branch of Society of Soil and Manure, Kyoto, on 6th Feb., 1960 and appeared in Mem.Res.Ins.Food Sci., Kyoto Univ., No.20, 22 — 30(1960)

$$D.F. = \frac{\text{Sr/Ca in plant (or milk)}}{\text{Sr/Ca in soil (or feed)}}$$

Mitsui et al (2) measured D.F. in rice plant by the determination of the contents of Sr and Ca in the plants and the exchangeable cations of soil, by activation analysis. However, some questions whether the plants absorbed them from the exchangable cations of soil alone were left unsolved. In this chapter, the author present the data on D.F. between ^{90}Sr and ^{45}Ca absorbed from the culture solution at the ear-forming and at the booting stages, with special references to the accumulation of these elements in the grains of rice plant, using the double label method which is based on the difference between β-ray energies of ^{90}Sr-^{90}Y (0.61 and 2.26 Mev) and ^{45}Ca (0.25 Mev).

EXPERIMENT

Culture of Rice Plant : Three seedlings of rice plant (Kyoto Asahi) were transplanted on 1st July, 1959 in a pot and were cultured by the water culture (4). The culture solution used is shown in Table 1. To study the effects of calcium nutrition upon the absorption and translocation of Sr and Ca, the plants were cultured at the low(10 ppm)

and high (30 ppm) calcium levels. The culture solution was prepared by the deionized water and was renewed once a week. The stage of growth of the plants was the same as that in Chapter III.

Table 1 Composition of Culture Solution

Elements	Forms	Concentration (ppm)
N	NH_4NO_3	70
P	Na_2HPO_4	10
K	KCl	16
Mg	$MgSO_4$	10
Ca	$CaCl_2$	10 (Low Ca treatment)
		30 (High Ca treatment)
Si	Na_2SiO_3	20
Fe	EDTA-Fe	2
(Trace elements)		

Table 2 Periods Treated with ^{90}Sr and ^{45}Ca and Time of Sampling

Exp. No.	Treated period of ^{90}Sr and ^{45}Ca	Time of sampling
A	20~26/VIII (Ear-Forming stage)	26/VIII
A′	20~26/VIII	24/X (Maturing stage)
B	7~13/IX (Booting stage)	13/IX
B′	7~13/IX	24/X

Application of ^{90}Sr and ^{45}Ca : During the period illustrated in Table 2, carrier free ^{90}Sr-^{90}Y (100 μc) and ^{45}Ca (60 μc) were supplied in each pot with the culture solution.

Sampling and Determination : The plants were sampled at the time after the application of radioisotopes and at the maturing, as shown in Table 2. The roots were washed to eliminate the radioisotopes adsorbed in the "outer space" region (5,6,7) at least for 20 min. (8) in running water, and the plants of A' and B' were cultured with the culture solution without the radioisotopes after that. The plants were divided into the roots, stems and leaf sheathes, leaf blades and ears. The ears obtained at the maturing were divided into the rachies, husks, bran and polished rice. The hulled rice was polished as the weight of bran was about 10% of the total, that is corresponding to the usual diet in Japan. The ash obtained from the samples was dissolved with nitric acid and the radioactivities of ^{90}Sr-^{90}Y and ^{45}Ca were determined, using a aliquot of it. The thickness of the ash in planchet was thin enough to neglect the self absorption of β-rays of the radioiosotpes, especially of ^{45}Ca. The radioactivity due to ^{90}Sr-^{90}Y was determined by a G.M. counter, using 80 mg/cm^2 aluminum filter, which was thicker than the maximum range of β-ray of ^{45}Ca, as

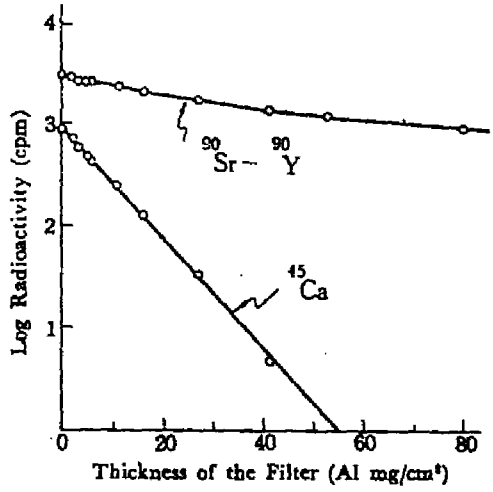


Fig. 1. Absorption of β-Rays of ^{45}Ca and ^{90}Sr-^{90}Y by Aluminium. (without correction of air and G.M. tube's mica window thickness)

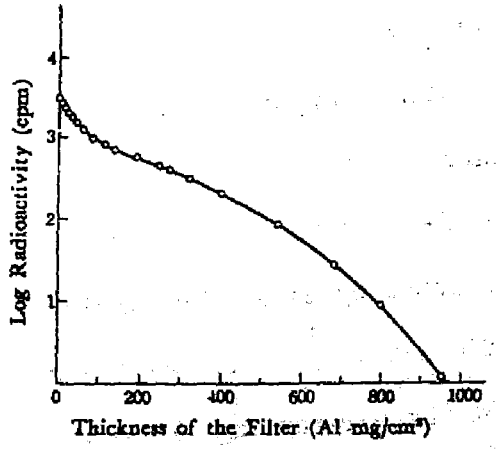


Fig. 2. Absorption of β-Rays of ^{90}Sr-^{90}Y by Aluminium. (without correction of air and G.M. tube's mica window thickness)

shown in Fig. 1. The filter absorbed the part of the β-ray spectrum of ^{90}Sr-^{90}Y (Figs. 1 and 2), the radioactivity of ^{90}Sr-^{90}Y was obtained by mutiplying 2.66. The total count from ^{90}Sr-^{90}Y and ^{45}Ca (without filter) minus ^{90}Sr-^{90}Y (filter x 2.66) gives the count due to ^{45}Ca. Since the determination of the radioactivity was performed one month after the preparation of the samples, radiation equilibrium of ^{90}Sr was attained with ^{90}Y. And the portion absorbed as ^{90}Y from the culture solution was not concerned in this determination, because its short half life comparing with the period between the application of ^{90}Sr-^{90}Y and the measurement of the radioactivity (9).

RESULTS

Distribution of ^{90}Sr and ^{45}Ca absorbed at the ear-forming or at the booting stages in each part of the rice plant and of the ears are shown in Figs. 3, 4, 5 and 6 and Tables 3 and 4. In these figures, the amounts of ^{45}Ca absorbed were illustrated by mg of the calcium labeled with ^{45}Ca (Ca*). Specific activity, that was the ratio of ^{90}Sr and ^{45}Ca absorbed at the ear-forming or at the booting stages to calcium absorbed at the other stages of growth, is shown in Figs. 7 and 8 by ^{90}Sr%/Ca % and ^{45}Ca %/Ca %, only in the

case of the low calcium treatment, since the high calcium treatment had the same tendency.

Discrimination factor between ^{90}Sr and ^{45}Ca abosrbed at the two stages of growth was calculated according to the following equation :

$$D.F. = \frac{^{90}Sr/^{45}Ca \text{ in plant}}{^{90}Sr/^{45}Ca \text{ in the culture solution}}$$

D.F. on the whole plant, that is D.F. on the absorption, is shown in Fig. 9. D.F. in each part of the plants is shown in Figs. 10 and 11.

Fig. 3 Distribution of ^{90}Sr and ^{45}Ca Absorbed at Ear-forming or at Booting Stages in Each Part of Rice Plant (Low Ca Treatment)

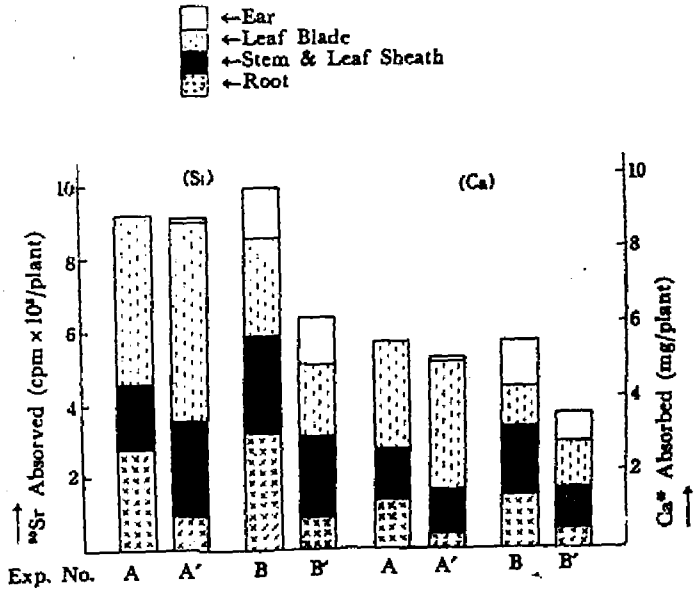
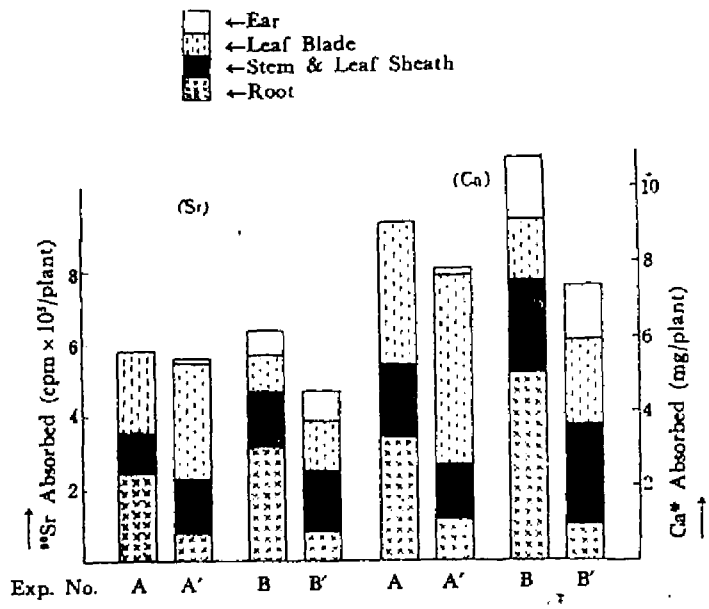


Fig. 4 Distribution of ^{90}Sr and ^{45}Ca Absorbed at Ear-forming or at Booting Stages in Each Part of Rice Plant (High Ca Treatment)



DISCUSSION

Distribution of ^{90}Sr and ^{45}Ca

Though the difference in the amounts of ^{90}Sr and ^{45}Ca absorbed between the two stages of growth was not observed, it was affected by the calcium nutrition, as shown in Figs. 3 and 4. The amounts of ^{45}Ca absorbed increased in the high calcium treatment, but that of ^{90}Sr was depressed and, on the contrary, in the low calcium treatment it increased. The decrease of the absorption of Sr by the supply of

calcium have been observed in the other experiments of water culture and soil liming (1, 10, 11, 12). Distribution of ^{90}Sr and ^{45}Ca in each part of the plant was affected by the time of the application and of the sampling, but was not affected by the calcium nutrition. About half of the ^{90}Sr and ^{45}Ca were found in the roots when the plants were sampled after the supply of the radioisotopes, and two thirds of them were transported to the shoots till the maturing. The distribution ratios of ^{90}Sr and of ^{45}Ca in the ear were largely affected by the time of the application of the radioisotopes. From Figs. 3 and 4 and Tables 3 and 4, it is observed that the accumulation of ^{90}Sr and ^{45}Ca absorbed at the ear-forming stage in the ears was one-tenth of that absorbed at the booting stage. This is confirmed from the low specific activity of ^{90}Sr and ^{45}Ca absorbed at the ear-forming stage in the ears (Figs. 7 and 8). This observation agrees with the results obtained by Sakaguchi et al with rice and wheat (3, 13). From these results, it is supposed that Sr and Ca of the ears have its origin in Sr and Ca absorbed at the booting stage, and the translocation of these elements absorbed at the other stages to the ears is not carried out. Lower specific activities of root, stem and leaf sheath and ear at the maturing than at the time after

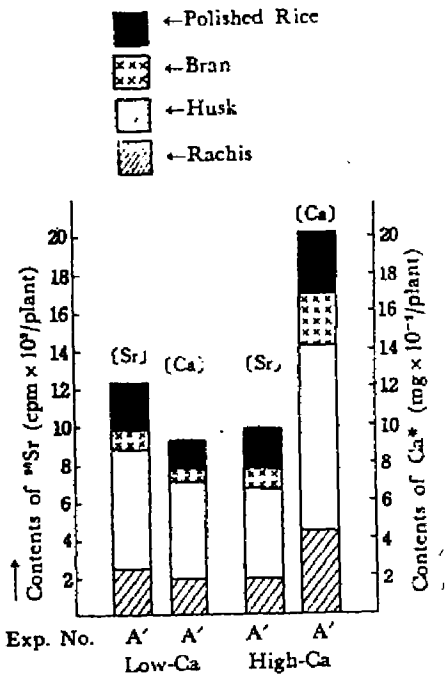
Table 3 Distribution Ratio of ^{90}Sr and ^{45}Ca Absorbed at Ear-forming or at Booting Stages and of Ca in Each Part of Rice Plant (Low Ca Treatment)

Exp. No. (Low-Ca)	Parts of Plant	Dry Wt. (g)	Ca Content (%, Dry Wt. Basis)	Distribution Ratio (%)		
				^{90}Sr	^{45}Ca	Ca
A	Roots	7.47	0.102	30.0	22.9	19.8
	Stem & Leaf Sheath	5.60	0.0950	19.4	25.1	13.3
	Leaf Blades	9.16	0.295	50.6	52.0	67.7
	Total			100	100	100
A′	Roots	15.50	0.0890	10.5	6.86	12.3
	Stem & Leaf Sheath	19.67	0.138	28.6	23.0	24.2
	Leaf Blades	12.84	0.437	59.6	68.4	50.0
	Rachis	1.32	0.165	0.267	0.378	1.94
	Husks	10.92	0.0727	0.694	0.975	6.68
	Bran	2.31	0.0952	0.122	0.147	1.96
	Polished Rice	20.51	0.0156	0.262	0.275	2.85
	Total			100	100	100
B	Roots	10.14	0.103	32.0	24.7	12.4
	Stem & Leaf Sheath	18.69	0.0770	27.0	33.1	17.1
	Leaf Blades	16.85	0.372	27.3	19.4	65.7
	Ears	4.06	0.0983	13.7	22.8	4.75
	Total			100	100	100
B′	Roots	16.39	0.0860	13.3	13.2	13.4
	Stem & Leaf Sheath	20.65	0.134	36.8	29.1	26.4
	Leaf Blades	13.26	0.367	29.2	34.7	46.4
	Rachis	1.35	0.617	3.49	3.80	2.16
	Husks	6.82	0.0899	8.79	10.1	5.85
	Bran	2.59	0.0719	1.76	1.83	1.74
	Polished Rice	25.47	0.0163	6.62	7.14	3.95
	Total			100	100	100

Table 4 Distribution Ratio of ^{90}Sr and ^{45}Ca Absorbed at Ear-forming or Booting Stages and of Ca in Each Part of Rice Plant (High Ca Treatment)

Exp. No. (High-Ca)	Parts of Plant	Dry Wt. (g)	Ca Content (%, Dry Wt. Basis)	Distribution Ratio (%)		
				^{90}Sr	^{45}Ca	Ca
A	Roots	10.44	1.122	41.3	36.1	20.8
	Stem & Leaf Sheath	8.65	0.0832	19.6	21.5	11.9
	Leaf Blades	11.06	0.372	39.1	42.4	67.3
	Total			100	100	100
A'	Roots	10.87	0.250	13.2	13.7	17.7
	Stem & Leaf Sheath	20.68	0.199	28.1	18.0	26.8
	Leaf Blades	12.35	0.552	56.9	65.1	44.4
	Rachis	1.41	0.203	0.341	0.553	1.86
	Husks	6.79	0.124	0.872	1.23	5.47
	Bran	3.27	0.0458	0.190	0.354	0.98
	Polished Rice	26.22	0.0162	0.370	0.403	2.77
	Total			100	100	100
B	Roots	13.42	0.157	49.2	46.5	19.0
	Stem & Leaf Sheath	20.26	0.0824	24.1	23.1	15.0
	Leaf Blades	16.40	0.418	16.3	15.1	61.6
	Ears	4.44	0.109	10.4	15.3	4.36
	Total			100	100	100
B'	Roots	12.45	0.164	16.6	12.9	15.1
	Stem & Leaf Sheath	20.56	0.195	36.4	36.4	29.6
	Leaf Blades	13.28	0.450	29.6	31.2	44.1
	Rachis	1.45	0.224	3.31	3.86	2.40
	Husks	9.08	0.0779	8.70	9.36	5.22
	Bran	3.22	0.0621	3.53	3.32	1.48
	Polished Rice	24.04	0.0120	1.90	2.95	2.13
	Total			100	100	100

Fig. 5 Distribution of ^{90}Sr and ^{45}Ca Absorbed at Ear-forming Stage in Each Part of Rice Ear at Maturing



the application of the radioisotopes were probably due to the translocation of ^{90}Sr and ^{45}Ca to the other parts and to the absorption of calcium after the application of the radioisotopes.

As described above, the quantities of ^{90}Sr and ^{45}Ca accumulated to the ears were influenced by the time of the application, but the distribution of each element in each part of the ears was not affected (Figs. 5 and 6). Distribution ratio of ^{90}Sr absorbed at the ear-forming or at

Fig. 6 Distribution of ^{90}Sr and ^{45}Ca Absorbed at Booting Stage in Each Part of Rice Ear at Maturing

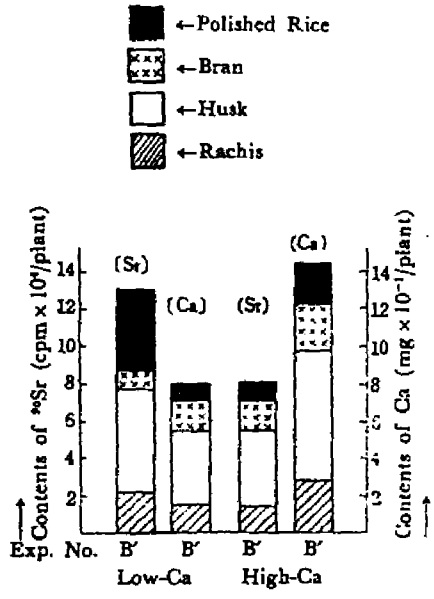


Fig. 7 Ratio of ^{90}Sr Absorbed at Ear-forming or at Booting Stages to Ca in Each Part of Rice Plant (Low Ca Treatment)

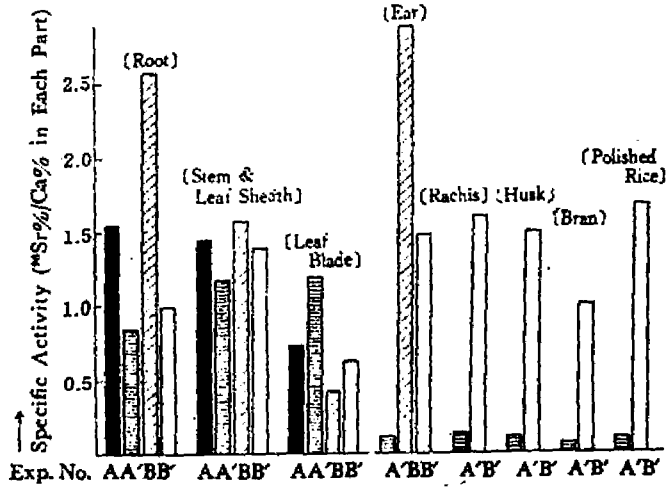
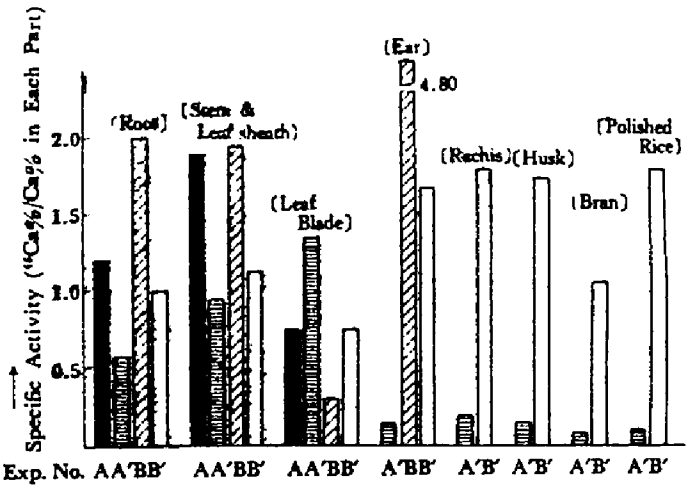


Fig. 8 Ratio of ^{45}Ca Absorbed at Ear-forming or at Booting Stages to Ca in Each Part of Rice Plant (Low Ca Treatment)



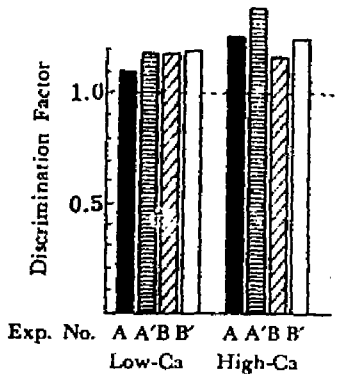
the booting stages in the hulled rice (bran and polished rice) was 0.384 and 8.37% (low Ca treatment), 0.560 and 5.43% (high Ca treatment) of total ^{90}Sr absorbed, respectively.

D.F. on the Absorption of Sr and Ca

D.F. between Sr and Ca on the absorption from the culture solution was about 1.20. This value showes that Sr is a little ready to be absorbed comparing with Ca. D.F. on the absorption was not affected by the calcium nutrition and by the time of the application (Fig. 9). From the viewpoint of the estimation of ^{90}Sr contamination in food chain, these values indicate that between two elements no selective

absorption by the rice plant exists and agrees with the results obtained by the other investigators with rice and

Fig. 9 Discrimination Factor on Absorption of ^{90}Sr and ^{45}Ca of Rice Plant



other kinds of plants (1). However, it must be taken into notice that these results were obtained in the two stages of growth and that on the whole life of the rice plant, other determination is required (2). Though we encounter many problems when the results obtained by the water culture experiments are applied to the relation between the soil and plant, D.F. between the exchangable Ca and Sr in soli and rice plant have similar value (2), so we can deduce that in the culture of rice plant in field, there are not selection between Sr and Ca.

D.F. in Each Part of Rice Plant

Comparison of D.F. in each part of the plants gives the information about the selection between Sr and Ca in the translocation. In Figs. 10 and 11, it is observed that D.F. of the roots, stems and leaf sheathes and leaf blades were higher than that of the ears, independently of the calcium nutrition, the times of the application of the radioisotopes and of the sampling. These data indicate the more ready translocation of Ca than Sr from the roots, leaves or stems to the ears. The low D.F. of the ears in A' than in B' except the polished rice may be due to the difference between the translocation from the leaves and from the roots. In A' most parts of the ^{90}Sr and ^{45}Ca originates from the leaves and stems, and in B', on the other hand, it originates from the roots directly, so it is supposed that from the leaves to the ears Ca was more readily translocated than Sr. Between the bran and polished rice, we could not find a definite discrimination between two elements, as Tensho et al reported (14).

SUMMARY

Discrimination factor, D.F., between Sr and Ca absorbed at the ear-forming or at the booting stages of rice plants cultured at the low and high calcium levels was determined

Fig. 10 Discrimination Factor in Each Part of Rice Plant (Low Ca Treatment)

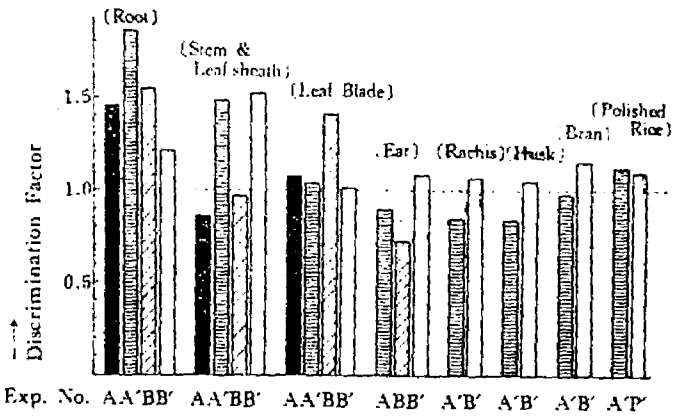
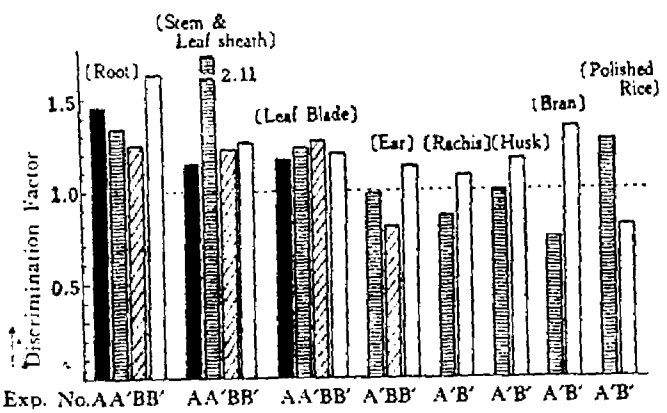


Fig. 11 Discrimination Factor in Each Part of Rice Plant (High Ca Treatment)



by the double label method of ^{90}Sr and ^{45}Ca. The results obtained are as follows;

1) Absorption of ^{90}Sr was depressed by the high calcium levels of the culture solution. ^{90}Sr and ^{45}Ca absorbed at

the booting stage was more accumulated in the ears than that at the ear-forming stage.

2) D.F. on the absorption was about 1.20, independent of the calcium nutrition and time of application.

3) D.F. of the ear was a little lower than that of the other parts, but we could not find any differences in D.F. of each part of the ears. From these values, it is supposed that there existed some selection between Sr and Ca on the translocation to the ears.

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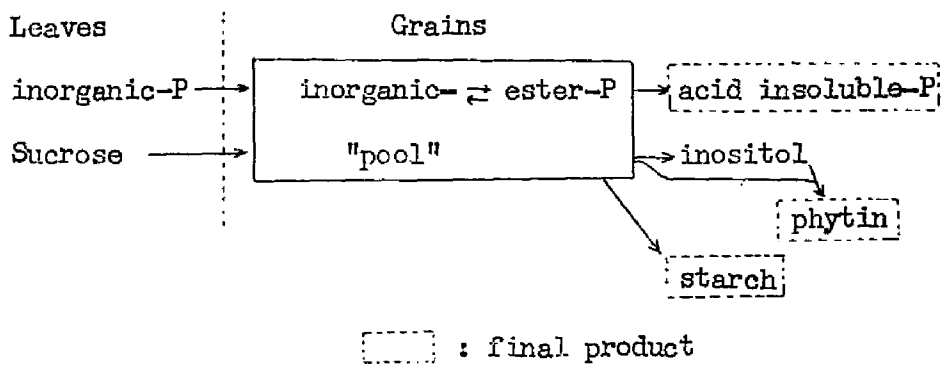
Chapter VII CONCLUDING REMARKS

From the studies on the accumulation of the nutrients into rice grains described above, by what growth stage the nutrients found in the grains at the end of maturing have been absorbed, the two types are found. The first type is represented by carbon (photosynthetic products) (Chapter II), calcium and strontium (Chapter VI). These nutrients accumulated in the grains have their origins chiefly in those absorbed during the ripening stage, that is, after flowering. The second type, represented by phosphorus (Chapter III), is characterized by the fact that the nutrient absorbed before the ripening stage is the main source of that found in the grains.

As shown in Chapter II, the carbon source of the grains is supplied in the form of sucrose from the leaf and from the sucrose thus transported into the grains inositol is formed (Chapter V). The determination of phosphorus compounds of rice grain in the ripening process and the feeding experiments of ^{32}P-phosphate and glucose-1-^{32}P in the grain show the presence of the "pool" of inorganic-ester-P and confirm that from the "pool" starch, acid insoluble-P and phytin, which do not turn over with the phosphorus of the "pool", are formed (Chapter IV, V). The determination

of inositol in different states in rice grains and the feeding experiment of inositol-^{3}H suggest that inositol functions as an acceptor of phosphorus in rice grains and does not undergo other reactions than phosphorylation.

From these results, the author proposed a metabolic pattern of phosphorus in rice grains, as follows:



According to this pattern, as discussed in Chapter IV and V, inositol plays a role as an acceptor of phosphorus to lower the level of inorganic phosphate in favor of starch synthesis by phosphorylase.

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