# STUDIES ON THE METABOLISM OF PHOSPHORUS IN THE HIGHER PLANTS I. TRENDS IN THE DISTRIBUTION OF PHOSPHORUS IN THE RICE AND THE BARLEY SEEDLINGS

# STUDIES ON THE METABOLISM OF PHOSPHORUS IN THE HIGHER PLANTS

# I. TRENDS IN THE DISTRIBUTION OF PHOSPHORUS IN THE RICE AND THE BARLEY SEEDLINGS

By

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*(Received June 8, 1957)*

## Introduction

As the physiology of phosphorus in plants and animals has been gradually advanced in recent years, the high phosphorus compounds have been given remarkable attention from the metablic stand points.

There are many works about the high energy compounds in the animal tissues and microorganisms, but in the higher plants many problems yet remained unsolved. Especially there are but few studies on phosphorus metabolism in many kinds of plants considered from the points of comparative biochemistry.

For example, it is recognized that the necessity of phosphorus is greater in the barley than in the rice plant. It is also distinct that there are considerable differences between the barley and the rice plant in response to the phosphorus supply when they are raised by means of water culture to eliminate the effects of soil.

Thus we have attempted to clarify the distribution of organic phosphorus in various plants from the stand point of comparative biochemistry.

High energy phosphorylated compounds such as adenosinetriphosphate (ATP), and adenosinediphosphate (ADP) are found in the fraction extracted with cold acid. The fractionation procedure treating this cold acid soluble part has been practised in plants and animals by many workers.

However such an extraction procedure is usually able to remove only about one-half of the total P contained in many tissues.

As the results of recent work applying $P^{32}$, it has become quite apparent that the acid insoluble P is also subjected to rapid turnover.

For example, Gest and Kamen (1) have shown the rapid turnover of the

acid insoluble P treating Chlorella. Wildman, Campbell and Bonner (2) reported that auxin influences the rate of turnover of the acid insoluble P during the growth of oat coleoptiles. They also examined the acid insoluble P in tobacco leaves, oat coleoptiles and the rat liver.

The facts have been revealed that this acid insoluble P is extracted by 1*N* hot hydrochloric acid within 2 minutes and contains organic phosphorus which is bound to protein similar to ATP. Therefore, they have indicated that energy rich phosphorylated compounds exist in the acid insoluble fraction as well as in the cold acid soluble fraction, and play physiologically important roles.

We (3), have followed the change of the acid soluble phosphorus and labile phosphorus (hydrolyzable in 7 min. at 100°C in *N* hydrochloric acid) for a week per 24 hours in the course of germination and development of rice and barley seeds. The result obtained is that the existence of labile P is found in barley seedlings but not in rice seedlings. Both are monocotyledonous plants, but different in their requirement of phosphorus.

It is considered to be physiologically interesting to reveal such differences. So we have examined what differences are to be found in both seedlings after the procedure used by Bonner. In addition, it is indispensable to examine not only leaves but also roots from the point of the plant. In this respect, we attempted to extract and determine the nucleic acids that may play an important role in the function of roots of the both seedings in connection with the whole plant.

The results of experiments with regard to the physiological process of P metabolism are reported as follows.

## Materials and methods

The seeds of the rice (Norin 16) and the barley (Aizu-Shoki) were used as the experimental materials. The germination was allowed to take place under dark at 25°C for 5 days in the rice and for 3 days in the barley.

The water culture method was adopted for bringing up the plants under the illumination of 5000 Lux at 25°C for the rice, but for the barley the same culture method was done in the green house under room temperature. These experiments were made as follows.

The composition of the culture solution is indicated in Table 1.

The reason why we did not adopt the same nutrient solution in both seedings was that we have choosed the most adequate composition of the culture solution to enable maximum growth. The culture solution was renewed every 5 days and culture was carried for about a month. Sampling was done every 7 days.

**Table 1.** Composition of the nutrient solution

| Rice | | Barley |
|---|---|---|
| 10 ppm ($Na_2HPO_4 12H_2O$) | $P_2O_5$ | 100 ppm ($Na_2HPO_4 12H_2O$) |
| 40 〃 ($(NH_4)_2SO_4$) | N | 25 〃 ($(NH_4)_2SO_4$)<br>30 〃 ($NaNO_3$) |
| 40 〃 (KCl) | $K_2O$ | 100 〃 (KCl) |
| 4 〃 ($CaCl_2 2H_2O$) | CaO | 75 〃 ($CaCl_2 2H_2O$) |
| 6 〃 ($MgCl_2 2H_2O$) | MgO | 75 〃 ($MgCl_2 2H_2O$) |
| 2 〃 ($MnCl_2$) | Mn | — |
| 2.5 〃 (EDTA-Fe) | Fe | 2.5 EDTA-Fe |
| — | $SO_3$ | 90 |

Preparation of tissue····Freshly cut plant tissue was frozen in liquid air and dried in vacuo. When throughly dried, the tissue was ground to pass 40 mesh screen and stored in vacuo over $P_2O_5$ in darkness until analysis.

P distribution analysis····100 mg of dried tissue powder is weighed and transferreed to a sintered glass funnel. 5 *ml* of ice cold 1*N* trichloroacetic acid (TCA) was added to the sample, the mixture is stirred for several minutes, and the liquid is drawn through with suction. This filtrate is called as supernatant I. The process is repeated with another 5 *ml* of cold TCA; the filtrate is called as supernatant IA.

These filtrates are evaporated to a small volume at 98°C, and then subjected to total digestion with 60 per cent perchloric acid and $H_2O_2$. Inorganic phosphate is determined by the method of Allen (4).

The cold acid-extracted tissue is quantitatively transferred to a test tube by washing with 1*N* HCl and made to 5 *ml* volume. The tube is then placed in a boiling water bath for exactly 2 minutes with vigorous stirring, plunged into an ice-bath and shaken to insure rapid cooling. The tissue is separeted from the extract by filtering on sintered glass. The tissue is washed with an additional 5 *ml* of cold HCl. The combined filtrates (Supernatant II) are evaporated to a small volume, totally digested and analyzed for P. The residue is retrieved from the sintered glass funnel and also analyzed for total P.

At the same time, extraction and estimation of nucleic acids in both primary roots attempted. 100–150 primary roots are are sampled and 3 mm segments of root tips are cut off.

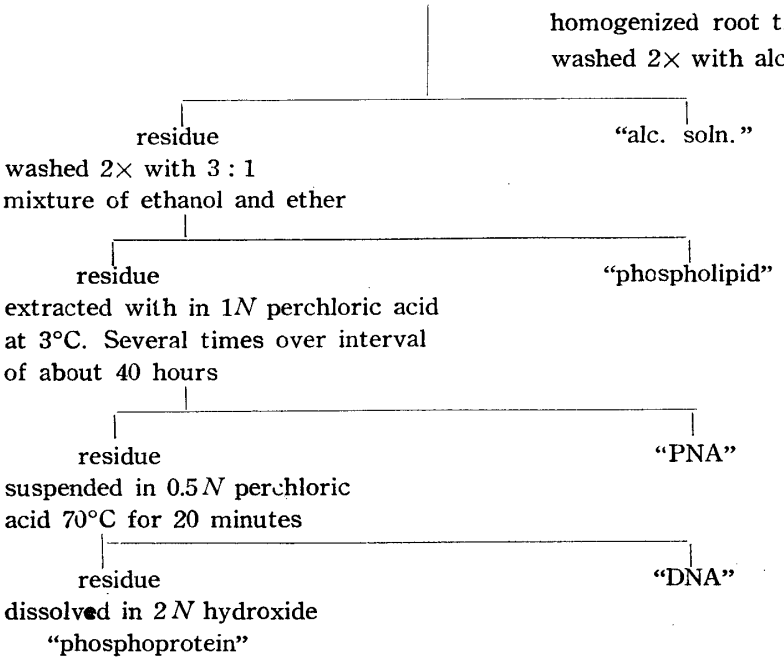
The nucleic acids of these materials are extracted and estimated according to the method of Ogur and Rosen (5).

The method briefly consists of treating plants roots first with alcohol to remove soluble nucleotides, then with a mixture of alcohol-ether to extract

lipids. This is followed by extraction with 1*N* perchloric acids in the cold, and then by 0.5 perchloric acid at 70°C.

The extraction with cold perchloric acid removes the pentose nucleic acid (PNA). The treatment with the warm perchloric acid removes the desoxypentose nucleic acid (DNA). Estimation of the PNA and DNA is based on ultraviolet absorption by Beckman Spectrophotometer. The above results are outlined in schma I.

Schema 1.




## Experimental Results

The distribution of phosphorus in the seedlings is indicated in Table 2.

**Table 2.** Distribution of phosphorus in the rice seedlings

| Tissue fraction | Treatment | Total (γ) per mg tissue | | | | |
|---|---|---|---|---|---|---|
| | | 5 days | 7 days | 14 days | 21 days | 28 days |
| Whole leaves | Total digestion | 6.01 | 5.97 | 6.23 | 9.18 | 9.87 |
| Supernatant I | Cold TCA extract | 2.56 | 3.67 | 4.15 | 6.42 | 7.17 |
| 〃 IA | 2nd cold TCA extract | 0.19 | 0.36 | 0.37 | 0.54 | 0.56 |
| 〃 II | Hot HCl extract for 2 minutes | 1.95 | 1.51 | 1.26 | 1.04 | 1.39 |
| Residue | Tissue remaining after the cold and hot acid extraction | 1.31 | 0.43 | 0.45 | 1.18 | 0.75 |

The distribution of phosphorus in the barley seedlings is indicated in Table 3.

**Table 3.** Distribution of phosphorus in the barley seedlings

| Tissue fraction | Treatment | Total (γ) per my tissue | | | | |
|---|---|---|---|---|---|---|
| | | 3 days | 7 days | 14 days | 21 days | 28 days |
| Whole leaves | Total digestion | 8.12 | 6.13 | 6.64 | 6.12 | 7.13 |
| Supernatant I | Cold TCA extract | 5.43 | 3.82 | 4.05 | 4.43 | 3.85 |
| 〃 IA | 2nd cold TCA extract | 0.71 | 0.69 | 0.49 | 0.34 | 0.45 |
| 〃 II | Hot HCl extract for 2 minutes | 1.89 | 1.29 | 1.16 | 0.75 | 1.44 |
| Residue | Tissue remaining after the cold and hot acid extraction | 0.09 | 0.51 | 0.94 | 0.60 | 1.34 |

The per cent of P in each fraction figured against the total P in whole leaves as 100 per cent is as follows. P per cent of Sup I and Sup IA are combined and indicated.

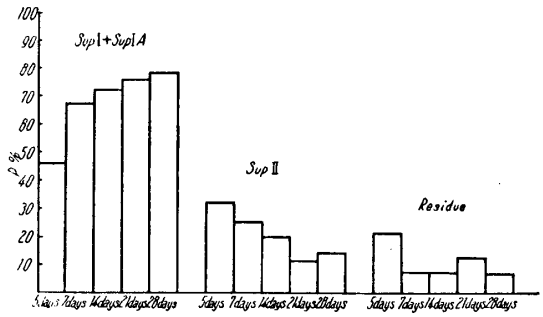



Fig. 1. P per cent of each fraction with the lapse of days (rice seedlings)

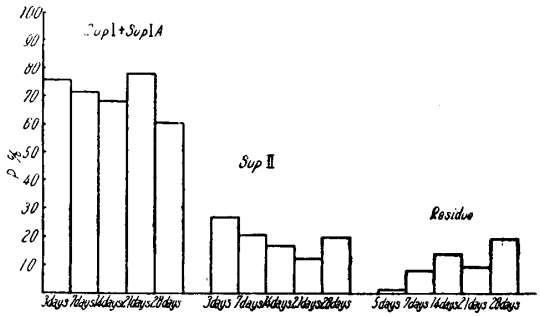



Fig. 2. Pper cent of each fraction with the lapse of days (barley seedlings)

From the Tables 2, 3, Figs 1 and 2, it is concluded that the total P in the tissue of the rice seedlings increases rapidly after 21 days. The cold acid soluble P reached to 78 per cent after 28 days gradually. On the other hand the total P in the tissue of the barley seedlings showed the maximum value after 3 days. The increase of acid soluble P as seen in the rice seedlings was not found.

Supernatant II in both seedlings shows the same trend.

Absorption spectra of nucleic acids extracted from root meristems are indicated in Figs. 3 and 4. PH control in perchloric acid extraction is not carried on. From Fig. 3, the absorption spectrum of pentose nucleic acid reaches the minimum at 230 mμ and the maximum at 260 mμ. Absorption spectrum of desoxypentose nucleic acid reaches the maximum at 260 mμ as seen in Fig. 4. Pentose nucleic acid phosphorus and desoxypentose nucleic acid phesphorus calculated from the optical densities at 260 mμ are indicated in Tables 4 and 5.

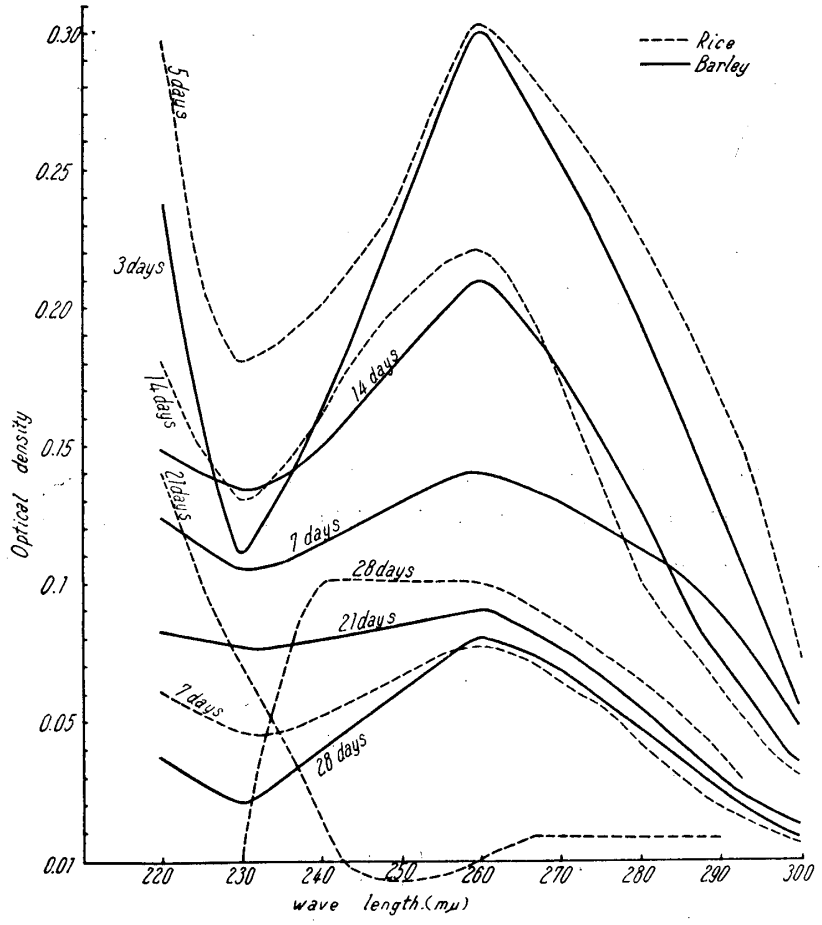



Fig. 3. The absorption spectra of PNA in rice and barley roots

Standard curves of the PNA and DNA are figured by thecommercial product of Minophagen Co. From the values observed at 260 mμ PNA-P and DNA-P are calculated. Fresh weight data used for the calculation of the results in Tables 4 and 5 are obtained by weighing 100–150 apical 3 mm root segment on a Shimazu torsion balance.

**Table 4.** Pentose nucleic acid phosphorus and desoxy pentose nucleic acid phosphorus of rice root tips.

| Treatment days | PNA-P | | DNA-P | |
|---|---|---|---|---|
| | $\gamma$ in 3 mm segment | $\gamma\times10^2$ per mg fresh wt. | $\gamma$ in 3 min segment | $\gamma\times10^2$ per mg fresh wt. |
| 5 days | 0.85 | 1.30 | 1.82 | 2.79 |
| 7 〃 | 0.21 | 0.39 | 1.18 | 2.16 |
| 14 〃 | 1.69 | 4.33 | 3.91 | 10.00 |
| 21 〃 | — | — | — | — |
| 28 〃 | — | — | — | — |

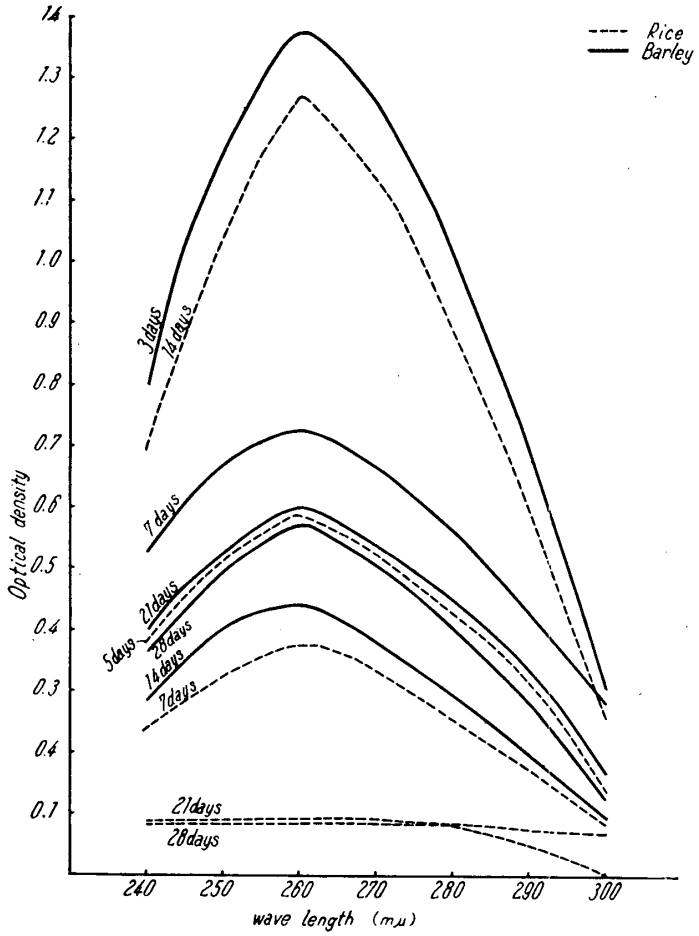



Fig. 4. The absorption spectra of DNA in rice and barley roots

**Table 5.** Pentose nucleic acid phosphorus and desoxy pentose nucleic acid phosphorus of barley roots tips

| Treatment days | PNA-P | | DNA-P | |
|---|---|---|---|---|
| | γ in 3 mm segment | γ×10² per mg fresh wt. | γ in 3 mm segment | γ×10² per mg fresh wt. |
| 3 days | 0.57 | 0.85 | 2.85 | 4.25 |
| 7 | 0.27 | 0.38 | 1.51 | 2.12 |
| 14 | 0.61 | 0.95 | 1.35 | 2.10 |
| 21 | 0.26 | 0.48 | 1.85 | 3.42 |
| 28 | 0.24 | 0.36 | 1.77 | 2.68 |

## Discussion

It is evident that energy rich phosphorylated compounds play important roles in the animals and microorganisms. It has been assumed that these compounds occur in plants. But, owing to the small quantities and unstable

condition, it was difficult to determine these.

Recently, Albaum (6) has determined the phosphorus compounds in Euglena after the fractionation by Lepage (7).

With regard to higher plants, Holden (8) has fractionated with tobacco leaves and investigated the enzymatic breakdown of some phosphorus compounds. Albaum and Ogur (9) have prepared ATP from the mung bean seedlings, Ohmura (10) has purified a nucleotide from the cold acid extract of soybeans which is similar to energy rich phosphate such as ATP.

Our results obtained after Bonner's method indicate that the total phosphorus in the whole leaves of the rice seedlings changes to high during the period from 14 days to 21 days.

But, in the barley seedings such remarkable changes cannot be seen during the whole period.

In the rice seedlings P per cent of the combined filtrate (Supernatant 1 and Supernatant IA) increases gradually from 47 per cent and reaches to 78 per cent after 28 days. But, in the barley seedlings, P per cent of the above combined filtrates indicates the reverse trend and decreases after 28 days.

Bonner has found that the Supernatant II which is acid insoluble and extracted by hot acid contains organic P bound to protein. Both seedlings indicate almost the same trend in the Supernatant II. The P per cent in the Supernatant II is to some degree more abundant in the rice seedlings than in the barley seedlings. Therefore, from the above results, in both seedling we recognize no differences in the Supernatant II similar to ADP. It seems that that the differences in both seedlings are in the acid soluble phosphorus compounds.

As the separate determination of phosphorus compounds in this fraction is not practised, further experiments may solve the problem of differences in the phosphorus compounds. As seen in the absorption spectra of nucleic acids in the roots the values of PNA and DNA in the rice roots reach the maximum in about 14 days but the normal curves of PNA and DNA could not be figured after 21 days. In this period the yellow parts of the root apex are not recognized by the naked eye.

From the above results the function of primary roots seems to be lost at this period. Moreover the growth of secondary roots is observed remarkably. At the same time, when the augumentation of the total phosphorus in the leaves is considered, it appears that the function of primary roots reaches the highest after two weeks and that the absorption of phosphorus after that period makes rapid progress owing to the secondary roots instead of the primary roots.

In the barley seedling PNA and DNA are recognized during the whole

period. It is thought that the function of the primary roots in the barley continues longer than that of the primary roots in the rice.

We would suggest that there are not only differences in acid soluble fraction of the leaves but also in the function of roots in the rice and the barley seedings.

## Summary

Studies were made on the distribution of phosphorus in rice and barley seedlings during about thirty days after germination. The results obtained may be summarized as follows.

1. In the rice seedlings, cold acid could extract 46, 68, 72, 75 and 78 per cent each and in the barley seedlings, 75, 71, 68, 78, and 60 per cent each of the total tissue P in the plants, respectively 3 or 5, 7, 14, 21 and 28 days after germination.

2. The bound P released by the 2 minute 1*N* hat acid treatment showed the same trends in both seedlings.

3. As the results of the estimation of PNA and DNA in the primary roots tips of the both plants, the function of the secondary roots in the rice seedlings exceodied that of the primary roots, after about 21 days, and therefore a large amount of P contained in the leaves at this stage was considered to be due to the vigorous absorption by the functional augumentation of the secondary roots. From the above results authors would suggest that there are large differences between the rice and barley seedlings in relation to P fraction extracted by cold acid.

## References

1) Gest, H., and M. D. Kamen (1948). J. Biol. Chem., **176**, 299.
2) Wildman, S. G., J. Campbell, and J. Bonner (1949). J. Biol. Chem., **180**, 273.
3) Fujiwara, A., and M. Kadowaki (Manuscript)
4) Allen, R, J. (1940). Biochem. J., **34**, 858.
5) Ogur, M. and G. Rosen (1950). Arch. Biochem., **25**, 262.
6) Albaum, H. G., A. Schatz., S. H. Hutner, and A. Hirshfeld (1950). Arch. Biochem., **29**, 210.
7) LePage, G. A., in W. W. Umbreit., R. H. Burris. and J. F. Stauffer (1949). Manometric Techniques, p. 193. Burgess, Minneapolis.
8) Holden, M. (1952). Biochem. J., **51**, 28.
9) Albaum, H. G., M. Ogur, and A. Hirshfeld (1950). Arch. Biochem., **27**, 130.
10) Ohmura, T. (1951). J. Agr. Chem. Soc. Japan., **24**, 371.