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BIOCHEMICAL AND NUTRITIONAL STUDIES ON POTASSIUM* III. ON THE LEACHING EXTRACTION OF POTASSIUM FROM THE HIGHER PLANTS

By

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Introduction

The authors attempt to give an explanation with regard to the biochemical function of potassium through the metabolic process in the standpoint of the higher plants nutrition.

Previously we (1) have reported on the respiration rate in relation to potassium status of the higher plants, and assumed that potassium is directly connected with the respiratory process. In part II(2), treating the carbohydrate metabolism of the rice plant and the barley, we confirmed the positive effect of potassium nutrient upon the increase of the content of the starch and total carbohydrate in the plants. In this report, forwardly, the problem with regard to the mobile property of potassium relating to the outer conditions and the inner metabolic process is proposed.

It has long been discussed in respect to the absorption of potassium and sodium by Nitella and Valonia. Nitella growing in fresh water and Valonia in the sea water accumulate a large amount of potassium in their body, although potassium concentration in the surrounding solution is quite low. Valonia, on the other hand, takes up a relatively small amount of sodium from the sea water, which contains a higher level of sodium as compared with potassium.

According to D. R. Hoagland(3), the inner cations were hardly depleted to the outside, when intact Nittella was immersed in the distilled water.

H. Jenny and R. Overstreet(4) observed that the low salt barley roots are able to retain their potassium against the distilled water, but the dilute clay suspensions which adsorbed the other exchangeable cations can pull out potassium from the normal low salt roots in the form of exchange with other outer cations during the brief interval. In this case the potassium level of roots is severely reduced dependent on some environmental conditions.

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T. C. Broyer and R. Overstreet (5) reported that when the barley roots containing isotopic potassium was dipped in the salt solution, isotoic potassium was replaced by the cations in the medium.

Then, the translocation of potassium takes place in the animal tissues and so many phenomena (6) have been observed and reported.

- K. C. Dixon (7) made researches on the behavior of potassium in the brain cortex. He found that in the presence of glucose during the anaerobic metabolism, there was no increase in the concentration of potassium, in some cases, rather there was a decrease in the cerebral cortex. While in the absence of glucose or when the glycolysis process was inhibited by the addition of 0.05 M fluoride, there was always a significant potassium loss from the brain slices into the surrounding fluid.
- G. T. Scott *et al* (8) have demonstrated the influences of glycolytic factors on the potassium and sodium content of *Saccharomyces cerevisiae*, that is in the absence of the substrate in the medium or at low temperature such as 5°C, the yeast cells lose about 30 per cent of its potassium, and the glycolytic inhibitors as jodoacetate and sodium fluoride effect progressive losses of potassium.

Also they have investigated the exchange of potassium ion in the green algae $Ulva\ lactuca$ using the isotopic K^{42} , and made the observations that stronger illumination or higher temperature markedly increased the rate of the exchange of K^{42} .

F. Scheffer *et al* (10) and W. Rathje (11) have made experiments on the relation between the carbon dioxide evolution and the intake of potassium using the yeast and observed that when the respiration of the yeast was highly increased by the addition of glucose, potassium concentration in the outer solution decreased rapidly. By the elapse of time, when the respiration rate decreased according to the consumption of the substrate, then potassium came out from the yeast to the surrounding solution. The translocation of potassium from the yeast cells to the outer solution also occured when the yeast cells were brought into the anaerobic condition.

The same tendency was also observed in *Lemna minor* by the treatments of light and darkness.

- S. Mitui and K. Kumajawa (12), S. Mitui *et al* (13) found out that by the addition of the respiratory inhibitors to the nutrient solution, potassium was hardly absorbed by the rice plant roots, or in cases, was depleted from the roots into the outer solution.
- G. T. Scott and H. R. Hayward (14) went on to seek information of the mechanisms regulating potassium and sodium distribution in Ulva, and confirmed as following, that 10^{-3} M phenylurethane and 3.3×10^{-5} M 4,6-dinitro-ocresol caused a marked progressive loss of potassium and a gain of sodium, but when the inhibited samples were transferred to the running sea water, then

the potassium content recovered completely until the normal level. Pyruvate added to the inhibited samples in the darkness for 5 hours, afforded the less protection against potassium loss, but phosphoglycerate and ATP on the other hand, offered more or sigificant protection.

In recent years, thus, the many investigations in this field have been performed from the biological standpoint with yeast, alga and animal tissues, but few in higher plants.

Although we have not yet succeeded in isolating or identifying the organic potassium compound from the vital materials and not achived the biochemical fractionation of potassium, it might be necessary to try the various research methods in our experimental plan.

At first the fractionation by means of the water extraction was adopted for convenience of our purposes.

Materials and Methods

The experiments consist of six parts. In the experiment (1), the rice plant (Norin 16) and the tomato were grown in the Wagner's pots (1/200000 acre) filled with alluvial soil, the leaves of the rice plant just before heading and of the tomato grown about 50 cm in the top length were harvested carefully, and immediately after their cut ends were sealed with parraffin, the fresh samples were immersed in the distilled water kept in the glass tube and then the leaching treatment was continued for 4 hours at room temperature (mean 23°C). The leaching water was renewed by using an automatic fraction collector, which was prepared for a constant volume of distilled water to pour into and out of the glass tube containing the plant leaves.

The changes of potassium and sodium concentration and the changes of hydrogen ion concentration of extracted solution during the extraction were measured by the flamephotometer and the glass electorode method.

To compare with the fresh leaves, the measurement of extraction was extended by the same method to the fully and partly dried leaves (dried for 5 min at 70°C).

In the experiment (2), the rice plant was raised by means of the water culture with normal nutrient level as shown in Table 2. The extraction experiment was done with the same methods using the fresh leaves as materials. In this case, the various inhibitory solutions besides the distilled water were prepared for leaching solution. The kinds, the concentration of inhibitory solutions as well as their hydrogen ion concentration are fully shown in Table 1, and these solutions were also applied in the later experiments of (3), (4), (5), (6). Beside this leaching experiment, the respiration rate which was inhibited by the addition of these substances, was measured by using Warburg's manometer, and the data was expressed initial respiration rate before inhibition as 100 percent.

Inhibitor		Concentration	pН
Phloridzin	(Phl)	$5 \times 10^{-4} \text{M}$	6.6
Sodium iodoacetic acid	(I.A.A.)	10^{-2}	5.3
Sodium fluoride	(F)	5×10^{-2}	6.3
Sodium arsenate	(As)	10-2	6.8
2,4-Dinitrophenol	(D.Ń.P.)	10^{-4}	6.9
Malonic acid	(M)	10^{-2}	5.3
Sodium azide	(Azide)	10^{-2}	6.9
3-Hydroxyquinoline	(8.H.Q.)	2×10^{-3}	7.1
Salicylaldoxime	(S.A.)	10-2	6.6
Urethane	(U)	10^{-2}	6.1

Table 1. The kinds of inhibitor, its concentration and pH, used in experiments (2), (3), (4), (5), (6).

In the experiment (3) the tomato leaves were used as the materials and the experimental procedures were the same as adopted in the experiments (1) and (2).

For the experimets (4), (6), the barley plant (Aizu Shoki) were cultured for three months by the following nutrient solution (Table 2), in the experiment (4) low potassium plants (N: 100 ppm, P_2O_5 : 100, K_2O : 5) were sampled and dipped in the flasks containing 50 ml of the inhibitory solutions for three hours, in stead of applying the fraction collector. Then the other experimental procedures were similar to the above experiments.

Table 2.	The composition of the nutrient solution applied in the experiment (2) for
	the rice plant and in the experiments (4), (6) for the barley, unit:ppm.

			Barley						
Salts		Rice plant	N ₁₀₀ K ₁₀₀ Series	N ₁₀₀ K ₅	N ₅ P ₁₀₀ K ₁₀₀	$N_{100}P_{100}K_{100}$			
NH ₄ NO ₃ ((NH ₄) ₂ SO ₄)	N	(50)	100	100	5	100			
Na ₂ HPO ₄ ·12H ₂ O	P_2O_5	50	100, 5, 0	100, 5, 0	100	100			
KCl	K_2O	50	100	5	100	0			
CaCl ₂ ·2H ₂ O	CaO	20	30	,,	,,	,,			
MgSO ₄ ·7H ₂ O	MgO	20	30	٠,	,,	,,			
MnCl ₂ ·4H ₂ O	MnO	1	2	,,	,,	,,			
FeCl ₃	Fe_2O_3	2	2	,,	,,	,,			

In the experiment (5) the materials of the wheat seedling were grown by means of sand culture for a month, and similar procedures were adopted.

In the experiment (6), the materials, as shown in Table 2, were grown with different level of the nitrogen, the phosphorus and potassium nutrient, only the potassium leaching due to the difference of nutrient levels was fully examined by the above mentioned method.

Experiments and Results

1. The leaching of potassium and sodium from the fresh and the prostrated materials immersed in the distilled water.

The fresh materials of the rice plant leaves and the tomato leaves were adopted as the samples. It was already reported that potassium was contained in the cuticular guttation sap(15), but it was hardly considered that potassium moves out from the fresh materials to the outer mediums in the normal cases. We found out accidentally from the previous analysis the decrease of the potassium content in the rice plant leaves after heavy rainfall, so the leaching experiment was performed systematically, and the results obtained are shown in Figs. 1 and 2, Table 3.

In the rice plant leaves (Fig. 1), a considerable amount of potassium was leached out within the first hour, and for the first four hours leached potassium came up to 10.6 per cent of total potassium, sodium to 8.8 per cent, pH value of leached solution rose fairly high as compared with the distilled water.

In the tomato leaves (Fig. 2) leached potassium came up to a higher level in the first 50 minutes. After 160 minutes it increased again continuously, and amounted to 8.4 per cent for the first four hours. The changes of leached sodium and pH curve were similar to that of the rice plant leaves.

While the rate of the potassium leaching, as shown in Table 3 was more rapid than that of sodium and went up to 50 per cent in partly dried leaves, and to $80\sim90$ per cent in dried leaves.

So it was confirmed from these experiments that potassium and sodium in the fresh leaves of higher plants move out easily according to the outer conditions such as the immersion or the heavy rain fall and that the rate of the potassium leaching was much increased by the prostration of cells on the drying. This cause will be considered in detail in the discussion.

2. The leaching of potassium from the fresh rice plant immersed in the various inhibitory solutions.

Applying the various metabolic inhibitory solutions besides distilled water, the leaching of potassium was examined by the similar methods as to the fresh materials of the rice plant leaves and roots.

To compare with the amount of depleted potassium, the rate of inhibition in the metabolic process was measured and estimated from the depressed rate of the oxygen uptake.

The respiratory inhibition in the leaves are shown in Fig 3, the data are expressed against 100 per cent of the initial respiration rate. The respiration rate was decreased to zero by the addition of 10^{-2} M azide, 70 per cent by 10^{-2} M monoiodoacetic acid, 80 per cent by 10^{-2} M arsenate.

The rate of potassium leaching from leaves (Fig 4, Table 4) was highly

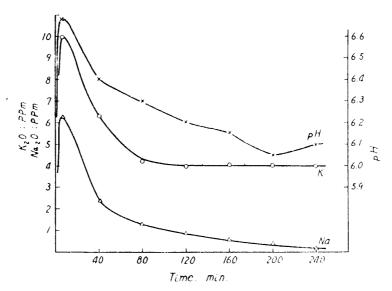


Fig. 1. Potassium and sodium leaching from the fresh rice plant leaves $(f.w.\ 5.5\,g)$ immersed in distilled water (initial pH 5.8)

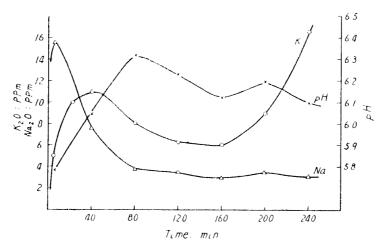


Fig. 2. Potasoium and sodium leaching from the fresh tomato leaves (f.w.~10.0~g) immersed in distilled water (initial pH 5.7)

Table 3. The leaching rate of potassium and sodium from the rice plant leaves and tomato leaves immersed into distilled water for 4 hrs.

Variety	Treat.	Content of K ₂ O (mg)	Leached K ₂ O (mg)	Rate of leaching (%)	Rate of sodium leaching (%)
	fresh	26.5	2.8	10.6	8.8
Rice plant	70°, 5min, dry	,,	14.5	54.6	10.0
leaves (f.w. 5.5 g)	dry	,,	21.4	81.0	55.9
	fresh	47.4	4.0	8.4	6.8
Tomato	70°, 5min, dry	"	24.9	52.5	
leaves (f.w. 10 g)	dry	. ,,	44.4	93.7	

increased by treatment of the inhibitors than by the distilled water, namely 24 per cent of inner potassium was depleted by iodoacetate treatment, 20 per cent by arsenate, and 14 per cent by fluoride.

The same tendencies were observed in the roots, and the respiration rate was decreased to 0 per cent by azide, 60 per cent by iodoacetate and arsenate, and 85 per cent by malonate. Potassium leaching, on the other hand, was 53 per cent by iodoacetate, 40 per cent by arsenate, 38 per cent by azide and fluoride, as shown in Fig. 6, Table 4.

In the both leaves and roots, when the oxygen respiration was strongly inhibited, the rate of potassium leaching was not always more increased, and it varied highly in relation to the kinds of the inhibitors, possibly, with the difference of speificity of its inhibitory mechanism.

M. Nagao, et al (23) have already confirmed that glycolytic inhibition due to sodium bisulphite and 1-amino-2-naphthol-4-sulphonic acid affect sensitively on the growth of the rice plant coleoptil. To correspondence with his opinion, it may be concluded from the present results that potassium retained in both rice leaves and roots was depleted out more strongly and sensitively by the addition of iodoacetate, arsenate and fluoride inhibitors than by others.

3. The leaching potassium from the fresh tomato leaves immersed in various inhibitory solutions

There may be question as to whether the similar specific leaching phenomena occur with the tomato leaves, which are distinguised in biological and morphological points from the rice plant.

The results obtained are shown in Figs. 7, 8, and Table 5. The respiration rate was diminished at 20 per cent level by azide inhibition, 30 per cent by arsenate and 60 per cent by fluoride, 70 per cent by malonate as shown in Fig. 7. While the rate of potassium leaching (Fig. 8, Table 5) was in the order as follows, 34 per cent by azide, 23 per cent by malonate, 20 per cent by fluoride, and 19 per cent by arsenate. These results differed from that of the cases of rice plant, namely potassium was leached out from the fresh tomato leaves abundantly by the azide and malonate inhibition.

4. The leaching of potassium from the fresh barley plant immersed in various inhibitory solutions

Similar experiments were extended to the barley leaves and roots. To avoid complicacy, in the first step the experiments were carried on the difference of the respiratory inhibition between the leaves and roots.

At first, the respiration rate of the roots were more strongly inhibited than that of the leaves, secondly, under the salicylaldoxime inhibition the respiration of the roots were depressed about to a 10 per cent level. On the other hand, the respiration rate of the leaves under the same treatment was accelerate to

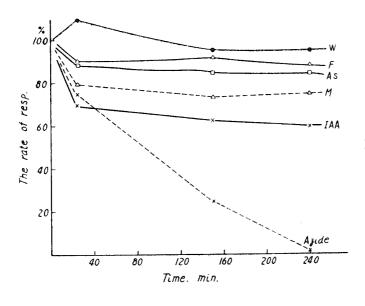


Fig. 3. Respiratory inhibition of the rice plant leaves, data are expressed taking the initial respiration as 100 per cent.

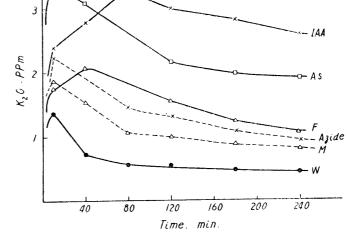


Fig. 4. The potassium leaching from the rice plant leaves (f. w. 0.7g) immersed in various inhibitory solutions.

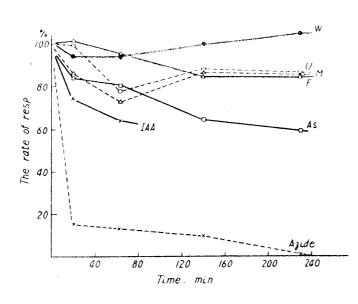


Fig. 5. Respiratory inhibition of the rice plant roots immersed in various inhibitory solutions.

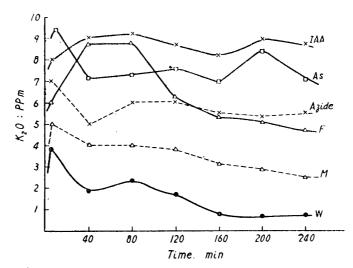


Fig. 6. The potassium leaching from the rice plant roots (f.w. 3.8g) immersed in various inhibitory solutions.

Table 4. The rate of potassium leaching from the rice plant leaves and roots immersed into various inhibitory solutions for 4 hrs. (the method of experiment (4) was applied to the roots)

Inhibitors	Le	aves (f. w. 0.	7g)	Roots (f.w. 0.4g)			
	Content of K ₂ O (mg)	Leached K ₂ O (mg)	Rate of leaching(%)	Content of K ₂ O (mg)	Leached K ₂ O (mg)	Rate of leaching (%)	
Water	5.0	0.3	6.0	2.6	0.4	15.4	
Malonate	,,	0.5	10.0	2.8	0.5	18.0	
Urethane	,,	0.4	8.0	2.5	0.4	16.0	
Arsenate	,,	1.0	20.0	2.5	1.0	40.0	
Azide	,,	0.6	12.0	2.1	0.8	38.1	
Fluoride	,,	0.7	14.0	2.1	0.8	38.1	
Iodoacetate	,,	1.2	24.0	1.9	1.0	52.6	

some extent, and in cases of the phloridzin inhibition, the reverse results were obtained. These phenomena are in accord with the following experimental data in cases of the wheat leaves and roots.

Next, the respiration rate of the roots (Fig. 10) was lowered to the 10 per cent level by the salicylaldoxime inhibition and to the 30 per cent level by iodoacetate, fluoride, azide. While the rate of potassium leaching was not parallel to the rate of the respiratory inhibition, as shown in Table 7, it went down to 59 per cent by iodoacetate, 50 per cent by arsenate, 46 per cent by fluoride, 42 per cent by salicylaldoxime, and to 36 per cent by azide.

The rate of the inhibited respiration in the leaves (Fig. 9) was in the order of azide, arsenate, malonate, and iodoacetate, but the magnitude of the rate of potassium leaching (Table 6) was in order of arsenate, iodoacetate, malonate, fluoride and azide, being in general smaller than that of the root.

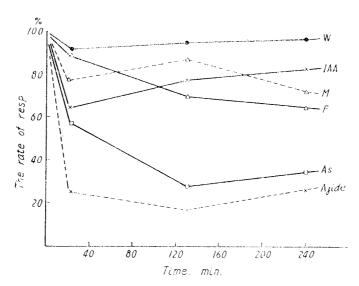


Fig. 7. The respiratory inhibition of the tomato leaves immersed in various inhibitory solutions.

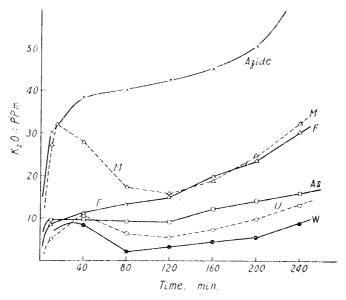


Fig. 8. The potassium leaching from the fresh tomato leaves (f.w. 10.0g) immersed in various inhibitory solutions.

Table 5. The rate of potassium leaching from the fresh tomato leaves (f.w. 10.0g) immersed in various inhibitory solutions for 4 hrs.

Inhibitors	Content of K ₂ O (mg)	Leached K ₂ O (mg)	Rate of leaching (%)	
Water	47.8	4.0	8.4	
Malonate	,,	10.8	22.6	
Urethane	,,	7.8	16.4	
Arsenate	,,	9.0	18.8	
Azide	,,	15.7	34.1	
Fluoride	,,	10.5	19.9	

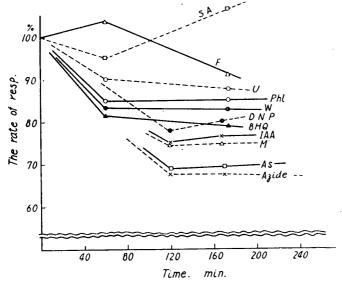


Fig. 9. The respiratory inhibition of the barley leaves immersed in various inhibitory solutions.

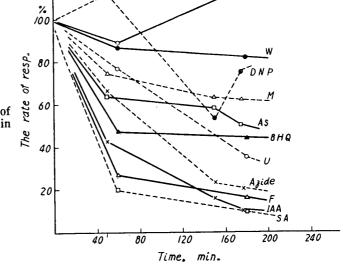


Fig. 10. The respiratory inhibition of the barley roots immersed in various inhibitory solutions.

Table 6. The rate of potassium leaching from the fresh barley leaves and roots immersed in various inhitory solutions, for 3 hrs.

	Lea	ives (f.w. 1.	0g)	Roots (f.w. 0.5g)			
Inhibitors	Content of K ₂ O (mg)	Leached K ₂ O (mg)	Rate of leaching(%)	Content of K ₂ O (mg)	Leached K ₂ O (mg)	Rate of leaching(%)	
Water	2.8	0.08	2.6	0.70	0.08	10.7	
Phloridzin	,,	0.08	2.6	,,	0.08	10.7	
Salicylaldoxime	,,	0.13	4.5	,,	0.30	42.0	
Urethane	,,	0.13	4.5	,,	0.08	10.7	
8-Hydroxyquinoline	,,	0.15	5.5	,,	0.24	34.0	
Fluoride	,,	0.15	5.5	,,	0.34	45.6	
Dinitrophenol	,,	0.05	1.7	,,	0.22	30.7	
Azide "		0.15	5.5	,,	0.25	35.7	
Malonate	,,,	0.19	6.8	,,	0.17	23.6	
Arsenate	,,	0.24	8.6	,,	0.35	50.0	
Iodoa'cetate	,,	0.23	8.0	,,	0.42	59.3	

There were some difficulties to make these experiments as to the leaves, because of the cuticular membrane was hardly wetted with the leaching solution. At any rate, the results obtained showed the similar tendencies in both leaves and roots, resembling with the movement of potassium in the rice plant leaves and roots.

It may be also concluded that potassium maintained in the fresh barley leaves and roots was depleted out more strongly and sensitively by the inhibition of iodoacetate, arsenate, and fluoride, being similar as in cases of the rice plants.

5. The leaching of potassium from the fresh wheat immersed in various inhibitory solutions.

The results obtained are shown in Fig 11, and Fig. 12, the differences of the respiratory inhibition between the wheat leaves and the roots were similarly observed under the salicylaldoxime and phloridzin treatment, other aspects of respiratory inhibition also resembled that of the barley.

Potassium leaching from the leaves (Table 7) was highly sensitive to the inhibition of salicylaldoxime, iodoacetate, arsenate and fluoride. But the aspect of the potassium leaching in the roots (Table 7) differed from that of the leaves, and this difference was observed in only the case of the wheat, but not in either the rice plant and barley, in this case potassium was depleted from the roots more sensitively and strongly by the salicylaldoxime, dinitrophenol, 8-hydroxyquinoline treatments than by others.

The wheat materials applied in this case were the seedling grown in sand for a month, while the rice plant and the barley were the matured materials grown by the water culture method, thus it is difficult to conclude whether it depends upon the difference in the original nature of the plant or in the culture condition and the growth stage that the tendency of the potassium leaching in the root was dissimilar to that of the leaves in the case of the wheat. This point should be investigated in the future.

6. Potassium leaching from the leaves of the barley cultivated with the nutrient solutions having different levels of phosphorus, nitrogen, potassium

In recent years, there were many knowledges about the exchange absorption or the replacement of potassium in the root depending upon the environmental cations level. In this experiment, using the barley leaves, a study was made as to how the potassium retention or the rate of leaching would vary according to the difference of the nutrients levels such as phosphorus or nitrogen.

This experiment was made to obtain some prospects which suggest the existence of some organic compounds consisting of nitrogen or phosphorus, and by which potassium might be retained as an organic complex in the plant tissue.

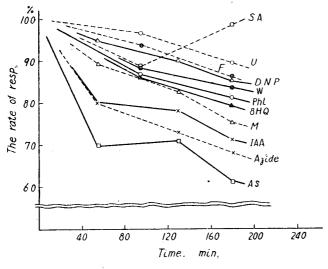


Fig. 11. The respiratory inhibition of the fresh wheat leaves immersed in various inhibitory solutions.

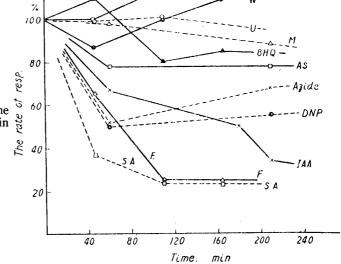


Fig. 12. The respiratory inhibition of the fresh wheat roots immersed in various inhibitory solutions.

Table 7. The rate of potassium leaching from the fresh wheat leaves and roots immersed in various inhibitory solutions for 4 hrs.

*	Lea	ves (f.w. 1.0	g)	Roots (f.w. 0.5g)			
Inhibitors	Content of K ₂ O (mg)	Leached K ₂ O (mg)	Rate of leaching(%)	Content of K ₂ O (mg)	Leached K ₂ O (mg)	Rate of leaching(%)	
Water	6.60	0.13	1.9	2.08	0.25	12.0	
Phloridzin	,,	0.10	1.5	,,	0.45	24	
Salicylaldoxime	,,	0.70	10.6	,,	1.34	65.7	
Urethane	,,	0.23	3.4	,,	0.55	26.5	
8-Hydroxyquinoline	,,	0.18	2.7	,,	0.85	43.3	
Fluoride	,,	0.25	3.7	,,	0.75	36.1	
Dinitrophenol	,,	0.15	2.3	,,	0.90	43.5	
Azide	,,	0.30	4.5	,,	0.75	36.1	
Malonate	,,	0.33	4.9	,,	0.65	31.3	
Arsenate	,,	0.48	7.1	,,	0.45	21.6	
Iodoacetate	,,	0.43	6.4	,,	0.60	28.9	

The results obtained are shown in Table 8.

Table 8. The potassium content, amount of leaching of the fresh barley leaves cultured with different levels of nitrogen, phosphorus, potassium. The samples were immersed in various inhibitory solutions for 3 hrs. Unit: K_2O 1/20 mg. Sample: f. w. 1.0 g.

	Inhibitors	N ₁₀₀ K ₁₀₀ Series		N ₁₀₀ K ₅ Series			low N	non K	
		P ₁₀₀	P ₅	P ₀	N ₁₀₀	P_5	P ₀	$N_5 P_{100} K_{100}$	N ₁₀₀ P ₁₀₀ K ₀
Potassium content		144.0	96.5	95.0	112.0	78.0	79.0	80.0	37.0
Potassium leaching	Water Phloridzin Salicylaldoxime Urethane 8-Hydroxyquinoline	1.6 2.0 3.0 2.8 2.6 2.6 2.4 5.0 6.0	3.5 3.5 5.8 4.0 3.5 4.5 3.0 6.5 7.5 8.5 8.0	8.0 3.3 9.5 5.0 7.0 5.3 4.3 8.0 8.0 17.0	1.5 1.5 2.5 2.5 3.0 3.0 3.0 3.8 4.8 4.5	3.0 2.5 3.0 2.0 2.0 3.0 4.5 6.0 5.0	5.5 2.0 2.5 3.5 3.5 7.8 3.3 4.0 6.3 8.0	2.4 2.9 5.3 3.2 2.8 3.2 2.4 8.5 8.3 7.2 6.4	0.4 0.3 0.8 2.5 0.5 1.0 0.6 3.5 3.5 3.5

The potassium level retained in the plant was decreased, and the rate of potassium leaching from the fresh leaves dipped in the various inhibitory solutions were increased by diminishing the phosphorus level in the culture solution from 100 ppm to 5 and 0 in $N_{100}K_{100}$ series of the normal nitrogen and potassum supply. In $N_{100}K_5$ series of normal nitrogen and low potassium supply similar tendencies were observed with less magnitude. Also the same result was extended in the low nitrogen plant N_5P_{100} K_{100} compared with the normal plant $N_{100}P_{100}K_{100}$.

Discussion

In dealing with the leaching of potassium from the fresh and prostrated materials by drying of several plant species, the leaching phenomena and causes were investigated. In an attempt to explain the biochemical function of potassium, the results obtained are discussed.

According to H. B. Steinbach (6), body fluids are in general poorer in potassium and richer in sodium as compared with the protoplasm. Higher content of potassium and lower concentration of sodium inside the cell have been reported for yeast, algae, other plants, and such phenomena have been considered as the so-colled selective absorption.

Up to the present, various theories on the absorption mechanism (6), (16) have been suggested. The living thing, at any rate, will develop their selective properties with respect to sodium and potassium at a very early stage in the life history.

In recent years, as already described, algae (9, 11, 14), yeast (8, 10, 11), animal tissue (7) would deplete potassium easily under some conditions and the explanation for this phenomena has been undertaken from the view of metabolic process relating to the glucose content.

In higher plants, it has already been reported that the root has the capacity to exchange potassium with other cations (4, 5), and that the cuticular guttation sap contains more or less potassium (15), but it has not been fully investigated whether some amount of potassium in the fresh higher plants is able to be leached out easily into distilled water.

The same opinion for leaching property of potassium can be supported from our results. The leaching of potassium, depending on the lowering of nitrogen, phosphorus nuturient level in the culture solution as stated in experiment (6), came up to 2 to 10 per cent in the leaves and more in the roots. While the rate of potassium leaching was far more increased with the prostration of cells due to the drying of the materials, we could not conclusively discuss whether this cause depends on the deformation of the cell membrane, the depression and interruption of metabolic process supplying the biological energy, or the degeneration of important biochemical substance.

Except for the problem of the deformation of cell membrane, the force, which could retain potassium inside, may relate with the respiratory process of the cells. This suggestion was considered in experiments (2), (3), (4), (5), using the various respiratory inhibitors such as glycolysis, Krebs cycle, terminal oxidase system, and taking the samples of four species from the comparative biochemical stand.

These fresh materials were immersed into the inhibitory solutions, then the rate of potossium leaching, and the inhibited rate of respiration was simultaneously measured. As to the results mentioned above, the rate of potassium leaching was not always parallel with the inhibited rate of respiration, the rate of potassium leaching varied highly with the kinds of inhibitors perhaps depending upon the difference of the inhibitor's specific action.

An interesting aspect was also observed from the comparative biochemical stand, that is, in the rice plant leaves and roots, the barley leaves and roots, the leaching of potassium was sensitive to the inhibition of glycolysis stage such as iodoacetate, arsenate, fluoride (17, 18, 19). While the tomato leaves were sensitive to the inhibition of terminal oxidase or Krebs cycle system, azide and malonate (17, 18).

As the inhibitory mechanism or the specificity of the inhibitors have been studied and applied in many biological and biochemical researches (17-23), the problem is not yet fully solved. In this study the fresh intact materials possessing the multiple enzyme system were used instead of the biochemical preparations, though we can not define the places or the stages to which

potassium is able to be connected through the respiratory process, the following suggestion may be allowed.

There may be places to which potassium relate with the respiratory process, and if the places is inhibited or weakened, potassium may be leached out easily. The places may differ according to the varieties of the plants, in this case such as "rice plant type" or "tomato type". It was not ascertained whether these types are distinguished by the difference of mono- or di- cotyledonous plants, or the upland plants requiring oxygen preferably or the marsh plants requiring oxygen not so much.

Secondly, experiment (6) was done to advance the assumption further that the organic substance—the metabolic intermediate, some kind of protein relating the metabolic reaction or high energy compound introduced by metabolic process,—by which potassium is retained inside the cell, may be present in the place of potassium fixation or as the complex compound. We might also suggest the presence of such organic substance, considering from the experimental results.

Potassium may be retained selectively by the organic substance relating to the respiratory process. Further investigation is needed to identify such organic compound and to make clear why it could retain potassium.

Summary

In this report, the fresh materials or in some cases prostrated materials by the drying of the rice plant, tomato, barley, and wheat were used, and the phenomena of the potassium leaching and its causes were investigated.

When the fresh materials were immersed in distilled water, the rate of potassium leaching was 2 to 10 per cent in the leaves and larger in the roots. The rate reached 50 per cent by prostration by drying and increased to the 93 per cent level in the tomato leaves by complete dryness.

In the fresh materials, potassium was depleted out more strongly by the treatment of the inhibitors than by the distilled water, but the rate of potassium leachig was not always parallel to the degree of the respiratory inhibition. In the leaves of the rice plant and roots, barley leaves and roots, potassium was leached sensitively and highly by the inhibition of iodoacetate, arsenate and fluoride. While in the tomato leaves potassium was leached out by azide and malonate inhibitions.

The rate of leaching and also the rate of potassium retention varied distinctly according to the nutrients level supplied in the culture solution such as nitrogen and phosphorus.

From these experimental results, it was emphasized that there may be a force to retain potassium within the higher plant tissues, and the source of this force might be placed in some stages of metabolic process, and which

difer with the varieties of higher plants such as rice plant or tomato, and still this force might be coupled with the some organic compound in the stage of metabolic process.

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