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Biochemical and nutritional studies on potassium III. On the leaching extraction of potassium from the higher plants

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3rd part

Introduction

The authors endeavour to explain the biochemical function of potassium by the metabolic process from the standpoint of higher plants nutrition.

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

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

According to *Hoagland (3)*, the inner cations were hardly depleted outwards, when intact *Nitella* was immersed in the distilled water.

Jenny and *Overstreet (4)* observed that the low salt barley roots were able to retain their potassium against the distilled water, but the diluted clay suspensions which adsorbed the other exchangeable cations can abstract potassium from the normal low salt roots by exchange with other outer cations during the brief interval. In this case the potassium level of roots is severely reduced under the influence of certain environmental conditions.

Broyer and *Overstreet (5)* reported that when the barley roots containing isotopic potassium were dipped in the salt solution, isotopic 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.

Dixon (7) investigated the behaviour of potassium in the brain cortex. He found that when glucose was present 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.

Scott et al. (8) have demonstrated the influence of glycolytic factors on the potassium and sodium content of *Saccharomyces cerevisiae:* in the absence of the substrate in the medium or at low temperatures such as 5° C, the yeast cells lose about 30 per cent of its potassium, and the glycolytic inhibitors as iodoacetate 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 observed that stronger illumination or higher temperatures markedly increased the rate of the exchange of K^{42}.

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

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

Mitui and *Kumazawa (12), Mitui et al. (13)* discovered that by the addition of the respiratory inhibitors to the nutrient solution, potassium was hardly absorbed by the rice plant roots, or in some cases, was depleted from the roots into the outer solution.

Scott and *Hayward (14)* went on to seek information on the mechanisms regulating potassium and sodium distribution in *Ulva,* and confirmed, that $10^{-3}M$ phenylurethane and $3.3 \times 10^{-5}M$ 4.6-dinitro-o-cresol caused a marked progressive loss of potassium and a gain in sodium, but when the inhibited samples were transferred to running sea water, the potassium content recovered completely up to the normal level. Pyruvate added to the inhibited samples in the darkness for 5 hours, afforded less protection against potassium loss, but phosphoglycerate and ATP, on the other hand, offered more or significant protection.

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

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

First of all fractionation by means of water extraction was adopted for convenience.

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Materials and methods

The experiments consist of six parts. In experiment 1, the rice plant *(Norin [16])* and the tomato were grown in *Wagner*'s pots (1/200,000 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 carefully collected, and immediately afterwards their cut ends were sealed with paraffin, 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 (average of 23° C). The leaching water was renewed by using an automatic fraction collector, which was prepared for a constant volume of distilled water to be poured into and out of the glass tube containing the plant leaves.

The changes of potassium and sodium concentration and those of hydrogen ion concentration of the extracted solution during extraction were measured by the flame photometer and the glass electrode method.

For comparison 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 experiment 2, the rice plant was raised by means of water culture with normal nutrient level as shown in table 2. The extraction experiment was done by 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 types of inhibitor, the concentration of inhibitory solutions and their hydrogen ion concentration are fully shown in table 1, and these solutions were also applied in the later experiments 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 expressed by taking the initial respiration rate before inhibition as 100 per cent.

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

Table 1 The types of inhibitor, their concentration and pH, used in experiments 2, 3, 4, 5, 6

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

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For experiments 4 and 6, the barley plants (Aizu Shoki) were cultured for three months with the following nutrient solutions (table 2), in 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, instead of applying the fraction collector. The other experimental procedures were similar to the above experiments.

Table 2 The composition of the nutrient solutions applied in experiment 2 to the rice plant and in experiments 4 and 6 to barley; unit: ppm

Salts		Rice plant	Barley			
			$N_{100}K_{100}$ series	$N_{100}K_5$ series	$N_5P_{100}K_{100}$ series	$N_{100}P_{100}K_0$ series
NH_4NO_3 $[(NH_4)_2SO_4]$	N	(50)	100	100	5	100
Na_2HPO_4 $12H_2O$	P_2O_5	50	100, 5, 0	100, 5, 0	100	100
KCl	K_2O	50	100	5	100	0
$CaCl_2$ $2H_2O$	CaO	20	30	30	30	30
$MgSO_4$ $7H_2O$	MgO	20	30	30	30	30
$MnCl_2$ $4H_2O$	MnO	1	2	2	2	2
$FeCl_3$	Fe_2O_3	2	2	2	2	2

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

In experiment 6, the materials, as shown in table 2, were grown at different levels of the nitrogen, phosphorus and potassium nutrients; 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 alterated materials immersed in the distilled water.

The fresh materials of the rice plant leaves and of the tomato leaves were adopted as samples. It had already been reported that potassium was contained in the cuticular guttation sap *(15)*, but it was not thought that potassium moved out from the fresh materials to the outer media in normal cases. We discovered accidentally from the previous analysis the decrease of the potassium content in the rice plant leaves after heavy rainfall, so that the leaching experiment was carried out systematically; the results obtained are shown in figures 1 and 2, and in 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 amounted to 10.6 per cent of total potassium and sodium to 8.8 per cent, while pH value of leached solution rose fairly high as compared with the distilled water.

In the tomato leaves (fig. 2) leached potassium reached a higher level in the first 50 minutes. After 160 minutes it increased again continuously, and

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amounted to 8.4 per cent for the first four hours. The changes in leached sodium and pH curve were similar to those of the rice plant leaves.

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 to 90 per cent in dried leaves.

It was thus confirmed from these experiments that potassium and sodium in the fresh leaves of higher plants move out easily according to external conditions such as immersion or heavy rainfall and that the rate of the potassium leaching was much increased by the alteration of cells by 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 similar methods as to the fresh materials of the rice plant leaves and roots.

For comparison 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 is shown in figure 3, the data are expressed with reference to 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.

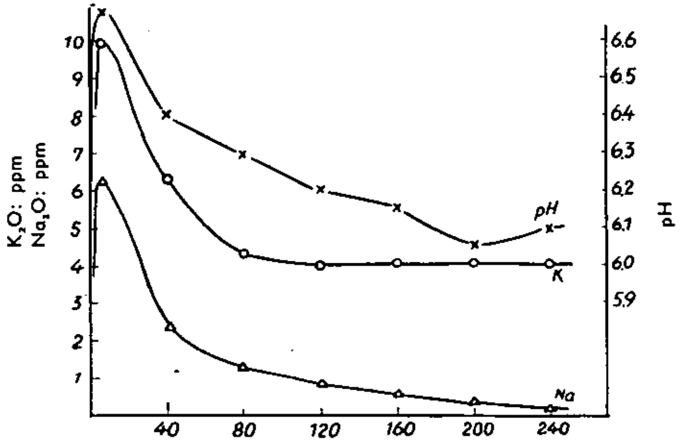

Figure 1

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

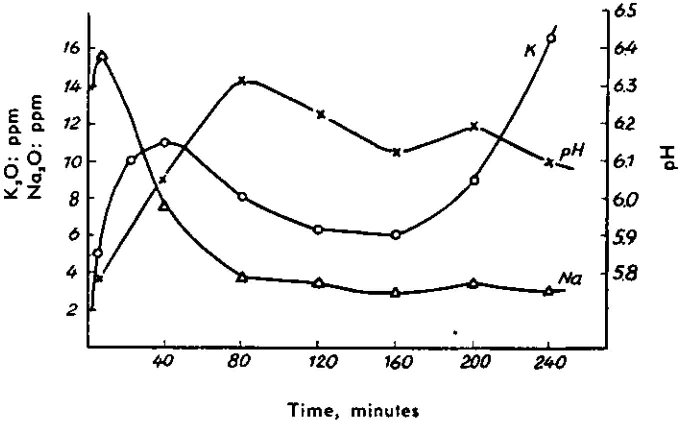

Figure 2
Potassium and sodium leaching from the fresh tomato leaves (f.w. 10.0 g) immersed in distilled water (initial pH 5.7)

Table 3 The leaching rate of potassium and sodium from the rice plant leaves and tomato leaves immersed in distilled water for four hours

Variety	Treatment	Content of K_2O (mg)	Leached K_2O (mg)	Rate of leaching (per cent)	Rate of sodium leaching (per cent)
Rice plant leaves (f. w. 5.5 g)	fresh .... 70°, 5 min., dry . dry	26.5 26.5 26.5	2.8 14.5 21.4	10.6 54.6 81.0	8.8 10.0 55.9
Tomato leaves (f.w. 10 g)	fresh .... 70°, 5 min., dry . dry	47.4 47.4 47.4	4.0 24.9 44.4	8.4 52.5 93.7	6.8 — —

The rate of potassium leaching from leaves (fig. 4, table 4) was more greatly increased by treatment with 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 (fig. 5). Potassium leaching, on the other hand, amounted to 53 per cent by iodoacetate, 40 per cent by arsenate and 38 per cent by azide and fluoride, as shown in figure 6 and table 4.

In both leaves and roots, when the oxygen respiration was strongly inhibited, the rate of potassium leaching was not always more greatly increased, and varied considerably with the type of inhibitor and possibly, with the difference of specificity of the inhibitory mechanisms.

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

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

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

The results obtained are shown in figures 7 and 8, and table 5. The respiration rate was reduced to 20 per cent level by azide inhibition, to 30 per cent level by arsenate, to 60 per cent level by fluoride and to 70 per cent level by malonate as shown in figure 7, while the rate of potassium leaching (fig. 8, table 5) was 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 those in the case of rice plant; namely potassium was abundantly leached out from the fresh tomato leaves 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. For the sake of simplicity the experiments in the first place applied to the difference of the respiratory inhibition between the leaves and roots.

Firstly, the respiration rate of the roots was more strongly inhibited than that of the leaves, secondly, under the salicylaldoxime inhibition the respiration of the roots was depressed about to a 10 per cent level. On the other hand, the respiration rate of the leaves under the same treatment was accelerated to some extent, and in the case of the phloridzin inhibition, the opposite results were obtained. These phenomena are in accordance with the following experimental data in the case of wheat leaves and roots.

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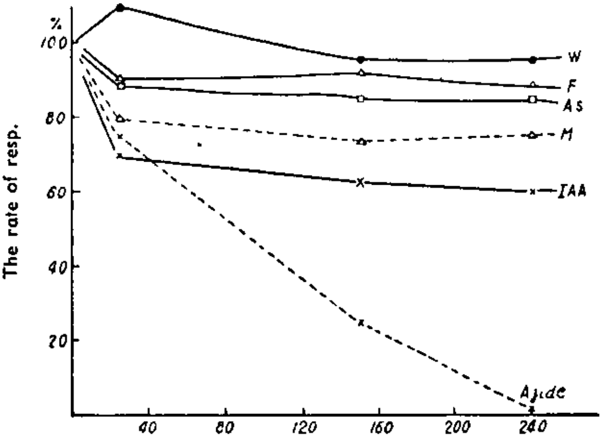
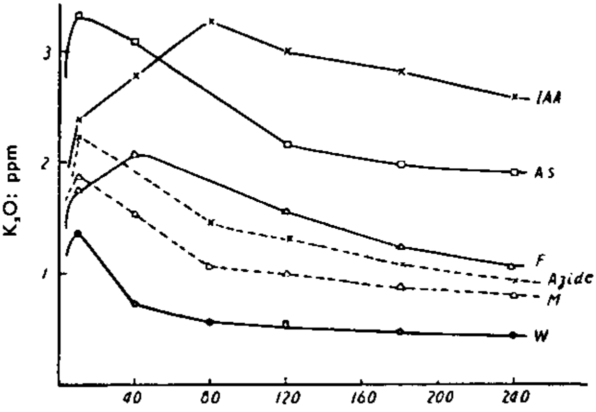


Time, minutes

Figure 3

Respiratory inhibition of the rice plant leaves; the data are expressed on the basis of an initial respiration of 100 per cent



Time, minutes

Figure 4

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

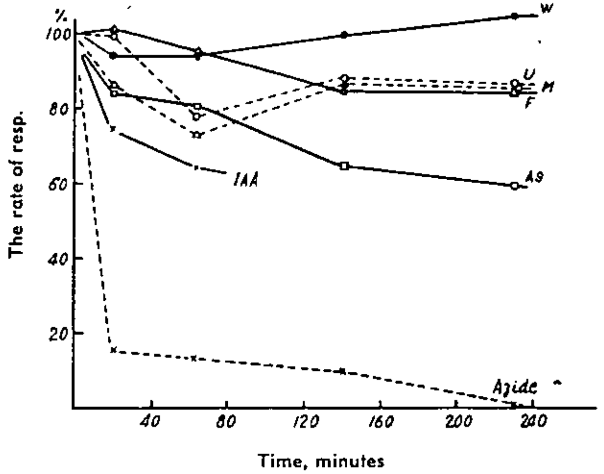


Figure 5
Respiratory inhibition of the rice plant roots immersed in various inhibitory solutions

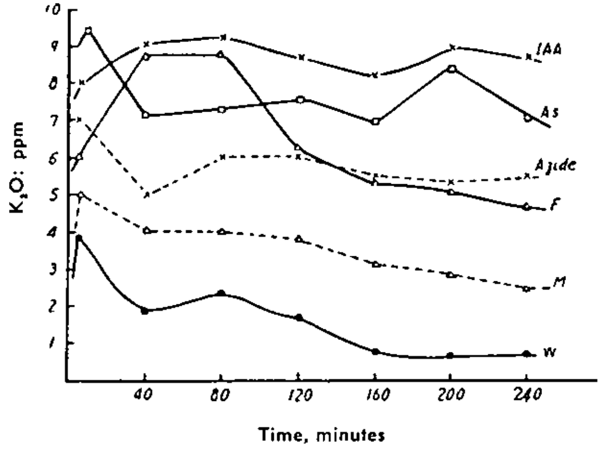


Figure 6
The potassium leaching from the rice plant roots (f. w. 3.8 g) immersed in various inhibitory solutions

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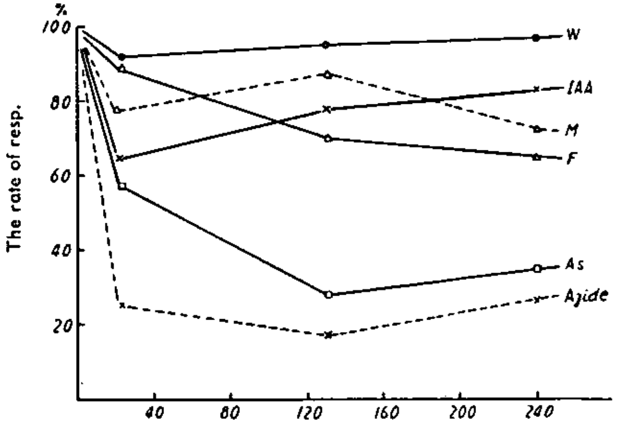
Table 4 The rate of potassium leaching from the rice plant leaves and roots immersed in various inhibitory solutions for four hours (the method of experiment 4 was applied to the roots)

Inhibitors	Leaves (f.w. 0.7 g)			Roots (f.w. 0.4 g)		
	Content of K_2O (mg)	Leached K_2O (mg)	Rate of leaching per cent	Content of K_2O (mg)	Leached K_2O (mg)	Rate of leaching per cent
Water	5.0	0.3	6.0	2.6	0.4	15.4
Malonate	5.0	0.5	10.0	2.8	0.5	18.0
Urethane	5.0	0.4	8.0	2.5	0.4	16.0
Arsenate	5.0	1.0	20.0	2.5	1.0	40.0
Azide	5.5	0.6	12.0	2.1	0.8	38.1
Fluoride	5.0	0.7	14.0	2.1	0.8	38.1
Iodoacetate	5.0	1.2	24.0	1.9	1.0	52.6

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 and azide. While the rate of potassium leaching was not parallel to the rate of the respiratory inhibition, as shown in table 6, it amounted to 59 per cent by iodoacetate, 50 per cent by arsenate, 46 per cent by fluoride, 42 per cent by salicylaldoxime and 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 the order of arsenate, iodoacetate, malonate, fluoride and azide, being in general smaller than that of the root.



Time, minutes

Figure 7

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

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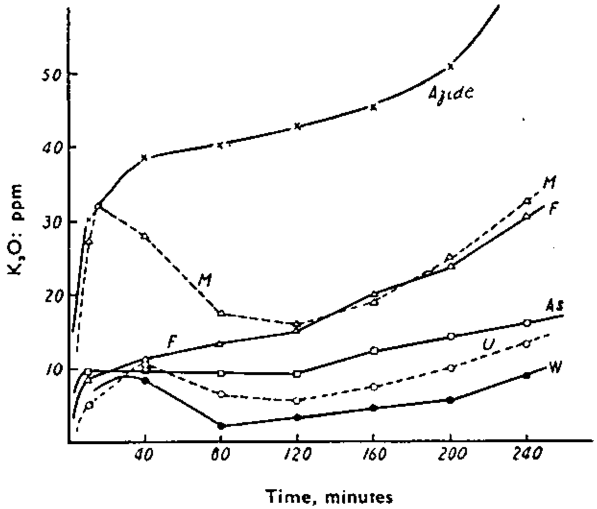

Figure 8

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

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

Inhibitors	Content of K_2O (mg)	Leached K_2O (mg)	Rate of leaching per cent
Water	47.8	4.0	8.4
Malonate	47.8	10.8	22.6
Urethane	47.8	7.8	16.4
Arsenate	47.8	9.0	18.8
Azide	47.8	15.7	34.1
Fluoride	47.8	10.5	19.9

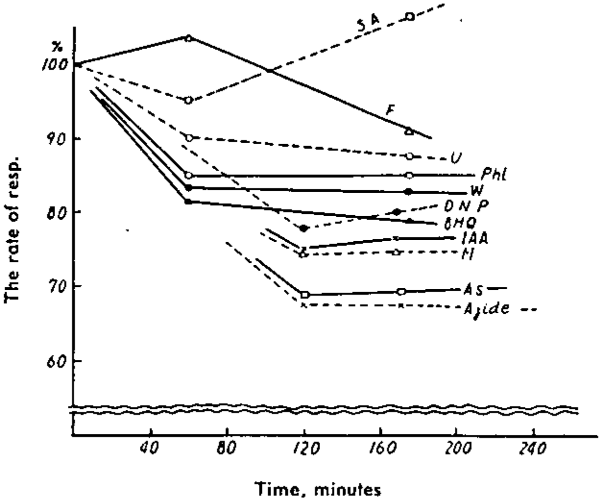

Figure 9
The respiratory inhibition of the barley leaves immersed in various inhibitory solutions

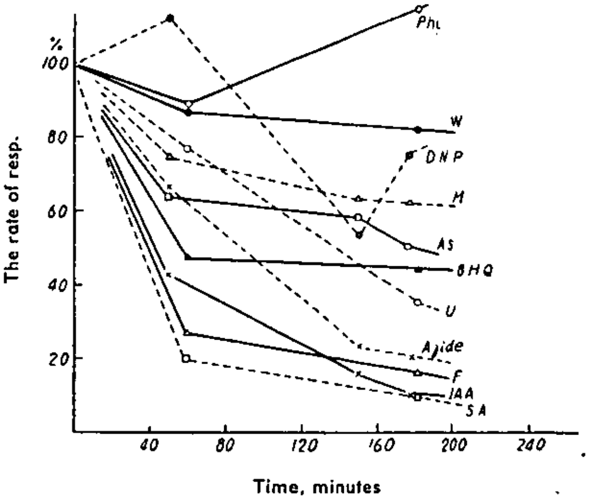

Figure 10
The respiratory inhibition of the barley roots immersed in various inhibitory solutions

Table 6 The rate of potassium leaching from the fresh barley leaves and roots immersed in various inhibitory solutions for three hours

Inhibitors	Leaves (f.w. 1.0 g)			Roots (f.w. 0.5 g)		
	Content of K_2O (mg)	Leached K_2O (mg)	Rate of leaching per cent	Content of K_2O (mg)	Leached K_2O (mg)	Rate of leaching per cent
Water	2.8	0.08	2.6	0.70	0.08	10.7
Phloridzin	2.8	0.08	2.6	0.70	0.08	10.7
Salicylaldoxime	2.8	0.13	4.5	0.70	0.30	42.0
Urethane	2.8	0.13	4.5	0.70	0.08	10.7
8-Hydroxyquinoline	2.8	0.15	5.5	0.70	0.24	34.0
Fluoride	2.8	0.15	5.5	0.70	0.34	45.6
Dinitrophenol	2.8	0.05	1.7	0.70	0.22	30.7
Azide	2.8	0.15	5.5	0.70	0.25	35.7
Malonate	2.8	0.19	6.8	0.70	0.17	23.6
Arsenate	2.8	0.24	8.6	0.70	0.35	50.0
Iodoacetate	2.8	0.23	8.0	0.70	0.42	59.3

There was some difficulty in making these experiments with the leaves as the cuticular membrane was scarcely wetted with the leaching solution. At any rate, the results obtained showed the same tendencies in both leaves and roots, resembling the movement of potassium in the rice plant leaves and roots.

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

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

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

Potassium leaching from the leaves (table 7) was highly sensitive to the inhibition by 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 only in the case of the wheat, but not in that of either the rice plant or barley, where potassium was depleted from the roots more sensitively and strongly by the salicylaldoxime, dinitrophenol, 8-hydroxyquinoline treatments than by others.

The wheat material applied in this case was 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 was due to the difference in the original nature of the plant or to that of the

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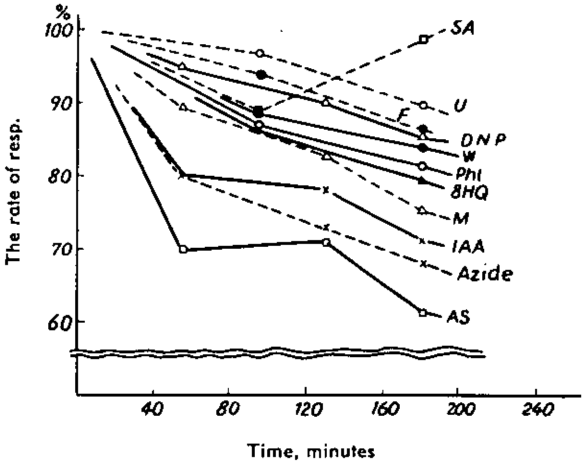

Figure 11
The respiratory inhibition of the fresh wheat leaves immersed in various inhibitory solutions

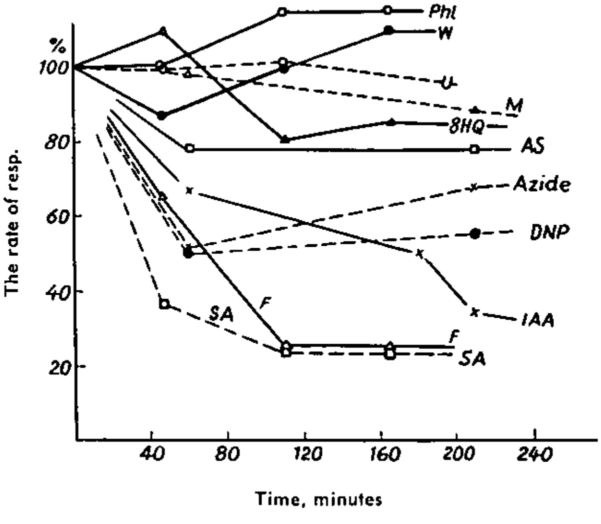

Figure 12
The respiratory inhibition of the fresh wheat roots immersed in various inhibitory solutions

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culture condition and the stage of growth 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 further investigated.

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

In recent years, a great deal has been learnt about the exchange absorption and the replacement of potassium in the root depending upon the environmental cation levels. In this experiment, using the barley leaves, it was sought to ascertain how the potassium retention or the rate of leaching would vary according to the difference of the nutrient levels such as phosphorus or nitrogen.

This experiment was made in order to obtain indications of the existence of organic compounds consisting of nitrogen or phosphorus, whereby potassium might be retained as an organic complex in the plant tissue (table 8).

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

Inhibitors	Leaves (f.w. 1.0 g)			Roots (f.w. 0.5 g)		
	Content of K_2O (mg)	Leached K_2O (mg)	Rate of leaching per cent	Content of K_2O (mg)	Leached K_2O (mg)	Rate of leaching per cent
Water	6.60	0.13	1.9	2.08	0.25	12.0
Phloridzin	6.60	0.10	1.5	2.08	0.45	24.0
Salicylaldoxime	6.60	0.70	10.6	2.08	1.34	65.7
Urethane	6.60	0.23	3.4	2.08	0.55	26.5
8-Hydroxyquinoline	6.60	0.18	2.7	2.08	0.85	43.3
Fluoride	6.60	0.25	3.7	2.08	0.75	36.1
Dinitrophenol	6.60	0.15	2.3	2.08	0.90	43.5
Azide	6.60	0.30	4.5	2.08	0.75	36.1
Malonate	6.60	0.33	4.9	2.08	0.65	31.3
Arsenate	6.60	0.48	7.1	2.08	0.45	21.6
Iodoacetate	6.60	0.43	6.4	2.08	0.60	28.9

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 was 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 potassium supply. In $N_{100}K_5$ series of normal nitrogen and low potassium supply, similar tendencies were observed with less magnitude. The same result was arrived at in the case of the low nitrogen plant $N_5P_{100}K_{100}$ as compared with the normal plant $N_{100}P_{100}K_{100}$.

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Table 8 The potassium content and amount of leaching of the fresh barley leaves cultured with different levels of nitrogen, phosphorus and potassium. The samples were immersed in various inhibitory solutions for three hours. 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	P_{100}	P_5	P_0	$N_5P_{100}K_{100}$	$N_{100}P_{100}K_0$
Potassium content		144.0	96.5	95.0	112.0	78.0	79.0	80.0	37.0
Potassium leaching	Water	1.6	3.5	8.0	1.5	3.0	5.5	2.4	0.4
	Phloridzin	2.0	3.5	3.3	1.5	2.5	2.0	2.9	0.3
	Salicylaldoxime.	3.0	5.8	9.5	2.5	3.0	2.5	5.3	0.8
	Urethane	2.8	4.0	5.0	2.5	2.0	3.5	3.2	2.5
	8-Hydroxyquinoline	2.6	3.5	7.0	3.0	2.0	3.5	2.8	0.5
	Fluoride	2.6	4.5	5.3	3.0	3.0	7.8	3.2	1.0
	Dinitrophenol . .	2.4	3.0	4.3	1.0	3.0	3.3	2.4	0.6
	Azide	5.0	6.5	8.0	3.0	4.5	4.0	8.5	3.5
	Malonate	6.0	7.5	8.0	3.8	6.0	6.3	8.3	3.5
	Arsenate	6.0	8.5	17.0	4.8	5.0	8.0	7.2	3.5
	Iodoacetate	6.4	8.0	12.5	4.5	5.0	8.0	6.4	3.5

Discussion

In connection with the leaching of potassium from the fresh and alterated 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 *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 regarded as the so-called selective absorption.

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

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

As regards 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 established whether certain amount of potassium in fresh higher plants can be leached out easily into distilled water.

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This opinion concerning the leaching property of potassium is confirmed by our results. The leaching of potassium, depending on the lowering of nitrogen and phosphorus nutrient level in the culture solution as stated in experiment 6, amounted to 2 to 10 per cent in the leaves and more in the roots. While the rate of potassium leaching was far greater with the alteration of cells due to the drying of the materials, we could not state definitely whether this 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 substances.

Except the case of the deformation of cell membrane, the force which could retain potassium inside, may relate to the respiratory process of the cells. This suggestion was considered in experiments 2, 3, 4 and 5, using the various respiratory inhibitors such as glycolysis, *Krebs* cycle, terminal oxidase system, and taking samples of four plant species being comparable with respect to their biochemical behaviour.

These fresh materials were immersed in the inhibitory solutions, then the rate of potassium leaching, and the inhibited rate of respiration were simultaneously measured. As to the results mentioned above, the rate of potassium leaching was not always parallel to the inhibited rate of respiration; the rate of potassium leaching varied greatly depending upon the difference in the inhibitor's specific action.

An interesting fact was also observed with respect to the biochemical behaviour of the different plant species; in the rice plant leaves and roots and 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 oxydase system or *Krebs* cycle such as azide and malonate *(17, 18)*.

As the inhibitory mechanism or the specificity of the inhibitors have been studied and applied in the course of much biological and biochemical research work (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 cannot determine the places or the stages with which potassium can be connected through the respiratory process, the following suggestion may be allowed.

There may be places where potassium relates to the respiratory process, and if the places are inhibited or weakened, potassium may be leached out easily. The places may differ according to the plant species, 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 mainly oxygen or the marsh plants requiring less oxygen.

Secondly, experiment 6 was carried out to strengthen the assumption that the organic substance—the metabolic intermediate, some kind of protein relating to 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, in the light of the experimental results.

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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 show why it could retain potassium.

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