THE POSSIBLE ROLE OF ADENOSINE TRIPHOSPHATE IN RUBIDIUM ABSORPTION AS REVEALED BY THE INFLUENCE OF EXTERNAL PHOSPHATE, DINITROPHENOL AND ARSENATE[1]

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Ion accumulation in plant cells requires aerobic respiration. According to Lundegardh's theory of anion absorption, the driving mechanism for the process is the cytochrome system which transports anions inward as electrons move outward in the steps of terminal oxidation (9). DNP (2,4-dinitrophenol)—which in animal mitochondria is known to interfere with metabolic steps involving adenosine triphosphate (ATP) (7, 8)—inhibits salt accumulation by plant tissue while maintaining or increasing the rate of respiratory oxygen consumption (11). This suggests that ATP, an energy source for many reactions, may be rather directly linked with the ion accumulation process. The present study deals with some experiments designed to obtain further evidence on the hypothesis that active salt uptake may be closely related to ATP.

If ATP is closely linked with ion accumulation, then several possible consequences may be useful in testing this hypothesis. One is that inorganic phosphate deficiency may limit the amount of ATP and thus lower ion uptake. Another possibility is that DNP depression of ion uptake is due to interference with phosphate transfers to or from ATP; the rate at which useful bond energy from ATP is made available should be related to amounts of ion accumulation. Finally the presence of ATP should be demonstrable.

Previous work has shown that Rb^+ is absorbed by excised tissues (3, 4, and 13) much as in the case of potassium and that after a more rapid initial uptake the amount of cation uptake parallels that of anion absorption (13). Also Rb^{86} appears to be a suitable tracer for K^+ in short term experiments (6). Auxin may enhance Rb^+ uptake in amounts related to the auxin induced increase in respiration (3); and, in turn, auxin activity has been reported to be related to oxidative phosphorylation (2) and, in fact, to raise the level of ATP (10).

MATERIALS AND METHODS

The tissues used for most tests were 5 mm segments of etiolated pea epicotyls, 3rd internode (Alaska var.); in addition, for some experiments, discs (approximately 1.2 mm thick × 7 mm diameter) of potato tubers (Katahdin var.), or of Jerusalem artichoke tubers were used. The pea seedlings were grown in complete darkness for 7 days. The upper 2 cm below the bud were cut into segments and used immediately. Potato or artichoke discs were aerated overnight in distilled water prior to tests.

Rubidium-86 was used as a tracer for Rb^+ and P^{32} for $H_2PO_4^-$. Specific activities were in the range of 0.01 to 0.05 μc/ml of experimental solution. Following the experimental treatment, the tissues were removed from the solution with a small sieve, rinsed briefly (about 30 sec) with distilled water, then blotted and weighed. In these experiments no attempt was made to distinguish the amount of the ion of "apparent free space" from that actively accumulated. All counting was done on dry samples which had been wet ashed with concentrated nitric acid as described previously (4). Assays of radioactive samples were made with a scaler and thin end window Geiger tube. The solutions were adjusted to pH 4.8 to 5.0 unless specified otherwise. Respiration measurements were made with a Warburg apparatus at 25° C in the salt solutions indicated, i.e., without added buffer.

The Rb H_2PO_4 was made by titrating H_3PO_4 with Rb_2CO_3 solution. In experiments in which KH_2PO_4 concentration was varied, the K^+ level was kept constant by adding the required amounts of KCl. RbCl and Rb^{86} levels were constant in all tests.

Phosphate assays were made with a colorimetric molybdate method described by Umbreit et al (14) and using a Cenco Photelometer.

RESULTS

EFFECT OF PHOSPHATE CONCENTRATION ON RUBIDIUM UPTAKE: Some experiments were conducted to see whether internal phosphate concentrations limit ion uptake and whether externally added phosphate might evoke greater Rb^+ absorption. In these experiments seedlings were grown in phosphate-deficient nutrient solutions and the 3rd internode segments excised for tests of the effect of external phosphate concentration on Rb^+ accumulation. The pea seeds used in these experiments came from plants grown in phosphate-deficient soils.

In general, the results of these experiments indicate that phosphate did not affect Rb^+ uptake ap-

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preciably. Results of one of the tests are shown in figure 1. In this test indole acetic acid (IAA) and sucrose were added to all solutions in an attempt to increase demand for phosphate. There is a suggestion in the data that at the lowest level of $H_2PO_4^-$ in the tissue (14.5 μM per g initial fresh weight) slightly less Rb^+ absorption occurs. Several unsuccessful attempts were made to obtain pea seedling tissue with less $H_2PO_4^-$ content, e.g., by excising cotyledons and by growing excised epicotyls in sucrose solution. These trials gave appreciably less stem growth and led to the surmise that the minimum amount of phosphate required for growth of tissue is also quite adequate for Rb^+ uptake.

It is significant that Rb^+ uptake was essentially the same in these experiments whether $H_2PO_4^-$ or Cl^- ion predominated. One might have expected that Rb^+ uptake would proceed at a faster rate with Cl^- than with $H_2PO_4^-$, but any difference was small or insignificant under the conditions of these experiments.

PHOSPHATE ABSORPTION AND EXCHANGE: The time courses of absorption and exchange (approximate values) of P^{32} labeled $H_2PO_4^-$ are shown in figure 2. In 2 such time course experiments a lag was noted during the first 8 hours in net accumulation of $H_2PO_4^-$, i.e., no net increase in tissue phosphate occurred; however labeled phosphate was taken up. Thus exchange of internal and external phosphate was taking place prior to increased net accumulation which followed. Approximations of this exchange in percentage were made as:

$$\frac{\text{Labeled } H_2PO_4^- \text{ uptake}}{\text{Total } H_2PO_4^- \text{ in tissue}} \times 100$$

In time course experiments with Rb^+ there was no lag in accumulation of this ion (in single salt solution); however, it is presumed to exchange with internal K^+ and thus the values reported here primarily reflect influx amounts not corrected for any efflux. It is of interest to note that, aside from exchange, $H_2P^{32}O_4^-$ uptake is essentially linear for 48 hours and internal labeling is essentially complete at the end of this period.

EFFECT OF DNP ON ABSORPTION AND RETENTION OF Rb^+ AND $H_2PO_4^-$: DNP has been shown to profoundly affect phosphate metabolism and to result in disruption of various energy consuming activities of living cells such as growth and ion accumulation. The action of DNP appears to be complex since some results suggest that it prevents ATP formation by uncoupling phosphorylation from aerobic respiration (8) possibly because it induces dephosphorylation (7). The latter data further indicates a possible degeneration of protoplasmic structure.

An interesting effect of DNP in this connection is that it may increase O_2 consumption while depressing growth and ion uptake (11). Thus it would appear that the cytochrome system proceeds at a faster pace while working energy of the cell is blocked. Under these conditions, if the Lundegardh theory of anion respiration and salt absorption holds, then salt

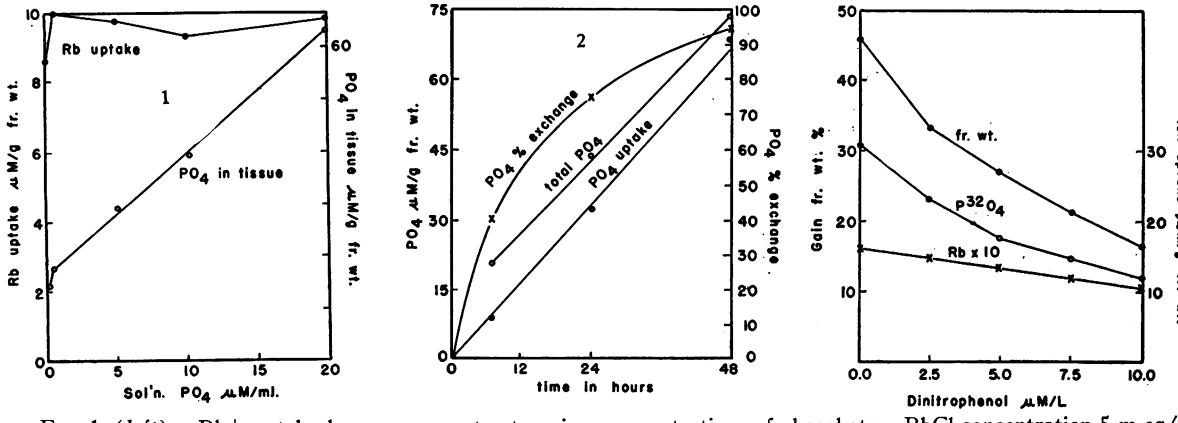


FIG. 1 (*left*). Rb^+ uptake by pea segments at various concentrations of phosphate. RbCl concentration 5 m eq/l. Duration of expt. 24 hr. All soln. contained 1 ppm indoleacetic acid and 1 % sucrose.

FIG. 2 (*center*). Time course of P^{32} labeled phosphate absorption showing total tissue phosphate content and percentage of exchange (of labeled phosphate with tissue phosphate). External solution 10 mM KH_2PO_4 per liter.

FIG. 3 (*right*). Inhibition of absorption of PO_4 and of Rb^+ by varying concentrations of DNP (2,4-dinitrophenol) and the effect of DNP on total phosphate retained by the tissue. External solution 10 μM KH_2PO_4 plus 0.5 μM RbCl per ml. The PO_4 curve represents one series, P^{32} labeled, the Rb^+ curve a parallel series, Rb^{86} labeled. Duration of experiment 24 hr.

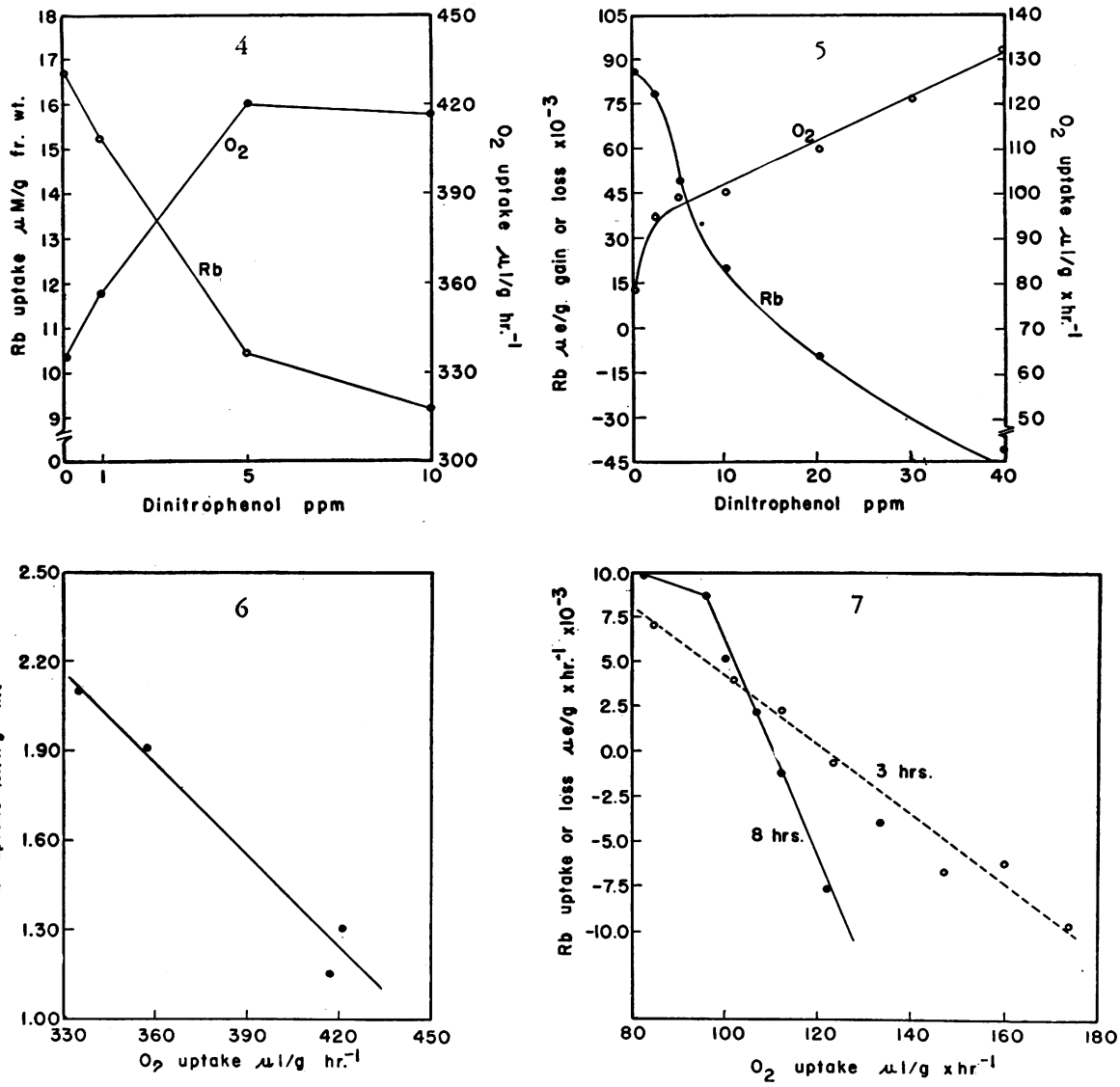
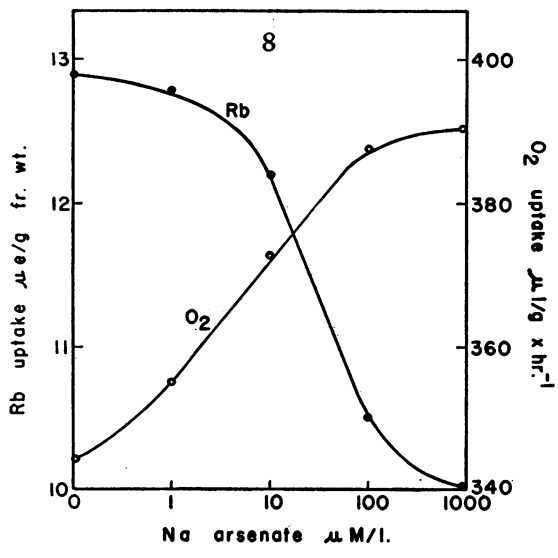


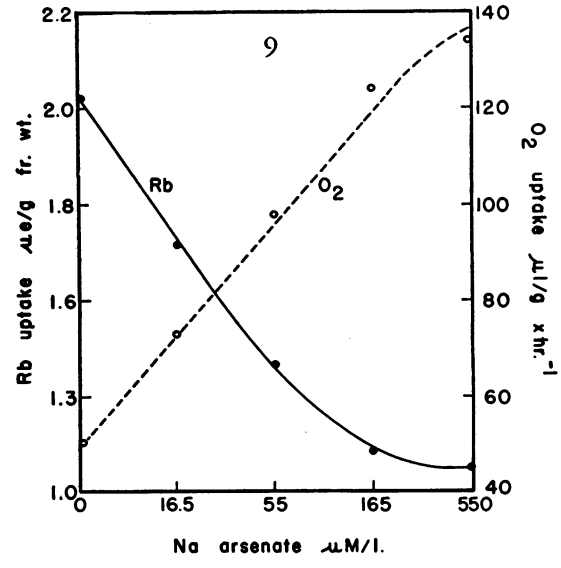
FIG. 4 (*upper left*). Dinitrophenol stimulation of respiration and depression of Rb^+ uptake in pea segments. Time of treatment 8 hrs. RbH_2PO_4 conc. 10 mM/l.

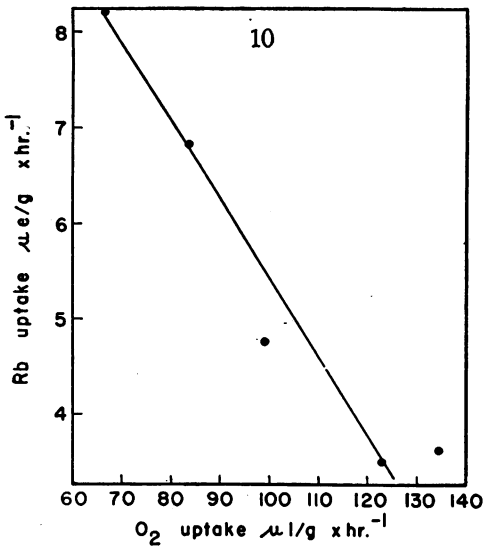
FIG. 5 (*upper right*). Dinitrophenol stimulation of respiration and inhibitions of Rb^+ uptake or induction of Rb^+ loss from tissue in potato discs. Discs were allowed to absorb Rb^+ 3 hrs prior to DNP addition. Time of DNP treatment 8 hrs. RbCl conc. 140 μM/l.

FIG. 6 (*lower left*). Data of experiment on pea stem (fig 4) showing the inverse linear relation of Rb^+ uptake to O_2 consumption when the latter is influenced by a range of DNP concentration.

FIG. 7 (*lower right*). Data on experiment with potato discs showing that Rb^+ uptake or loss is inversely related to O_2 consumption when the latter is influenced by DNP over a small concentration range.







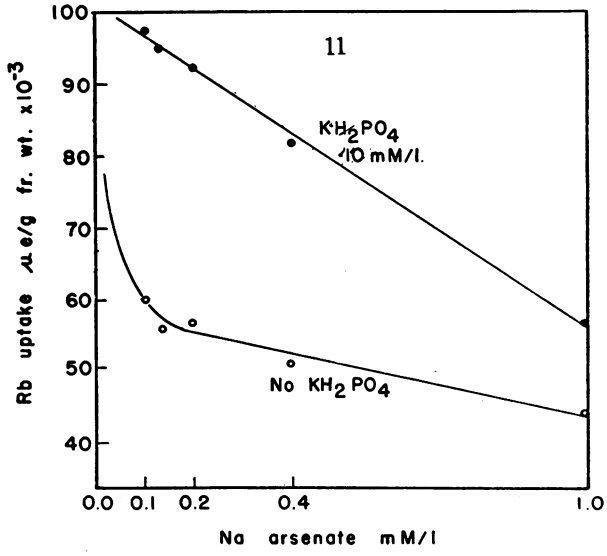


FIG. 8 (*upper left*). Arsenate stimulation of respiration and inhibition of Rb^+ uptake in pea stem tissue. Time of treatment 5 hr. RbCl conc. 10 mM/l.

FIG. 9 (*upper right*). Arsenate stimulation of respiration and inhibition of Rb^+ uptake in Jerusalem artichoke tissue discs. Time of treatment 22 hr. RbCl conc. 1 mM/l.

FIG. 10 (*lower left*). Data on experiment (fig 10) with Jerusalem artichoke tissue showing that Rb^+ uptake may be inversely related to O_2 consumption when the latter is increased by Na arsenate over a certain concentration range.

FIG. 11 (*lower right*). The effect of phosphate on arsenate inhibition of Rb^+ uptake in artichoke tissue. Time 3.5 hr. Rb^+ conc. 56.4 μ eq/l.

uptake might be expected to proceed at a faster rate, since, according to Lundegardh's theory, anions pass in via the cytochrome system as electrons move outward in the terminal oxidation system. Lundegardh's explanation of this apparent contradiction is that oxidation of cytochrome b is reversibly coupled to formation (or decomposition) of ATP; that DNP inactivates cytochrome b; that cytochrome b is involved with ATP in maintaining normal protoplasmic structure; and that high energy phosphate may be required in the formation of special carriers between b and the final site of accumulation (9). An alternative hypothesis is that ATP is required in salt absorption but that the cytochromes are not directly involved.

In a number of experiments testing the effect of DNP on Rb^+ uptake, it has been found that absorption is depressed. In general, there appears to be an inverse linear relation between the amount of Rb^+ uptake and the concentration of DNP under conditions in which the tissue is not obviously damaged by DNP. This effect is illustrated by figure 3 which shows the influence of DNP concentration on Rb^+ absorption by pea stem tissue and, in a parallel series of flasks, the influence on phosphate uptake and tissue content. Here the Rb^+ absorption was depressed in a linear fashion, whereas the response of phosphate absorption was a more complex function of DNP inhibition. However, there was a qualitative correlation of phosphate and Rb^+ uptake since absorption of each was depressed over the same concentration range of DNP.

Subsequent experiments have been made on absorption of Rb^+ and K^+ from solutions of RbCl, KCl, and mixtures of these using both Rb^{86} and K^{42} tracers. These indicate that Rb^{86}, within reasonable limits acts as a tracer for K^+ which is in accord with a previous report (6). Thus the Rb^+ absorption curve in figure 3 essentially reflects the K^+ absorption by the tissue.

The percentage gain in fresh weight in this experiment (fig 3) was closely parallel to the phosphate curves. At zero DNP the tissue gained 45 % in fresh weight and at 10 μM DNP, 17 %.

RELATION OF Rb^+ ABSORPTION TO INCREASED O_2 CONSUMPTION INDUCED BY DNP: In the effective concentration range of DNP, lower levels, about 10^{-5} to 10^{-6} molar, induce increased O_2 consumption in many plant and animal tissues; this effect reaches a maximum and at higher concentrations respiration is depressed (12).

The writer has investigated the effect of DNP on salt uptake and respiration of pea stem, potato and Jerusalem artichoke tissues. In each of these under appropriate conditions DNP induces an enhancement of respiration which is accompanied by a reduction of Rb^+ absorption. This is well illustrated by one of several tests with pea stem tissue, (fig 4), and by one of the experiments with potato discs, (fig 5); in each of these figures both oxygen consumption and Rb^+ uptake are shown and may be noted to be inverse to one another. This is in accord with the data of Robertson, et al (11) for carrot tissue.

If it is assumed that DNP interferes with the amount of high energy phosphate available for useful work, then the DNP effect at low concentration range in tissue may be proportional to the amount of increase in O_2 consumption induced by DNP. This appears to be the case for Rb^+ uptake and the DNP induced O_2 consumption by pea stem and potato tissue as shown in figures 6 and 7. Similar data were obtained with Jerusalem artichoke and carrot tissue.

EFFECT OF ARSENATE ON RESPIRATION AND UPTAKE OF Rb^+: Like DNP, arsenate interferes with phosphate metabolism and also may induce increased O_2 consumption in respiration. Arsenate is an effective inhibitor of growth (1) and ion uptake (5).

The action of arsenate on Rb^+ uptake and respiration is remarkably similar to that of DNP. The effect of a rather wide range of arsenate concentration on Rb^+ absorption and respiration by pea stem tissue and Jerusalem artichoke tissue is shown in figures 8 and 9 respectively. As with DNP treatment, terminal oxidation appears to be uncoupled from oxidative phosphate transfers. Under certain conditions, at least, Rb^+ uptake has an inverse linear relation to the increased O_2 consumption induced by arsenate as shown in an experiment with artichoke tissue (fig 10).

Addition of external phosphate greatly reduces or reverses arsenate inhibition of Rb^+ uptake (fig 11).

DISCUSSION

The data indicate that Rb^+ absorption is inversely related (probably proportionally) to the enhancement of O_2 consumption induced by DNP or arsenate. This may be construed as indicating a possible relation of ion uptake to oxidative phosphorylation or dephosphorylation. The lowest levels of DNP or arsenate which enhanced respiration also depressed Rb^+ uptake. This evidence allows the interpretation that the action of DNP may be to induce hydrolysis of ATP in a non-useful reaction rather than to block ATP formation. This is consistent with mitochondrial studies (7). This interpretation is contingent upon the ideas that the availability of phosphate acceptors, e.g., ADP, regulate aerobic respiration and that the induced increase in O_2 consumption with DNP is that amount tending to maintain the normal supply of high energy phosphates, e.g., ATP. Alternatively if ATP remained constant, and its energy expenditure were not impeded, ion uptake should not be depressed by threshold concentration levels of DNP; this was not the case. Arsenate appears to act in a manner similar to that of DNP.

These results appear not to conflict with Lundegardh's present concept of ion uptake since the cytochrome system is apparently coupled with oxidative phosphorylation in a manner which may require ATP energy in the sequence of steps leading to accumulation. It is interesting to note, however, that a theory

of ion absorption (not involving the cytochrome system) based on ATP utilization may equally well explain the data interpreted as indicating absorption via the cytochrome system.

Summary

Rubidium absorption and respiration of excised etiolated pea stem, potato and Jerusalem artichoke tuber were studied as influenced by inorganic phosphate, dinitrophenol (DNP) and arsenate. Within the limits of the present study the effect of added phosphate on Rb^+ uptake was negligible. DNP and arsenate, within the concentration range used, gave enhanced aerobic respiration, but depressed Rb^+ uptake. Under these conditions Rb^+ uptake was inversely related to O_2 consumption. The same concentration range of DNP enhancing respiration also depressed Rb^+ (and $H_2PO_4^-$) uptake. This is consistent with the hypothesis that DNP and arsenate block ion uptake by interfering with a necessary high energy phosphate hydrolysis.

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