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SIGNIFICANCE AND TECHNIQUE OF SHORT-TERM EXPERIMENTS ON SOLUTE ABSORPTION BY PLANT TISSUE

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The significance of short-term absorption periods in experiments on solute absorption by plant tissues is discussed. A technique is described for such short-term experiments. As applied in experiments on ion absorption by barley roots, the technique permits accurate absorption rate determinations to be made in absorption periods of ten minutes or even less. The heterogeneity of cation uptake by this tissue is demonstrated. A readily exchangeable fraction is often present which must be accounted for if rates of metabolically active transport are to be determined.

If the results of experiments on the absorption of ions and other solutes by plant tissues are to be amenable to quantitative kinetic interpretation, it is preferable, as a rule, to conduct them in such a way as to yield rates or velocities of absorption. The observed rates can then be inserted into kinetic equations or graphs to provide tests of the goodness of fit of the data with theoretical models of solute transport, and to yield values for theoretical maximal velocities at "infinite" substrate concentration, MICHAELIS constants, and others (EPSTEIN and HAGEN, *1*).

For a number of reasons it is desirable that the actual absorption periods be as brief as possible. These reasons are as follows.

1) During prolonged periods of absorption, rates of absorption will often not remain constant.
2) Respiratory and other metabolic patterns may change as a result of exposure of the tissue to the experimental solutions for long periods. This may make for quantitative and qualitative differences in its absorption behavior.
3) Inclusion of buffers in the experimental solutions is often inadvisable since the solutes used as buffers may have independent

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effects on the absorption of the solute under investigation, in addition to their buffering effect. Without buffer, however, the pH of the experimental solution is apt to change with time as a result of the metabolic activities of the tissue. The shorter the experimental period, the less pronounced will be the drift in pH of the experimental solutions.

4) At low concentrations of substrate solute, amounts absorbed by the tissue may be significant fractions of the total solute initially present in the experimental vessel. With the passage of time the experimental solution may become progressively depleted. In that case, absorption rates do not remain constant but change with time as a function of the declining concentration of substrate solute. Since other solutes present may be absorbed at different rates, the ratios of various solutes to each other may also change, further complicating the picture. Short absorption periods, combined with use of large volumes, minimize depletion of the experimental solutions through absorption by the tissue.

5) Short experiments are more economical of the investigator's time, and of materials, than are long ones.

Once evidence has been obtained that rates of absorption can be determined in short periods, there is no reason for actually using periods many times as long. On the contrary, the above considerations favor the shorter times. This paper reports on and discusses a technique for short-term experiments on the absorption of ions by excised barley roots. The absorption period routinely is ten minutes, but longer or shorter periods may be used. With appropriate modifications, the technique should lend itself to experiments with other plant tissues, and with solutes other than inorganic ions.

TECHNIQUE

Water, glassware, and chemicals

Laboratory procedure has been described earlier (*2*). Throughout, cleanliness is maintained to a degree comparable to that customary in micronutrient work. The ion whose rate of absorption is to be measured is radioactively labeled to give final counting rates of not less than several hundred counts per minute per sample.

Plant material

In our experiments, excised barley roots have been used most extensively. The technique of raising seedlings for these experiments is as described earlier (*2*).

Experimental solutions

When the experiment involves concentration ranges, the solutions are

not all of the same volume. Rather, for the lowest concentration customary in our experiments (0.002 *mM*), the volume is 2 *l*, and 2-*l* ERLENMEYER flasks are used. For progressively higher concentrations, smaller volumes are used, down to 250 *ml* in 500 *ml* wide-mouth ERLENMEYER flasks. Volumes are chosen in such a manner that depletion of the solutions during the absorption period does not cause the concentration of the substrate ion to drop by more than 5 per cent. In addition to the salt of the radioactively labeled ion whose absorption is to be studied (the substrate ion), the solution contains calcium, usually at a concentration of 0.5 *mM* (*3*). When necessary, the pH is adjusted by means of strong acid or base.

Experimental procedure: the "teabag" technique

The excised roots are gently but thoroughly mixed. An amount estimated as being a little over one gram is taken out, gently blotted on cheesecloth, and a 1.00 *g* sample is quickly weighed out on a torsion balance. The sample is transferred to a single-layer square of cheesecloth *20 cm* on the side. This cheesecloth is of a coarse weave, with openings roughly 1×2 *mm*. The edges of the cheesecloth are quickly gathered together, making a "teabag". The "teabag" is closed by means of a plastic bag closure (Kwik Lok Corporation, Yakima, Washington; Series A, No. 1 closures, white) to which is tied a length of white Nylon or cotton thread, and the "teabag" is transferred to an aerated "holding solution" (4 *l* of 0.5 *mM* $CaCl_2$ at the same temperature as the experimental solutions). This operation is repeated till all tissue samples for the experiment are suspended in "teabags" in the "holding solution".

The absorption period

The first sample is lifted by its thread from the "holding solution" and rinsed in two successive 150-*ml* aliquots of 0.5 *mM* $CaCl_2$ at the same temperature as the experimental solutions proper. Total rinsing time is one minute. The "teabag" is twirled rapidly in the air to spin out excess solution, and at time zero minutes is immersed in its vigorously aerated experimental solution. This procedure is repeated at 2-minute intervals with all the other samples.

The absorption period is discontinued by one of two methods, depending on whether or not it is necessary to desorb readily exchangeable ions which are associated with the tissue without having been actively accumulated.

Procedure A (without desorption exchange of labile, exchangeably bound ions)—When conditions are such that no appreciable labile fraction of the substrate ions is retained by the tissue after thorough rinsing in water, the absorption period is discontinued as follows. A few seconds before the end of the absorption period, the sample is drawn into the neck of the flask, and at the exact time, dropped into 200 *ml* water in a 250-*ml* beaker,

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whereupon it is quickly rinsed with several changes of water, the beaker being filled and the water decanted several times in rapid succession, for a total rinsing time of one minute.

Procedure B (with desorption of labile, exchangeably bound ions)—If conditions during the absorption period are such that at the end of the period the tissue contains in addition to the actively accumulated (and very sluggishly exchanged) fraction of ions a fraction that is held in readily exchangeable form, the absorption period is followed by a desorption period during which the readily exchangeable fraction of ions is desorbed. For example, in experiments with ^{86}Rb at fairly high concentrations (5 or 10 *mM* or higher), the absorption period is discontinued as follows. The "teabag" containing the tissue is drawn into the neck of the flask a few seconds before the end of the absorption period, and at the exact time, dropped into a volume of about 200 *ml* of cold (5°) solution 5 *mM* with respect to RbCl (non-labeled) and 0.5 *mM* $CaCl_2$. The tissue is rinsed in four successive fresh aliquots of identical solution, for a total rinsing time of one minute. It is next suspended in 4 liters of aerated identical solution, at 5°, for 30 minutes, to effect complete removal of readily exchangeable labeled rubidium from the tissue. Several samples can be suspended in the same desorbing solution. Finally the tissue is rinsed several times with water, for one minute.

Radioactive assay

The samples are ashed and assayed as described earlier (*2*).

COMMENT AND DOCUMENTATION

Procedure A (no desorption)—This procedure is valid under conditions where simple rinsing with water will remove all labeled ions on the surface of the tissue and in the "outer space" (*4*) where they are present in readily diffusible and elutable form. With barley roots, this applies when the substrate solute is a halide, or a monovalent cation at relatively low concentration, up to about 0.3 *mM*, in the presence of excess calcium at, say 0.5 *mM*. Under these conditions, calcium ions occupy the general, non-selective exchange capacity of the tissue, and any labile (non-accumulated) monovalent cations are present only on the surface of the tissue and in the "outer space", and are readily eluted by water rinses. After rinsing, only those ions actively accumulated into "inner" spaces and thereby rendered very slowly exchangeable remain in the tissue. Amounts of rubidium absorbed under these conditions are a strictly linear function of time (*3*, Fig. 1).

Procedure B (with desorption)—Labile (non-accumulated) ions may be present in the tissue which are not removed by simple rinsings with water. This may be the case if the substrate ion is a divalent cation (*5*) or a monovalent cation present at a concentration so high that measurable ex-

change adsorption takes place. Figure 1 shows the results of an experiment in which 1.00 *g* samples of barley roots absorbed labeled rubidium from 500 *ml* volumes of aerated solutions. The temperature was 30° and the pH 5.8. The samples used for following the time-course of the absorption were rinsed with four 250-*ml* aliquots of water, for a total rinsing time of one minute, *i.e.*, procedure A was followed. The black dots (Fig. 1) show that amounts of ^{86}Rb absorbed were not a linear function of time.

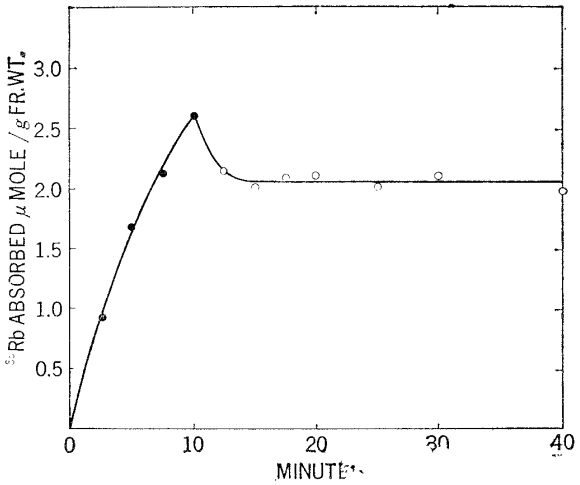

Fig. 1. Absorption and desorption of ^{86}Rb by excised barley roots as a function of time. Black dots: tissue in 5 *mM* $^{86}RbCl$, 0.5 *mM* $CaCl_2$, 30°, rinsed with water at the end of the absorption period. Open circles: tissue in 5 *mM* KCl, 0.5 *mM* $CaCl_2$ for periods indicated.

The reason for this is revealed by the results of the second period of the experiment. After the initial 10-minute absorption period roots were transferred for various periods to a cold (5°) aerated desorbing solution 0.5 *mM* with respect to $CaCl_2$ and 5 *mM* with respect of KCl. (For the sake of economy, KCl was used instead of RbCl during the desorption period. Results are identical whether RbCl or KCl is used.) In the desorbing solution, a fraction of the ^{86}Rb still associated with the roots after the water rinse was lost by exchange with K^+. Loss of the readily exchangeable fraction was complete in five minutes, and there was no further loss of ^{86}Rb. The ^{86}Rb retained by the tissue against the desorbing solution is the fraction that has been transported into "inner" spaces and rendered non-exchangeable or very slowly exchangeable. The non-linearity of the graph depicting uptake *vs.* time during the first ten minutes is due to the fact that the values for absorption obtained under these conditions represent summations of the rapidly equilibrating exchangeable fraction and the "inner" space fraction which is not subject to rapid exchange. Figure 2 shows that amounts transported into the "inner" spaces are a linear function of time. In this experiment, procedure B was used, *i.e.*, the absorption period was followed in each case by a 30-minute period of desorption in cold (5°) 5 *mM* KCl, 0.5 *mM* $CaCl_2$ solution. This procedure resulted in the removal of the readily exchangeable fraction of

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^{86}Rb, leaving for final assay the "inner" space fraction only.

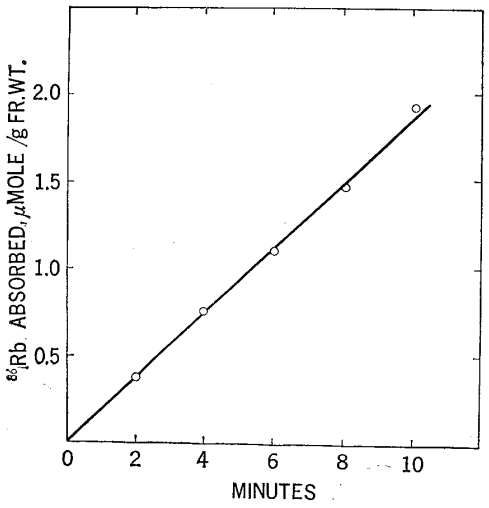

Fig. 2. Absorption of ^{86}Rb by excised barley roots as a function of time. Tissue in 5 *mM* ^{86}RbCl, 0.5 *mM* $CaCl_2$, 30°, for periods indicated, then rinsed with cold (5°) 5 *mM* KCl, 0.5 *mM* $CaCl_2$ desorbing solution for 1 minute, followed by transfer to identical desorbing solution for 30 minutes.

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