

METABOLIC AND NON-METABOLIC UPTAKE OF SODIUM IN ROOTS OF ZEA MAYS^{1,2}

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INTRODUCTION

It has been recognized for many years that part of the ions that enter plant tissue bathed in a salt solution are not irreversibly sequestered but enter and are free to leave again in accordance with Fick's law of diffusion and principles pertaining to Donnan or ionic exchange equilibria (8, 9, 13, 16). This phenomenon is generally ascribed to the non-metabolic diffusive movement of salt into a part of the tissue (the free space) which presents little if any resistance to the entry of dissolved materials. Some importance has been attached to the measurement of free space in terms of the relative volume of tissue involved. The difficulties inherent in such measurements have been discussed thoroughly by Briggs and Robertson (3) and need not be considered here. In this article the authors have followed Jacobson et al (13) and have sought to avoid questions of volume and space by using the term "non-metabolic uptake". Arbitrarily we have included under this heading ion uptake at low temperatures, in an atmosphere of N₂, or in the presence of metabolic inhibitors.

Studies of non-metabolic absorption have been carried out with tissue slices and whole roots (16). Most of the tissue comprising these materials consists of mature cells since even where whole roots are used the meristematic portion amounts to only a small fraction of the sample taken. Over and above their capacity for non-metabolic uptake of salt such tissues exhibit a pronounced ability to absorb ions metabolically by a process that is relatively irreversible. This phenomenon makes necessary the postulation of a barrier either within the cell or at its surface which is impermeable to ions in the medium. According to some evidence (1), the barrier is perhaps the tonoplast, the vacuoles representing a sink or dead end in which ions are trapped as they move through the symplast. The possibility is not ruled out that the plasmalemma may represent the barrier, but if this were the case, it would become necessary to limit non-metabolic uptake of ions to the cell walls and the intercellular spaces (16, 17). Yet a third conception is that the whole cytoplasm constitutes a barrier,

that is, there may be ionic exchange sites throughout the cytoplasm which are but slowly accessible to ions of the medium (19). In this view ultimate secretion of ions into the vacuole may result from a breakdown of protoplasmic structure.

Because of these general questions studies of ion absorption in immature cells are of interest, in particular cells in which the vacuole is either absent or at least minute and possibly not yet delimited by a tonoplast. For example, if the tonoplast constitutes the barrier, then such tissues should exhibit no ability for the metabolic accumulation of ions.

Root tips of maize proved to be excellent for this purpose. Microscopic examination indicates that the cells extending at least 1.8 mm from the tip are essentially non-vacuolated, although occasional cells are seen in which extremely small structures resembling vacuoles may be found. Most of the cells in this region display mitotic figures when suitably stained. The chief purpose of this work was to study the absorption characteristics of these undifferentiated cells and to compare them with those farther from the tip in which vacuolation is progressively more nearly complete.

EXPERIMENTS

Dark-grown (26° C) tips of maize roots (Peoria variety) were used in this investigation. The seed was soaked in aerated distilled water for 24 hours and then spread on cheese cloth supported by a well tinned screen. The screen was then supported in a pyrex baking dish filled with 0.25×10^{-3} M CaCl₂, the cheese cloth dipping into the solution. Another baking dish was inverted over the first to maintain a humid atmosphere. The roots were harvested on the 4th day.

After excision, the roots were blotted dry and the terminal portions were cut into segments, 0 to 1.8 mm, 1.8 to 3.8 mm, 3.8 to 7.8 mm, and 7.8 to 11.8 mm measured from the tip. A simple cutting device composed of four thin razor blades bolted to two machine screws and spaced with washers permitted sectioning of twenty roots simultaneously; a block of paraffine was used as a cutting board. Forty of each of the segments were used for each experiment after the fresh weights had been determined.

Microscopic examination of the segments indicated that physical cell damage occasioned by cutting was negligible. In a preliminary experiment absorption and retention of Na by sectioned and unsectioned root tips 11.8 mm long were studied using labeled NaCl.

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In 10 hours 40 root tips sectioned as described above absorbed 15.0 μe of Na whereas the value for unsectioned tissue was 14.0 μe . After 10 hours in non-labeled 0.005 M NaCl at 0° C, both sectioned and unsectioned root tips were found to have retained 73 % of the sodium previously taken up. Thus, sectioning had an appreciable effect neither upon the ability of this material to take up Na nor upon its ability to retain Na against isotopic exchange.

The initial phase of this research was devoted to studying Na uptake by the segments as influenced by the concentration of NaCl in the medium. For each uptake determination, a weighed batch of 40 segments was placed in 500 ml of the appropriate NaCl solution for 8 hours at 21° C. The initial pH of the culture solution was 6.0. At the end of the absorption period the segments were washed twice in 500 ml of distilled water for about five minutes and then filtered out each time on a nylon net. The washed segments were ashed and analyzed for Na by a Beckmann flame photometer with photomultiplier attachment. Each quantity plotted on the ordinate (fig 5) represents an average of the uptake values for three separate batches of 40 segments.

The second research phase dealt with metabolic and non-metabolic uptake of sodium by the root segments. The experiments were carried out with Na²² (half-life, 2.6 yr). Using NaCl solutions labeled with this isotope, it was possible to determine time curves on single batches of 40 segments. In these studies, however, the segments were not washed in distilled water after an absorption period. Instead, the segments were blotted free of adhering liquid and the sodium content of the fresh tissue was determined by counting the Na²² gamma emission with Berkeley Decimal Scaler (Model 2001) and a lead shielded scintillation detector. Since the blotting and counting of the segments were carried out in less than 10 minutes, the segments could be returned to the culture solution for another absorption period. While being counted the tissue was sealed in the planchet to avoid drying out.

This procedure was used to determine the sodium uptake versus time curves for the various sections of maize roots at 2° C and 26° C. For each curve 40 segments of tissue were placed in 500 ml of 0.005 N NaCl labeled with 0.02 μc of Na²² per ml. The

initial pH of the culture solution was 6.0; the pH did not vary appreciably throughout the experiment. The curves for 2° C and 26° C are presented in figure 1.

Experiments conducted in this way with different batches of roots were found to give consistent results as evidenced by the data of table I. Although only the 24 hour absorption data are presented, the data obtained for other absorption periods were similarly consistent.

The Na²² was also used in two isotopic exchange studies with the various root sections. These experiments were suggested by the general observation that isotopic exchange between ions of the culture medium and metabolically absorbed ions usually is slow whereas isotopic exchange between ions of the medium and non-metabolically absorbed ions often is rapid. In the first study 40 of each of the four segments were immersed for 11 hours at 26° C in 500 ml of a 0.005 N NaCl solution labeled with 0.02 μc of Na²² per ml. Then the sections were removed, blotted dry, and their radioactivity measured. Each batch of 40 segments was then placed in unlabeled 0.005 N NaCl at 2° C. The loss of Na²² by the segments with time was determined by removing the segments periodically and counting them as previously described (fig 3). In the second study, batches of the various segments each were first placed in 10 ml of 0.010 N NaCl labeled with 0.10 μc of Na²² per ml for 2 hours at 26° C. They were then removed, blotted dry, and their radioactivity was determined. Following this, one batch of each segment was placed directly in 10 ml of unlabeled 0.010 N NaCl at 0° C. The other batches were so treated after having been allowed to stand for periods up to 3 hours in a moist chamber at room temperature (~21° C). After 2 hours in cold NaCl solution the tissue was again blotted and counted, and the retained percentage of Na²² was calculated (fig 4).

Total nitrogen determinations were made on the root tissue by wet digestion with sulfuric acid followed by distillation of the ammonia using a micro-Kjeldahl still. The distilled ammonia was determined by titration with HCl. The total nitrogen content and the percentage of dry matter found for the various segments are shown in table II.

The water was laboratory distilled water further purified by passage over a Dowex-50 resin column.

TABLE I
24 HOUR UPTAKE OF SODIUM FROM 0.005 N NaCl

SEGMENT	NA UPTAKE, INDIVIDUAL VALUES, MEQ/KILO, WET WT			NA UPTAKE, MEAN, MEQ/KILO	STANDARD DEVIATION, σ^*	CONFIDENCE LIMITS, 95 %*
0-1.8 mm	61.0,	48.5,	50.1	53.2	6.80	36.3- 70.1
1.8-3.8 mm	122.6,	126.2,	119.3	122.7	3.45	114.1-131.3
3.8-7.8 mm	92.4,	96.5,	97.8	95.6	3.98	85.7-105.5
7.8-11.8 mm	70.5,	77.8,	72.8	73.7	3.72	64.4- 82.9

* Terminology of Brownlee (5).

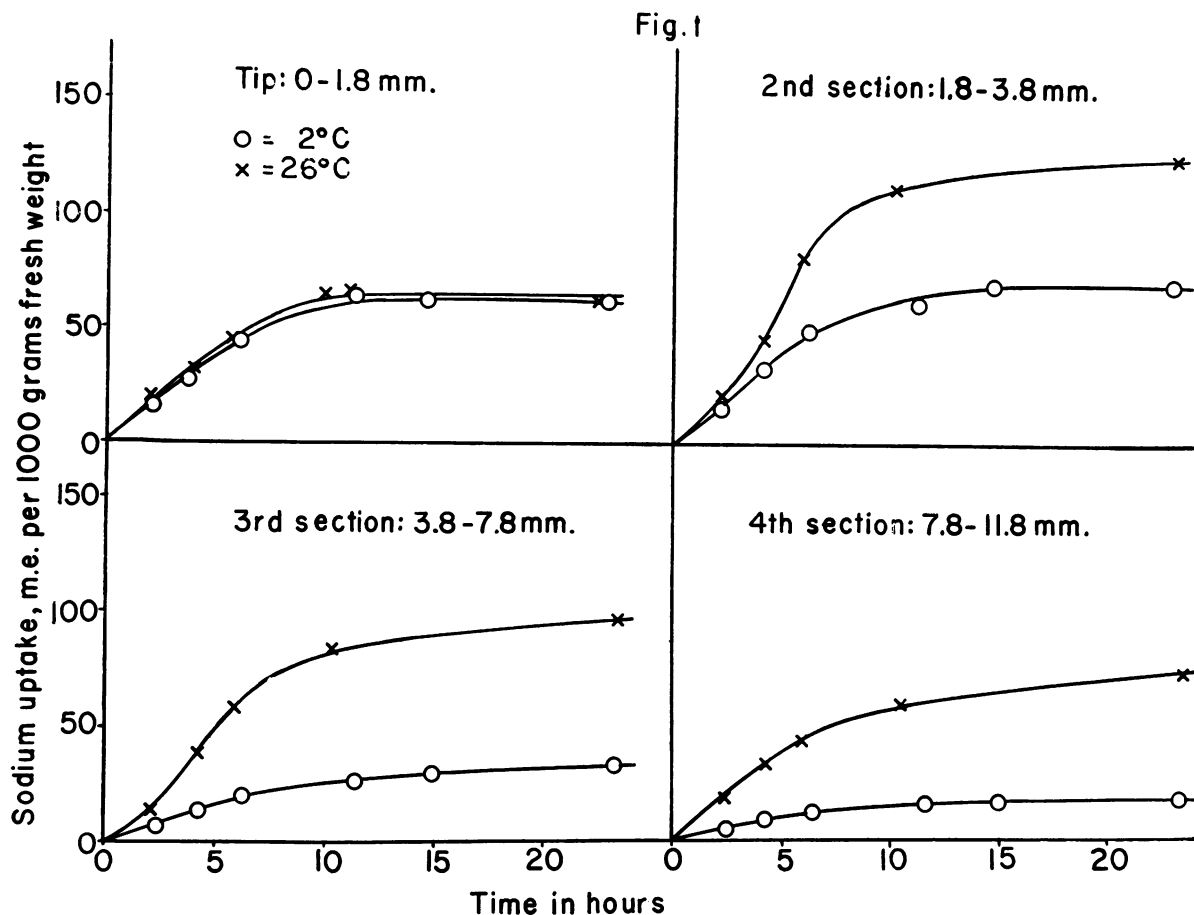


FIG. 1. Sodium uptake versus time curves for various sections of maize roots at 2° C and 26° C. For each curve, 40 segments of tissue were placed in 500 ml of 0.005 N NaCl labeled with 0.02 μ c of Na^{22} per ml. The pH of the culture solution was 6.0. At the intervals indicated in each curve, the segments were removed briefly from the medium, blotted dry, and counted. The uptake of sodium as meq/1,000 g fresh wt was then calculated using the known fresh weight of the segments.

DISCUSSION

The data of table II are in accord with those obtained by Brown and Cartwright (7) for root tips of maize. Although no respiratory measurements have yet been made here, it is reasonable to suppose on the basis of data obtained elsewhere that the first segment represents a region of relatively rapid me-

tabolism. Goddard and Meeuse (10) have found the most active respiration to occur in the apical millimeter of corn roots. The data of Brown and Broadbent (4) and of Lund, Vatter, and Hanson (18) indicate that regions of the root of pea and corn, respectively, which are highest in dry matter and protein nitrogen also respire most actively.

The presumed high metabolic activity of the first segment is, however, not associated with a parallel high rate of sodium uptake (fig 1). This apparent anomaly has been previously noted in studies dealing with the absorption of potassium by maize roots (5). As with K absorption, the first segment shows a relatively low rate of sodium uptake. Kramer and Wiebe (12) on the other hand report that phosphate is usually more actively absorbed by cells at the root tip than by those immediately behind it in the region of elongation. Of greater significance perhaps is the form of the time curve of absorption for the first segment (fig 1). Absorption by this segment has apparently

TABLE II
NITROGEN AND DRY MATTER CONTENTS OF VARIOUS SEGMENTS OF MAIZE ROOTS

SEGMENT	NITROGEN, MG/G, WET WT	DRY MATTER % WET WT	NITROGEN % DRY WT
0-1.8 mm	14.2	15.2	9.3
1.8-3.8 mm	5.9	8.3	7.1
3.8-7.8 mm	2.6	6.3	4.1
7.8-11.8 mm	2.0	5.6	3.6

ceased after about ten hours exposure to the solution whereas in the case of the more mature segments the process has gone on for at least 24 hours although at a reduced rate. There are at least two possible reasons for this behavior. The obvious possibility is that the rapidly respiring first segment has completely exhausted its reserves of substrate after ten hours and

therefore is unable any longer to provide energy for active accumulation although the barrier is still functional and preventing any appreciable loss to the surrounding medium. A second possibility is that absorption by the first segment is wholly or mostly non-metabolic and that the immature cells comprising this segment are incapable of irreversibly absorbing

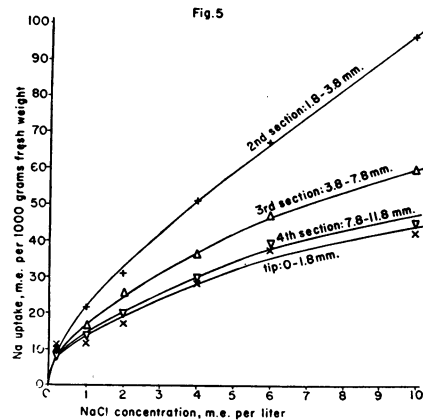
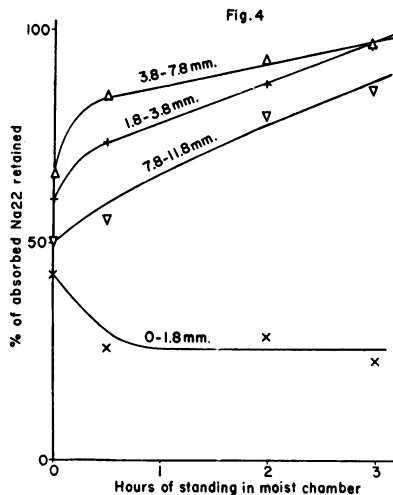
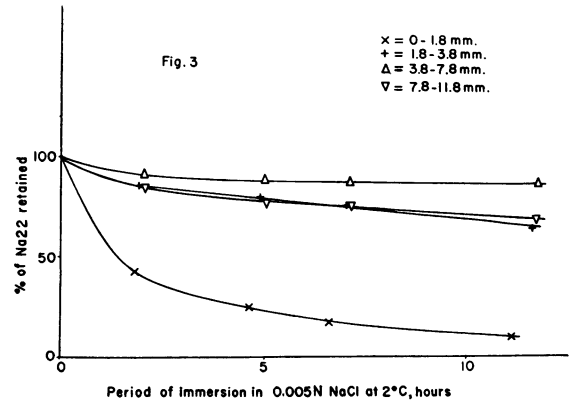
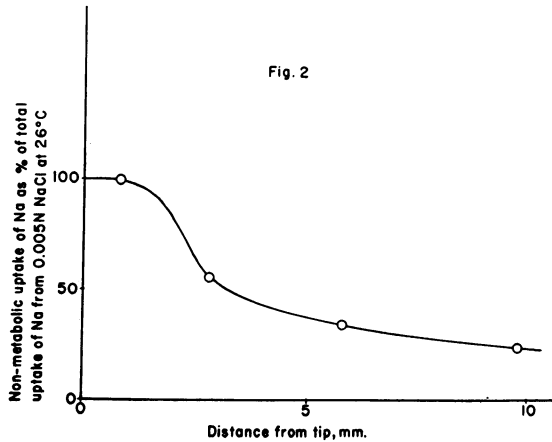


FIG. 2. Curve showing the importance of non-metabolic Na absorption as a function of distance from the tip in maize roots. The curve was derived from the data of figure 1 assuming that the uptake at 2°C is entirely non-metabolic. The values on the ordinate were obtained by dividing the Na uptake for 24 hours in 0.005 N NaCl at 26°C by the uptake for 24 hours in 0.005 N NaCl at 2°C .

FIG. 3. Curves showing the percentage of absorbed Na^{22} retained by maize root segments after various periods of immersion in 0.005 N NaCl at 2°C . Prior to the experiment, the segments were placed for 11 hours at 26°C in a 0.005 N NaCl solution labeled with $0.02\text{ }\mu\text{C}$ of Na^{22} per ml.

FIG. 4. Graph showing percentage of absorbed Na^{22} retained by maize segments against a 2 hour immersion in 0.01 N NaCl at 0°C following a period of standing in a moist atmosphere at 21°C . The Na^{22} was absorbed initially as the result of an immersion of the segments in 0.01 N NaCl labeled with $0.10\text{ }\mu\text{C}$ of Na^{22} per ml for 2 hours at 26°C .

FIG. 5. Na uptake versus concentration curves for various segments of maize roots. For each uptake determination, 40 segments were placed in 500 ml of the appropriate NaCl solution for 8 hours at 21°C . Each quantity plotted on the ordinate represents an average of the uptake values for three separate batches of 40 segments. The initial pH of the culture solutions was 6.0.

sodium. If this is the case we must assume that after about ten hours the tissue of the first segment has arrived at equilibrium with respect to the distribution of sodium between itself and the solution. The experimental results reported in figure 3 support the second possibility. At the end of the experiment less than 10% of the original activity remained in the first segment whereas about 88% had been retained by the third segment and 70% by the fourth. The behavior of the second segment requires some comment. While being much more retentive than the first segment, some 65% of the initial activity remaining after 11 hours, the second segment is not in equilibrium with the medium at the end of the experiment but is continuing to give up sodium. The reason for this is not clear but it seems most probable that this phenomenon is related to the presence of non-vacuolated tissue in the second segment. Repeitions of this experiment yielded essentially the same results.

From figure 1, where the absorption versus time curves for 2° C and 26° C are compared, it is apparent that metabolism plays a minor role in absorption of sodium by the first segment whereas in succeeding segments its part is considerable and increasingly so as the cells mature. In this connection, it is interesting that the increase in cell wall material and intercellular space taking place as the cells mature does not lead to an increase in the capacity for non-metabolic sodium absorption, but quite the reverse. This fact appears to indicate that something more than the outer reaches of the cell is involved in this type of uptake. Also, the relatively long period required for the first segment to come to equilibrium with the environment suggests strongly that the essentially non-metabolic uptake of sodium involves an attachment of the ion to sites within the protoplasm and perhaps an incorporation of sodium in protoplasmic structure as has been suggested elsewhere (19). If this latter idea is correct we must suppose, however, that a much lower level of metabolic activity is required for such incorporation than is needed for accumulation in vacuoles.

The uptake versus concentration curves of figure 5 are interesting in that the curve for the first segment does not appear to be different in kind from the curves for the other segments. For example, the curves for the first and fourth segments are quite similar even though a profound difference in the character of the uptake is indicated by other data presented in the paper.

Figure 2 was derived from the data of figure 1 by dividing the Na uptake value obtained after 24 hours at 2° C by that obtained after 24 hours at 26° C in 0.005 N labeled NaCl to obtain an estimate of the percentage of the total absorption which is non-metabolic in each of the four segments. Of course, the percentages obtained apply only to the particular concentration of sodium employed. The result indicates a rapid decline in the importance of non-metabolic uptake over the first 3 mm. Farther back the rate of decline is progressively slower. The form

of the curve indicates that the contribution of non-metabolic absorption may become fairly constant beyond 11 mm or so from the tip.

The ability of the various segments to transform non-metabolically absorbed sodium into a more difficultly exchangeable form while standing in a moist chamber at 21° C is depicted in the curves of figure 4. The results indicate that the first segment possesses no ability for this presumably metabolic transformation whereas the older tissues show considerable ability in this regard. Indeed, as a result of standing in the moist chamber the first segment showed a decrease in the retention of absorbed Na²² against the action of an eluting NaCl solution. Quite possibly the decrease was due to some loss of sodium by the first segments to the slightly damp filter paper used to support them in the moist chamber. Such losses, of course, may have occurred with the older segments but were more than counterbalanced by metabolic accumulation.

The experimental results indicate that meristematic tissue of maize roots possesses little if any ability to absorb sodium metabolically, but that this ability develops rather rapidly during maturation. Whether or not this is true for other cations or for anions has not been determined. The concurrence of this development with any other phase of maturation is not proven by these experiments. However, the development of vacuoles with well defined tonoplasts does seem to coincide and it is at least reasonable to believe that the connection may be causative and that the possibility of secretion to vacuoles is a prerequisite for metabolic uptake.

The question as to the location of the barrier region operative in metabolic absorption cannot be answered definitely on the basis of this research. Because of the evidence that at least part of the cytoplasm is involved in non-metabolic uptake, however, it may be presumed that the barrier is situated deeper in the cell than the plasmalemma. The writers are inclined to believe that the cytoplasm itself is rather resistant to the entry of sodium and probably to other ions and may actually constitute the barrier region.

SUMMARY

Using separate sections of the root tips of maize and NaCl solutions labeled with Na²², the uptake of sodium by essentially non-vacuolated tissue (0-1.8 mm from tip) has been compared with that in tissues farther from the tip in which vacuolation is progressively more nearly complete.

For the non-vacuolated tissue, the sodium uptake versus time curves at 2° C and 26° C were nearly identical. In the older tissues, however, a much greater sodium uptake was observed at 26° C than at 2° C.

Isotopic dilution experiments with Na²² showed in the case of the non-vacuolated tissue an almost complete isotopic equilibrium between sodium of the tissue and sodium of the medium after a period of

about ten hours whereas the vacuolated tissues were still far removed from isotopic equilibrium.

By means of Na^{22} it was found that vacuolated tissues are able to transform non-metabolically absorbed sodium into a more difficultly exchangeable form during standing at 21°C in a moist chamber. Non-vacuolated tissue is unable to accomplish this.

On the basis of the experiments it is concluded that sodium uptake in the non-vacuolated sections is purely non-metabolic. That is, the ability for the metabolic absorption of sodium appears to coincide with the development of well defined vacuoles.

Because of the relatively long time required for isotopic equilibrium between sodium of non-vacuolated tissue and sodium in the culture medium, it is concluded that at least part of the cytoplasm is involved in the non-metabolic uptake of that element.

The sodium uptake versus concentration curves were of essentially the same form for the various root sections. This was the case in spite of the profound differences in the character of sodium absorption in tissues at different stages of development.

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