

THE ABSORPTION OF BORON BY DISKS OF PLANT STORAGE TISSUES

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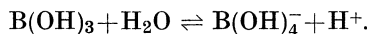
Abstract

Disks prepared from the storage tissues of carrot (*Daucus carota*) were able to absorb boron from boric acid solutions (0.1 mM). At 25°C, net influx ceased 3–4 hr after the tissue was placed in boric acid solution. At equilibrium the tissues were able to maintain an internal concentration of diffusible boron in excess of that in the external solution. Boron uptake was inhibited by anoxia, 2,4-dinitrophenol, and at 1°C; and, in the presence of these inhibitors, the equilibrium concentration of boron in the tissues only approached that in the external solution. The capacity of the tissues to accumulate boron developed 2–3 days after the beginning of a washing treatment. Boric acid (0.1 mM) in this washing solution inhibited the development of this capacity.

It is deduced that there are two components of boron uptake from boric acid solutions in these tissues: a passive diffusion and an active transport process. A model is proposed for the uptake of borate, incorporating the active transport of the $\text{B}(\text{OH})_4^-$ ion and the passive diffusion of $\text{B}(\text{OH})_3$.

I. INTRODUCTION

It is only recently that the mechanisms by which plant cells absorb boron have been investigated (Thellier and Le Guiel 1967; Tanaka 1967; Bowen 1968, 1969; Elseewi, Bingham, and Oertli 1968). Plants absorb boron as boric acid or borates. Boric acid $[\text{B}(\text{OH})_3]$ is a weak acid ($\text{p}K_a = 9.26$) and ionizes in solution to form the borate ion $[\text{B}(\text{OH})_4^-]$ in the following manner:



At a physiological pH the unionized $\text{B}(\text{OH})_3$ molecule predominates; thus, at pH 5.7, 99.9% of the boric acid is present as $\text{B}(\text{OH})_3$. Therefore two molecular species $[\text{B}(\text{OH})_3]$ and $[\text{B}(\text{OH})_4^-]$ must be considered when studying the absorption of boron by plant cells.

In the investigations referred to above, it has been proposed that boron is absorbed by plant tissues either as a result of the active transport of $\text{B}(\text{OH})_4^-$ (Thellier and Le Guiel 1967; Bowen 1968, 1969), or passively by diffusion of $\text{B}(\text{OH})_3$ (Elseewi, Bingham, and Oertli 1968). The conclusion that barley tissues absorb boron passively (Elseewi, Bingham, and Oertli 1968) depended upon the insensitivity of the process to KCN, 2,4-dinitrophenol (DNP), and also to temperature variations. The equilibrium concentration within the tissue equalled that in the external solution at acid pH. Also, the boron absorbed from a dilute boric acid solution was rapidly and almost completely desorbed when the tissue was transferred to a boron-free solution.

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In contrast, Bowen (1968, 1969) has shown that the uptake of boron by excised roots and slices of leaves of sugarcane occurs by the active transport of $B(OH)_4^-$. It can be inferred from Bowen's results that, unlike the cells of barley leaves, the cells of sugarcane leaves are impermeable to $B(OH)_3$. Boron absorption by the sugarcane tissue occurred in two phases; an initial rapid and reversible influx into the free spaces of the tissue that was completed within 20 min, followed by an irreversible accumulation of boron by the plant cells (Bowen 1968). Application of enzyme kinetics to the irreversible phase indicated the presence of multiple mechanisms for boron absorption.

The absorption of boron by *Lemna minor* plants also occurs in two phases (Thellier and Le Guel 1967). The mechanism responsible for the boron absorption during the linear phase was saturated between 0.5 and 1.0 mM boric acid. Furthermore, boric acid competitively inhibited the uptake of phosphate and chloride by *L. minor* (Thellier and Ayadi 1967; Thellier, Ayadi, and Tromeur 1967). These findings indicate that *L. minor* is able to actively accumulate boron.

The present paper describes an investigation of the mechanism by which the cells of carrot and red beet storage tissues absorb boron.

II. METHODS AND MATERIALS

(a) Plant Material

Carrot (*Daucus carota*) and red beet (*Beta vulgaris*) storage roots were obtained from commercial sources. The storage roots were peeled thickly and cut into slices 1.0 mm thick from which disks 1.0 cm in diameter were prepared with a cork-borer. The carrot disks were prepared from the phloem parenchyma and the red beet disks from the parenchyma between the vascular tissue.

The disks were washed for 5 days at room temperature in either aerated distilled water or 0.5 mM $CaCl_2$. During the course of this investigation it was found that the addition of 0.5 mM $CaCl_2$ to the washing medium helped maintain the viability of the disks during the washing pretreatment, and also maintained their ability to absorb boron.

(b) Boron-uptake Studies

Storage tissue disks were suspended in aerated 0.1 mM boric acid solutions that were normally maintained at 25°C. Uptake was calculated from the boron content of a subsample of disks withdrawn at various times. In the experiments in which the disks were washed in 0.5 mM $CaCl_2$, the uptake of boron was measured from solutions containing the same concentration of $CaCl_2$. The effect on boron uptake of including $CaCl_2$ in the uptake solution is reported in Section III. The uptake solutions were unbuffered at $pH\ 5.7 \pm 0.1$.

The disks, after being blotted dry and weighed, were incubated in the uptake solutions using one of the following two methods:

- (1) The disks were threaded on to 25-gauge nichrome wire in groups of 10 or 15, and spaced 3.0 mm apart with polyethylene catheter tubing (Intramedit PE 50). The disks on the wire were resuspended in the washing medium overnight before being transferred into 2-litre treatment solutions held in Perspex containers. At the required time intervals, replicate samples were removed. Each replicate consisted of the disks grouped together on one length of wire.
- (2) Alternatively, disks (10–30) were immersed in 50.0 ml of treatment solution in 250 ml polypropylene Erlenmeyer flasks. The flasks were agitated in a water-bath at 25°C by a Warburg shaker.

The absorption experiments were terminated by rinsing the disks three times for 30 sec with distilled water in a Buchner funnel. The disks were then dried and analysed for boron. This rinsing procedure was omitted in the desorption experiments.

(c) *Boron Determination*

After drying at 80°C the disks were ashed at 250°C for 2 hr in covered porcelain crucibles using a muffle furnace. Under these conditions there was no boron contamination from the walls of the muffle.

The ash was taken up in 0.1N HCl and the boron was determined in aliquots of this solution by the curcumin method of Dible, Truog, and Berger (1954). The curcumin was Koch-Light PRACT grade and the ethanol was redistilled.

The boron absorbed by the disks was calculated after allowing for the initial endogenous boron content of the tissue, which was approximately 0.1 μ mole per gram fresh weight. "Absorbed boron" thus excludes that originally present in the disks and also excludes that which was leached out in the rinsing procedure (mentioned above) which was used prior to tissue analysis.

III. RESULTS

(a) *Effect of CaCl₂ on Boron Absorption*

Calcium has been shown to have a beneficial effect on the absorption of ions by plant cells (e.g. Viets 1944; Epstein 1960; Pitman 1963; Rains, Schmid, and Epstein 1964), and to be necessary for the maintenance of the functional integrity of the cell membranes (e.g. Van Steveninck 1965; Jones and Lunt 1967). Consequently it has become a routine procedure to include calcium in studies of ion uptake. Bowen (1968) has shown that the presence of 0.5 mM CaCl₂ enhanced the absorption of boron by slices of sugarcane leaves from a 0.05 mM boron acid solution.

The inclusion of CaCl₂ in the "uptake" solution (as opposed to the washing solution) at concentrations between 0.01 mM and 10.0 mM did not significantly increase the absorption of boron by disks of carrot tissue, compared to the control treatment, as shown in the following tabulation. The disks had been washed in 0.5 mM CaCl₂.

CaCl ₂ concn. (mM):	0	0.01	0.1	1.0	10.0	
Boron uptake (μ mole/g fresh wt.):						
After 2 hr	0.142	0.163	0.162	0.145	0.145	} L.S.D. (<i>P</i> = 0.05) 0.026
After 5 hr	0.175	0.183	0.198	0.150	0.169	

The value for boron absorbed after 5 hr in the presence of 1.0 mM CaCl₂ is low compared to the other values. The reason for this is not known.

However, the presence of 0.5 mM CaCl₂ in the treatment (or "uptake") solution inhibited the uptake of boron over 5 hr by disks of carrot tissue that had not been washed in 0.5 mM CaCl₂, as indicated in the following tabulation:

Washing Pretreatment	Boron Uptake after 5 hr (μ mole/g fresh wt.)	
	From 0.1 mM Boric Acid + 0.5 mM CaCl ₂	From 0.1 mM Boric Acid
Distilled water	0.056	0.115
Chloramphenicol (50 p.p.m.)	0.069	0.125
0.5 mM CaCl ₂	0.132	0.119
L.S.D. (<i>P</i> = 0.05)	0.010	

It is not clear why the presence of CaCl₂ should inhibit the uptake of boron by carrot disks that had had no CaCl₂ pretreatment, but have no effect on the boron uptake by

those disks that had been washed in CaCl_2 . Bowen (1968) reported that chloride competitively inhibits the accumulation of boron by segments of sugarcane leaves. As a result of these experiments CaCl_2 was omitted from the uptake solution when the disks had not been washed in CaCl_2 .

(b) *Absorption and Desorption of Boron*

The time course of boron absorption (from 0.1 mM boric acid) by the disks of carrot and red beet storage tissues are shown in Figure 1. The red beet disks had been washed in 0.5 mM CaCl_2 and the treatment solution also contained 0.5 mM CaCl_2 . The pattern of absorption was similar in both beet and carrot tissues. Initially boron absorption was rapid, but it then declined steadily until a state of equilibrium was reached 3–4 hr after commencing the experiment. The time to reach 50% equilibration was about 30 min. There was no evidence of the period of constant net influx that is characteristic of ion absorption by plant tissues (Laties 1959), and which also occurred in the absorption of boron by slices of sugarcane leaves (Bowen 1968) and *L. minor* plants (Thellier and Le Guiel 1967).

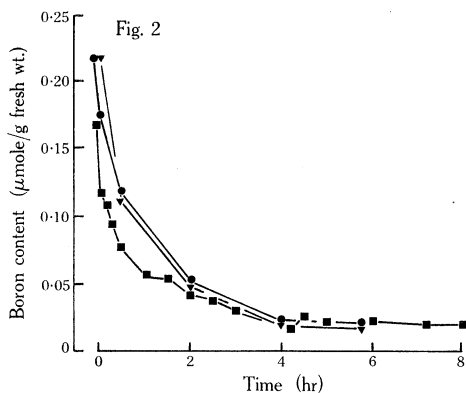
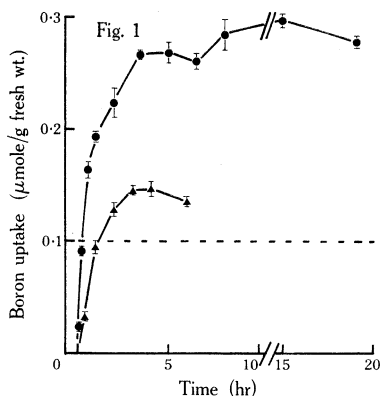


Fig. 1.—Time course of uptake of boron by carrot (●) and beet (▲) tissues from a solution of 0.1 mM boric acid + 0.5 mM CaCl_2 (pH 5.7) at 25°C. --- Concentration of boric acid in external solution.

Fig. 2.—Time course of desorption of boron (at 25°C) by carrot (▼ and ●) and beet tissue (■), which had previously been equilibrated with 0.1 mM boric acid (see Fig. 1). ● and ■ Desorption into distilled water. ▼ Desorption into 0.5 mM CaCl_2 .

The boron absorbed by the disks was readily desorbed when they were transferred to a minus-boron solution (Fig. 2). Carrot and red beet disks that had been washed in 0.5 mM CaCl_2 were allowed to absorb boron from a solution of 0.1 mM boric acid and 0.5 mM CaCl_2 for 220 and 180 min respectively, i.e. sufficient time to reach equilibrium. At the end of this period the disks (maximum total fresh weight = 35 g) were transferred to 2 litres of aerated solutions free of boron, and replicate samples of 10 disks were taken at various time intervals and analysed for boron. For these experiments the rinsing procedure was omitted and the disks were only blotted dry.

The desorption was rapid and, as for absorption, the half-time of the process was approximately 30 min. The presence of 0.5 mM CaCl_2 in the minus-boron solution did not affect either the rate or extent of the desorption (Fig. 2). Only a small proportion of the boron absorbed was not desorbed in the minus-boron solution. After desorption about 9 and 11% of the absorbed boron above that originally present in the disks remained in the carrot and red beet disks respectively. The concentration of previously absorbed boron remaining in the disks after 8 hr ($0.025 \mu\text{mole/g}$ fresh wt.; Fig. 2) was approximately eight times that in the 2 litres of solution ($0.003 \mu\text{mole/ml}$), also after 8 hr. This boron which was not desorbed therefore did not represent a new equilibrium state with that in the external solution and may have been removed from the pool of diffusible boron by metabolism or by complex formation with a tissue constituent, such as the absorption process described by Tanaka (1967).

In contrast to the boron absorbed from the external solution, the endogenous boron of the tissues, present before absorption and desorption, was not desorbed. Washing disks for up to 5 days in distilled water or 0.5 mM CaCl_2 caused little or no change in the boron content of the disks, as shown in the following tabulation. Each value represents the mean of three determinations, each of 10 disks:

Time of Washing (days)*	Boron Content ($\mu\text{mole/g}$ fresh wt.)	
	Carrot Disks	Beet Disks
0	0.108	0.122
4	—	0.124
5	0.104 0.102	—

* The washing solution contained 0.5 mM CaCl_2 .

Thus the endogenous boron was not present in the pool of freely diffusible boron, and can be ignored when comparing the concentration of boron within the disks with that in the external solution.

The concentrations of freely diffusible boron in the carrot and red beet disks, after uptake from 0.1 mM boric acid solutions, were 0.197 and $0.148 \mu\text{mole per gram}$ fresh weight respectively (Fig. 2). As the specific gravity of the carrot and red beet disks was approximately 1.0, it is apparent that, at equilibrium, the disks were able to maintain an internal concentration of freely diffusible boron in excess of that in the external solution ($0.1 \mu\text{mole/ml}$). The diffusible boron clearly could not have originated entirely from the intercellular spaces and cut cells, which have been estimated to account for about 20–25% of the disk volume (e.g. Pitman 1963; Cram 1968). It can be concluded therefore that the disks, after a washing pretreatment, are able to absorb boron against a concentration gradient, and also (from desorption experiments) that the cells, and consequently the cell membranes, are permeable to boric acid.

(c) *Effect of Metabolic Inhibitors on Boron Uptake*

The absorption of boron by carrot and red beet disks over 185 and 120 min respectively was decreased by anoxia, by a temperature of 1°C , and in the presence of

DNP (Table 1). A concentration of 10^{-4}M DNP was chosen, as it was the lowest concentration used on carrot root tissue by Robertson, Wilkins, and Weeks (1951) which gave the maximum stimulation of respiration and inhibition of potassium uptake from KCl solutions. Anoxia was achieved by gassing the treatment solutions with nitrogen.

TABLE 1
EFFECT OF METABOLIC INHIBITION ON THE ABSORPTION OF
BORON BY DISKS OF CARROT AND RED BEET
The concentration of boric acid in the treatment solutions
was 0.1 mM

Treatment†	Boron Uptake* ($\mu\text{mole/g wt.}$)	
	Carrot Disks (after 185 min)	Red Beet Disks (after 120 min)
Control	0.121	0.128
DNP, $5 \times 10^{-5}\text{M}$	—	0.047
DNP, $10 \times 10^{-5}\text{M}$	0.081	—
Aerated with nitrogen	0.072	0.037
Low temperature (1°C)	0.056	—
L.S.D. ($P = 0.01$)	0.012	0.017

* After subtraction of the initial endogenous boron content, which is not removed by washing treatments [see tabulation, Section III(b)].

† Treatment solution also contained 0.5 mM CaCl_2 .

The time courses of boron absorption by carrot disks under anaerobic conditions, and in the presence of DNP are shown in Figures 3 and 4. As in the previous

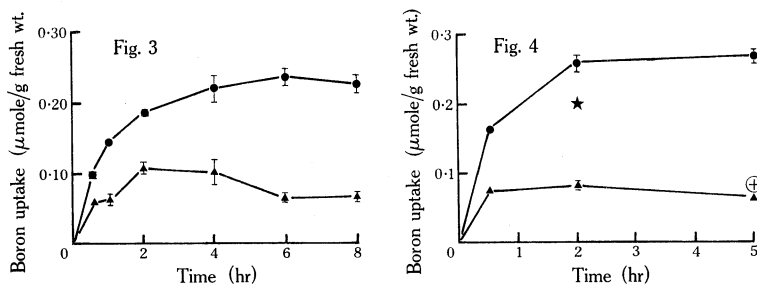


Fig. 3.—Effect of aeration (●) or gassing with nitrogen (▲) on the absorption of boron by carrot disks in 0.1 mM boric acid at 25°C .

Fig. 4.—Effect of 2,4-dinitrophenol (10^{-4}M , pH 4.2) on the uptake of boron from 0.1 mM boric acid. ● Control: 0.1 mM boric acid, pH 5.7; ★ 0.1 mM boric acid, pH 4.5; ▲ 0.1 mM boric acid + 10^{-4}M DNP, pH 4.2; ⊕ 2 hr uptake in the presence of 10^{-4}M DNP, followed by 3 hr in the presence of 0.1 mM boric acid alone.

experiment (Table 1) these treatments markedly reduce the absorption of boron. The equilibrium concentrations of boron in the disks were 0.060 and 0.069 $\mu\text{mole per gram fresh weight}$ in the presence of nitrogen and DNP respectively. When allow-

ance is made for the amount of boron leached from the tissue by the rinsing procedure (c. $0.04 \mu\text{mole/g}$ fresh wt.), it is seen that under these conditions of metabolic inhibition, the concentration of boron in the tissues approached $0.1 \mu\text{mole per gram}$ fresh weight, which is equal to the boron concentration in the external solution. Thus, the maintenance of an internal concentration of diffusible boron greater than that in the external solution was apparently dependent on the metabolic activity of the tissue.

The inhibition of the absorption by anoxia was reversible after 2 hr (Table 2). However, the inhibition of boron uptake by DNP (10^{-4}M) was not reversed by transfer to a DNP-free solution after 2 hr (Fig. 4). The pH of the 10^{-4}M DNP solution was 4.2, compared to that of 5.7 of the control solution. However, the inhibitory effect of DNP on boron uptake can only be partly ascribed to this pH difference because the decrease in uptake due to this pH difference alone was found to be only 32% of the decrease induced by 10^{-4}M DNP (Fig. 4).

TABLE 2
REVERSIBILITY OF THE NITROGEN INHIBITION OF BORON UPTAKE BY
CARROT DISKS

The treatment and pretreatment solutions both contained 0.5 mM CaCl_2 .
The disks were incubated sequentially in solutions gassed with nitrogen
and air for the indicated times

Duration of gassing treatment (hr):				
Nitrogen	—	0.5	2.0	5.0
Air	5.0	4.5	3.0	—
Boron uptake ($\mu\text{mole/g}$ fresh wt.):	0.146	0.147	0.154	0.110
L.S.D. ($P = 0.05$)	0.022			

(d) *Development of the Ability of Disks of Red Beet to Absorb Boron after Cutting and Whilst being Washed*

Disks prepared from dormant root storage organs only develop the ability to accumulate ions after a washing pretreatment (e.g. Van Steveninck 1961). Therefore, similar experiments were undertaken to investigate how the capacity of disks of red beet tissue to absorb boron varied with the duration of the washing pretreatment.

Disks were washed in a solution of 0.5 mM CaCl_2 and, at the times after cutting indicated in Figure 5, samples were taken to determine the ability of the disks to absorb boron in the presence or absence of $5 \times 10^{-5}\text{M}$ DNP. Due to an increase in fresh weight of the disks with time, the boron concentration at each sampling time was expressed on the basis of an estimate of the initial fresh weight. The freshly prepared disks absorbed very little boron in the presence or absence of DNP (Fig. 5). Presumably the rinsing procedure, used after the uptake period and before analysis, removed the boron from the free space. The total boron absorbed in 2 or 4 hr increased at each sampling period, up to 96 hr from the time the washing treatment began. The greatest increase occurred between 24 and 47 hr (Fig. 5).

It may be concluded from this experiment that the ability of this storage tissue to accumulate boron, to an internal concentration in excess of the external concen-

tration, develops over the period 20–96 hr from the beginning of the washing treatment (Fig. 5). Also, the ability of these tissues to absorb boric acid, in the presence of $5 \times 10^{-5}M$ DNP, increases steadily over the period 8–96 hr (Fig. 5). However, in the presence of DNP the internal concentration of boron never exceeded that of the external solution, as was also found in the previous experiments (Fig. 4; Table 1). These results point to the development, with time of washing, of a bipartite process: a passive uptake process, depending on diffusion, and an active process, inhibited in the presence of DNP.

The development of the ability of beet disks to absorb boron, with time of washing was investigated in a further experiment, the object of which was to ascertain the effect of including boric acid in the washing solution.

After cutting the disks of red beet were washed in distilled water for 50 min when a sample of disks was taken to determine their endogenous boron content. The remaining disks were distributed between the washing treatments tabulated below:

Treatment	Washing Solution	Procedures and Measurements
1	Boric acid (0.1 mM) + CaCl ₂ (0.5 mM)	Two samples (each of 20 disks) were periodically (Fig. 6) taken and rinsed in distilled water and analysed for boron. At 68 hr the remaining disks were transferred to a boron-free solution of 0.5 mM CaCl ₂ , and the desorption of boron studied [Fig. 6(a)]
2	CaCl ₂ (0.5 mM)	After 72 hr washing, disks were rinsed and placed in 0.1 mM boric acid + 0.5 mM CaCl ₂ and their uptake of boron measured after 2 and 5 hr, in the presence of $5 \times 10^{-5}M$ DNP, was also measured [Fig. 6(b)]

Disks washed in solutions containing 0.1 mM boric acid (treatment 1) did not develop the capacity to absorb boron to a concentration in excess of that in the external solution [Fig. 6(a)]. This may be contrasted to the results of Figure 5, for disks washed in the absence of boric acid. However, boron content, and therefore also the permeability of the disks to boric acid, did increase with time of washing. Also, the boron that was absorbed was almost completely desorbed in a period of 9 hr, into a boron-free solution [Fig. 6(a)]. The proportion of the boron that remained in the tissues after desorption was similar to that found in a previous experiment (Fig. 2).

In contrast to the behaviour of the disks washed in the presence of boric acid (treatment 1), a subsample of the same disks washed for 72 hr in the absence of boric acid (treatment 2), behaved as in the previous experiment (Fig. 5): they possessed the ability to accumulate boron to a concentration in excess of that in the external solution [Fig. 6(b)]. The "active" component of this uptake was, as before (Table 1 and Fig. 4), inhibited by DNP ($5 \times 10^{-5}M$).

It may be concluded from these two experiments (Figs. 5 and 6) that the capacity of washed disks of red beet to absorb boron from boric acid solutions develops over a period of 96 hr after cutting. Also, the capacity to accumulate boron to a concentration in excess of that in the external solution does not develop if the washing solution contains boric acid.

(e) *Summary of Experimental Evidence*

The experimental evidence that the uptake of boric acid by washed disks of carrot and red beet tissues involves both a passive and an active process is given below.

(i) *Evidence of an Active Uptake Process*

- (1) Disks of carrot and red beet absorb boron, supplied as boric acid, to an equilibrium internal concentration which is greater than that of the external solution (Figs. 1, 3, 4, 5; Tables 1 and 2).
- (2) This accumulation of boron in the tissues (assessed from the boron content of disks after three rapid rinses in boron-free distilled water) is inhibited by anoxia, by DNP, and also by low temperature (1°C) (Figs. 3, 4; Tables 1 and 2).
- (3) The capacity to accumulate boron against a concentration gradient is dependent upon a washing pretreatment of *c.* 20–40 hr duration. This capacity does not develop if the pretreatment washing solution contains boric acid (Figs. 5, 6).

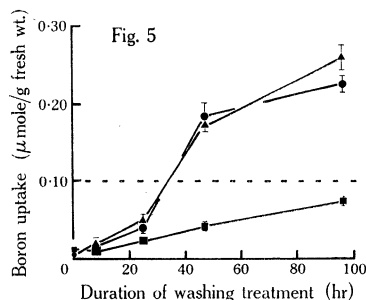


Fig. 5.—Effect of time of washing in 0.5 mM CaCl_2 of disks of beet tissue upon their subsequent ability to absorb boron from a solution of $0.1\text{ mM boric acid} + 0.5\text{ mM CaCl}_2$. ● Boron absorbed in 2 hr. ▲ Boron absorbed in 4 hr. ■ Boron absorbed in 2 hr in the presence of $5 \times 10^{-5}\text{ M DNP}$.

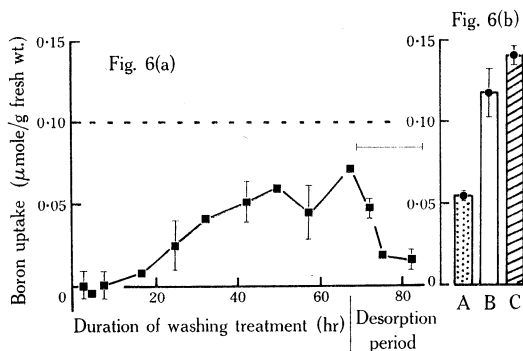


Fig. 6.—(a) Effect of time of washing in a solution of $0.1\text{ mM boric acid} + 0.5\text{ mM CaCl}_2$ on the boron content of beet tissue, followed by the desorptive loss of boron into a 0.5 mM CaCl_2 solution (68–83 hr). (b) Capacity of the same batch of disks, washed for 72 hr in 0.5 mM CaCl_2 alone, to absorb boron from $0.1\text{ mM boric acid} + 0.5\text{ mM CaCl}_2$ over 2 hr (histogram B), 5 hr (histogram C), and 2 hr in the presence of $5 \times 10^{-5}\text{ M DNP}$ (histogram A).

(ii) *Evidence of a Passive, Diffusion-type Process*

- (1) The boron content of storage tissue, after a period of absorption of boric acid, is rapidly and almost completely desorbed into a boron-free solution (Fig. 3).
- (2) In the presence of DNP, under conditions of anoxia or at a temperature of 1°C , the capacity of disks to absorb boron is apparent (Figs. 3 and 4), despite the fact uptake was assessed after the rinsing procedure, mentioned above. It may be concluded that the cells are permeable to boric acid.

- (3) The permeability of the tissues to boric acid increased with time of washing in pretreatment solutions either containing or devoid of boric acid (Figs. 5 and 6).

IV. DISCUSSION

(a) *Passive Uptake*

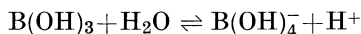
The passive uptake is a rapid process and equilibrium is reached within 30 min (Fig. 4). As this process results in the internal boron concentration at equilibrium approaching that in the external solution (Figs. 3 and 4), and since the cells of the tissues appeared to be permeable to boric acid (Fig. 2), it is concluded that this passive uptake occurs as a result of diffusion along a concentration gradient. The contribution of adsorption to this uptake was not specifically determined, but it would seem to be small, as only a small proportion of the absorbed boron was not free to diffuse out of the tissues (Fig. 2). At pH 5.7 99.968% of the boron is present in solution as B(OH)_3 , and it seems probable therefore that this is the form in which boron diffuses into the cells of the tissue. Elseewi, Bingham, and Oertli (1968) arrived at a similar conclusion with barley tissues, but from the results of Bowen (1968) it appears that the cells from sugarcane leaves are impermeable to this molecule.

The extent to which the cells of the storage tissues are permeable to B(OH)_4^- cannot be assessed from the present investigations. However, as cell membranes generally exhibit low permeability to ions (Collander 1959), it would seem probable that they are also relatively impermeable to B(OH)_4^- .

(b) *Uptake Dependent on Tissue Metabolism*

The second component of uptake, which is linked to the metabolic activity of the tissue, enables them to maintain an internal concentration of diffusible boron in excess of that in the external solution (Fig. 1). The role of metabolism in boron uptake then is not merely to remove boron from the solution within the tissue to an "insoluble" form, and thereby maintain a concentration gradient of "soluble" boron along which boron could diffuse. The inhibition of this component of boron uptake by anoxia and DNP indicates that it is dependent for its function on energy derived from aerobic respiration. This fact, along with the ability of this uptake component to transport boron against a concentration gradient, is consistent with the presence of a mechanism for actively transporting boron into the cells of carrot and red beet storage tissues. Bowen (1968) and Thellier and La Guie (1967) have also proposed mechanisms for actively transporting boron into plant tissues.

As the pH is lowered the equilibrium



is shifted to the left and the concentration of B(OH)_4^- is effectively reduced. Bowen (1969) has shown that the active uptake of boron by slices of sugarcane leaves at a given pH in the external solution, and the concentration of B(OH)_4^- at that pH, are correlated. He has concluded, therefore, that boron is actively accumulated as B(OH)_4^- in this tissue. The effect of pH on the absorption of boron by disks of storage tissue is also consistent with B(OH)_4^- being the form in which boron is actively transported into these tissues.

(c) *A Model for the Uptake of Borate by Plant Tissues*

Assuming that the uptake of borate involves two components, as already discussed, and assuming that the pH of the external solution (5.7) is similar to the internal pH of the cytoplasm and vacuole [Weast (1965) quotes a range of 5.0–5.5], then the ratio of B(OH)_3 to B(OH)_4^- in the tissues would be similar to that in the external solution. This follows from the equilibrium equation given in Section IV(b).

The scheme proposed in Figure 7 involves these assumptions and illustrates a possible model accommodating the observed experimental results on the exchange of boron (as boric acid) between external solutions and the cell. No distinction has been made between the cytoplasm and the vacuole.

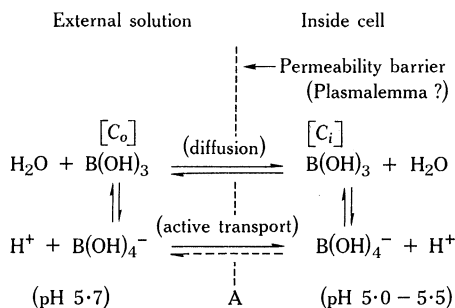
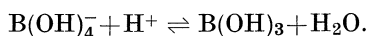


Fig. 7.—A model for the uptake of boric acid by root storage tissue of carrot and beet. The membrane A is considered to be permeable to B(OH)_3 but relatively impermeable to B(OH)_4^- . See text for further explanation.

It is proposed that the barrier between the interior of the cell and the external solution (most probably the plasmalemma—see Pitman 1963; Cram 1968, 1969) is permeable to B(OH)_3 but relatively impermeable to B(OH)_4^- , and that B(OH)_4^- is actively transported across this barrier into the cell. The following stages in boron absorption can now be envisaged.

When tissue is first immersed in a solution of boric acid, the internal boron concentration (C_i) is lower than that in the external solution (C_o). An uptake of boron will occur, therefore, as a result of the diffusive influx of B(OH)_3 and a slower active uptake of B(OH)_4^- . The contribution of diffusion to net uptake will subsequently decline, until, at $C_o = C_i$, it will be zero. At this stage net uptake is attributable solely to the active transport of B(OH)_4^- . As active transport of B(OH)_4^- continues, there will be a concomitant increase in the internal concentration of B(OH)_3 due to



A resultant diffusive loss of boron will ensue. The rate of this diffusive loss of boron will increase as the concentration gradient between the tissue and the external solution increases due to the active uptake of B(OH)_4^- . Finally a stage is reached where the uptake of B(OH)_4^- and the diffusive loss of B(OH)_3 are equal, with the result that a dynamic equilibrium is established.

This scheme, then, is able to explain the apparent contradiction of an active transport of boron into cells which appear to be freely permeable to boron. It also explains the absence of a steady state of net influx of boron into the tissues, which would be expected in the presence of a mechanism for active transport. Furthermore, it incorporates both the passive uptake proposed by Elseewi, Bingham, and Oertli (1968) and the active uptake proposed by Bowen (1968) and Thellier and Le Guiel (1967).

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