THE EFFECTS OF CALCIUM AND TRIS(HYDROXYMETHYL)AMINOMETHANE ON POTASSIUM UPTAKE DURING AND AFTER THE LAG PHASE IN RED BEET TISSUE

By R. F. M. Van Steveninck*

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Summary

Both Ca^{2+} and tris(hydroxymethyl)aminomethane (Tris) caused a shortening of the lag phase in net K^+ absorption in beetroot tissue. At pH 8 the effect of Tris was more immediate than that of Ca^{2+}, while the reverse was true at pH 6. At both pH values the effect of Ca^{2+} prevailed when Ca^{2+} and Tris were simultaneously present in the external solution.

Experiments with ^{42}K showed that Ca^{2+} caused an immediate depression of the apparent K^+ influx, which lasted from *c.* 6 to 20 hr depending on pH. It was followed by a rapid increase of apparent influx until values 3–4 times that of control treatments were reached. Ca^{2+} also caused an immediate decrease of apparent K^+ efflux which lasted from *c.* 20 to 40 hr.

I. Introduction

It was reported earlier that both Ca^{2+} and tris(hydroxymethyl)aminomethane (Tris) reduce the lag in net K^+ absorption, a common phenomenon in sliced storage tissues (Van Steveninck 1961). These experiments gave no indication whether the shortening of the lag phase was the result of an increase of influx or a decrease of efflux of K^+, or of both phenomena combined.

Experiments were carried out with ^{42}K, in which the tisues cells were considered as one single phase separated from the external solution, water free space, and ions associated with surface charges. This simple approach allowed the measurement of immediate changes or pattern of change of apparent fluxes induced as a result of the experimental treatments.

II. Methods

The methods employed were identical to those described by Van Steveninck (1964). ^{42}K, obtained as an isotonic KCl solution, was incorporated in 1 mM KCl solutions containing 10^{-2}M $CaCl_2$ or 10^{-2}M Tris hydrochloride, or both. These solutions were adjusted with NaOH or HCl during the experiment to maintain a pH of 6 or 8 as required. Radioactivity was approximately 500 μc/l when the disks were immersed. The freshly sliced disks of beetroot tissue (cv. Detroit Dark Red) were rinsed three times in distilled water for half an hour to remove cell debris before commencing the experimental treatment. They were divided into three batches: (1) for immediate use; (2) for use half-way through the lag phase (18–24 hr); (3) for use after the

* Crop Research Division, DSIR, Lincoln, N.Z.; present address: Botany Department, University of Adelaide.

completion of the lag phase (42–48) hr. Until they were required disks of groups (2) and (3) were kept in solutions identical to those used during later treatment except that standard KCl was used instead of ^{42}KCl.

Apparent influx (ρ_i) was calculated in accordance with:

$$\rho_i = \frac{[K_i^+]\, dS_i/dt}{(S_o - S_i)},$$

in which $[K_i^+]$ is the internal or non free space concentration of K^+, and S_i and S_o are the specific activities of K^+ in the non free space and external solution respectively. The apparent net flux (R) was calculated from the changes in concentration of the external solution, and the apparent efflux (ρ_o) from $\rho_o = R - \rho_i$. Also R was considered to assume a positive sign when directed inwards.

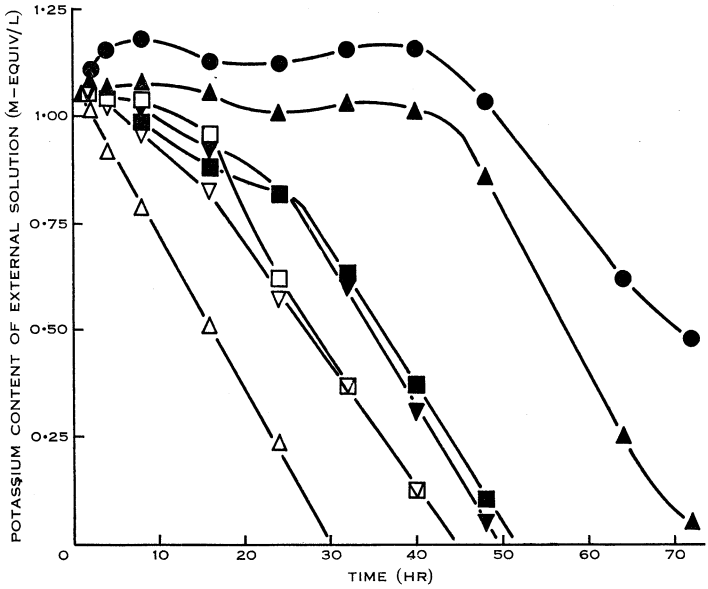

Fig. 1.—Change in potassium content of the external solution over 72 hr due to uptake or release of ions by freshly cut beetroot tissue placed in 1·0 mM KCl solution (4·6 g tissue/125 ml solution, temperature 24°C). Open symbols, pH approximately 8; closed symbols, pH approximately 6. ● Control; ▲, △ 10^{-2}M Tris.HCl; ▼, ▽ 10^{-2}M $CaCl_2$; ■, □ 10^{-2}M $CaCl_2$+10^{-2}M Tris.HCl.

III. Results

Figure 1 shows the effects which Ca^{2+} and Tris have on the duration of thel ag phase in net K^+ absorption. Although the Tris effect was much more immediate, at least at pH 8, Tris molecules did not appear to exert their effect when Ca^{2+} was present at the same time. That is, the effect of Ca^{2+} prevailed at pH 6 as well as at pH 8.

Apparent influx and efflux values are presented in Tables 1 and 2. The values for ρ_o, which are based on the differences of the observed net flux (R) and

influx (ρ_i), were inaccurate when both R and ρ_i became large with respect to ρ_o. This condition existed after completion of the lag phase, hence the above method cannot be recommended for the determination of apparent efflux in aged tissue.

TABLE 1

APPARENT INFLUX (ρ_i) AND APPARENT EFFLUX (ρ_o) OF K^+ IN BEETROOT TISSUE AT pH 6
Disks of various ages placed in 1·23 mM ^{42}KCl solution without further additions (control) or with further additions of calcium chloride or Tris or both at the concentrations indicated; incubation temperature 24°C

Time since Beginning of Incubation (hr)	ρ_i (m-equiv. kg^{-1} hr^{-1})				ρ_o (m-equiv. kg^{-1} hr^{-1})			
	Control	10^{-2}M $CaCl_2$	10^{-2}M $CaCl_2$ + 10^{-2}M Tris.HCl	10^{-2}M Tris.HCl	Control	10^{-2}M $CaCl_2$	10^{-2}M $CaCl_2$ + 10^{-2}M Tris.HCl	10^{-2}M Tris.HCl
Fresh disks								
0–½	0·53	0·04	0·02	0·16	−5·16	−8·49	−8·87	−8·10
½–2	0·12	0·02	0·03	0·08	−1·35	−0·09	−0·11	−0·58
2–5	0·13	0·10	0·13	0·06	−0·64	−0·06	−0·05	−0·10
5–8	0·13	0·10	0·16	0·05	−0·17	−0·06	−0·08	−0·01
8–21	0·12	0·08	0·12	0·05	−0·19	−0·04	−0·04	0·00
Disks 24¼ hr old								
0–½	0·66	0·33	0·23	0·22	−0·86	−1·54	−1·24	−1·83
½–2	0·13	0·17	0·31	0·07	−0·35	−0·10	−0·09	−0·44
2–5	0·17	0·27	0·35	0·14	−0·44	−0·11	−0·03	−0·18
5–8	0·20	0·77	0·56	0·14	−0·20	−0·16	−0·09	−0·01
8–21	0·24	0·94	0·51	0·29	−0·15	−0·10	+0·11	+0·02
Disks 48 hr old								
0–½	0·81	1·34	1·53	0·92	−1·11	−0·33	−0·33	−1·42
½–2	0·32	0·78	0·94	0·52	−0·06	−0·13	−0·07	−0·05
2–5	0·37	1·21	0·90	0·65	−0·09	−0·03	+0·01	−0·02
5–8	0·40	1·32	1·40	0·70	−0·10	−0·07	+0·03	−0·01
8–22	0·43	1·38	1·32	0·76	−0·10	+0·01	+0·07	+0·03

Values for ρ_i and ρ_o obtained during the first half hour of incubation in ^{42}KCl were influenced, in part, by the equilibration of the cytoplasmic phase with the external solution (cf. Pitman 1963: $t_{\frac{1}{2}}$ for K^+ at 25°C = 45 min), and by exchange of K^+ associated with the Donnan free space. The values for ρ_i then became quite steady for the control treatment. An increase of ρ_i could be observed during the process of aging; the advent of net uptake of K^+ was mainly determined by a diminishing apparent efflux.

When Ca^{2+} was present the apparent influx was almost entirely prevented in fresh tissue. However, ρ_i values rose rapidly and during the following period of 6 and 20 hr for pH 8 and 6, respectively, the apparent influxes of calcium-treated tissues and controls became equally large. This was followed by further increases until ρ_i values in the presence of Ca^{2+} were three times those of controls. The presence of Ca^{2+} caused a reduction in ρ_o values throughout the experimental period.

Tris at pH 6 caused a reduction in apparent influx for at least 32 hr. Following this period the influx increased rapidly, and became about twice as large as that of the control treatment. At pH 8 Tris caused an immediate increase in apparent influx with values up to four times those of the control treatment. Apparent efflux was reduced in the presence of Tris at both pH values, except where a condition approaching salt saturation was reached (Table 2).

TABLE 2

APPARENT INFLUX (ρ_i) AND APPARENT EFFLUX (ρ_o) OF K^+ IN BEETROOT TISSUE AT pH 8

Disks of various ages placed in 1 mM ^{42}KCl solution without further additions (control) or with further additions of calcium chloride or Tris or both at the concentrations indicated; incubation temperature 24°C

Time since Beginning of Incubation (hr)	ρ_i (m-equiv. kg^{-1} hr^{-1})				ρ_o (m-equiv. kg^{-1} hr^{-1})			
	Control*	10^{-2}M $CaCl_2$	10^{-2}M $CaCl_2$ + 10^{-2}M Tris.HCl	10^{-2}M Tris.HCl	Control*	10^{-2}M $CaCl_2$	10^{-2}M $CaCl_2$ + 10^{-2}M Tris.HCl	10^{-2}M Tris.HCl
Fresh disks								
0–½	0·79	0·06	0·07	0·80	−2·00	−6·63	−6·15	−3·71
½–2	0·20	0·04	0·04	0·27	−0·60	−0·45	−0·35	−0·09
2–4	0·24	0·09	0·07	0·48	−0·32	−0·09	−0·03	−0·06
4–6	0·22	0·21	0·12	0·91	−0·22	−0·08	−0·03	−0·05
6–16	0·22	0·41	0·17	1·76	−0·25	−0·05	−0·02	+0·05
Disks 18 hr old								
0–½	1·32	1·45	0·68	1·65	−2·17	−0·47	−0·08	−0·07
½–2	0·43	0·98	0·84	1·32	−0·29	−0·35	−0·16	+0·03
2–4	0·35	0·62	0·97	1·35	−0·23	−0·20	−0·13	−0·02
4–6	0·35	0·68	1·20	1·39	−0·27	−0·29	−0·16	−0·13
6–22	0·36	0·99	1·39	1·39	−0·33	−0·26	−0·19	−0·06
Disks 42 hr old								
0–½	1·20	1·44	1·76	1·62	−2·18	−0·35	−1·03	−0·28
½–2	0·45	1·52	1·65	1·40	−0·13	−0·16	−0·65	+0·09
2–4	0·43	1·43	1·65	1·48	−0·08	−0·10	−0·24	−0·11
4–6	0·46	1·32	1·65	1·86	−0·07	−0·10	−0·30	−0·30
6–23	0·47	1·42	1·71	2·01	−0·13	−0·10	−0·15	−0·41

* pH approximately 6.

When both Ca^{2+} and Tris were present ρ_i and ρ_o values followed a pattern similar to that of treatments containing Ca^{2+} only.

Amounts of K^+ associated with immobile charges at the cell surface show that Ca^{2+} treatment considerably reduced the amount of this K^+ fraction (Table 3). These values are based on the specific activities of K^+ present in 10^{-2}M $CaCl_2$ rinses of disks at 0°C. Naturally 10^{-2}M Tris at pH 6 was more effective in replacing K^+ than 10^{-2}M Tris at pH 8 (Tris: $pK_a = 8{\cdot}14$), but not nearly as effective as 10^{-2}M Ca^{2+}.

IV. Discussion

The stimulative effect which Ca^{2+} has on the uptake of monovalent ions is well known (Viets 1944; Jacobson, Moore, and Hannapel 1960; Waisel 1962; and many others). Preliminary experiments (Van Steveninck 1961) showed that Ca^{2+} will cause a shortening of the lag phase in net K^+ absorption which is a common phenomenon in most storage tissues. Although the effect of Ca^{2+} was less immediate than that of Tris, it was shown that the effect of Ca^{2+} prevailed when both agents were present together in the external solution. This seems to indicate that Ca^{2+} and Tris compete for a common site, the bivalent Ca^{2+} being much more efficient in this respect than the univalent Tris ions (cf. Rothstein and Hayes 1956).

Table 3

Amounts of K^+ originating from Donnan free space

Disks of various ages were rinsed in 10^{-2}M $CaCl_2$ at 0°C for 30 min after treatment with calcium chloride and Tris.HCl solutions at the concentrations and pH values indicated. Calculations based on water free space of 10% (Van Steveninck 1964) and on specific activities of external solution and disks before and after the calcium chloride rinse, assuming complete equilibration between the Donnan free space and calcium chloride solution after 30 min (Robertson 1960)

Treatment of Disks (pH = 6)	Age of Disks (hr)	K^+ (m-equiv/kg tissue)	Treatment of Disks (pH = 8)	Age of Disks (hr)	K^+ (m-equiv/kg tissue)
Control*	0	2·63	Control*	0	1·93
	24¼	2·67		18	2·06
	48	2·72		42	2·39
10^{-2}M $CaCl_2$	0	0·10	10^{-2}M $CaCl_2$ +	0	0·14
	24¼	0·19	$Ca(OH)_2$	18	0·33
	48	0·36		42	0·31
10^{-2}M $CaCl_2$ + 10^{-2}M Tris.HCl	0	0·08	10^{-2}M $CaCl_2$ +	0	0·10
	24¼	0·15	10^{-2}M Tris.HCl	18	0·13
	48	0·41		42	0·18
10^{-2}M Tris.HCl	0	0·71	10^{-2}M Tris.HCl	0	1·10
	24¼	0·82		18	1·17
	48	1·07		42	1·43

* pH approximately 6.

The more modern and elaborate flux analysis published by Pitman (1963) (see also Briggs, Hope, and Robertson 1961) was not applied because the rapid change in the physiological properties due to processes associated with the aging of the tissue made it impossible to comply with the first and foremost requirement of Pitman's technique, i.e. the attainment of a steady state of fluxes between the external solution, cytoplasmic phase, and vacuole. The method presented here made no distinction between fluxes across the plasmalemma and tonoplast, and hence the movement of K^+ from the external solution into the cell as a whole and vice versa, measured by means of $^{42}K^+$, was referred to as apparent influx and apparent efflux respectively. It was considered that the more immediate changes in apparent fluxes induced by the experimental treatments might be more informative than the end results when a steady state would have been reached.

Thus it was found that Ca^{2+} caused a marked decrease in the apparent K^+ influx during the initial 6–20 hr, depending on the pH of the external solutions. This is a much larger period than that required for the equilibration of K^+ between the cytoplasmic phase and the external solution (cf. Pitman 1963: $t_{\frac{1}{2}}$ for K^+ equilibration at 25°C = 45 min). The decrease in apparent influx seems logical because Donnan relations imply that in the presence of Ca^{2+} much less K^+ is associated with anionic surface charges (Table 3).

The elevated values of apparent efflux during the first half hour in solution were another indication that a considerable amount of K^+ was being exchanged for Ca^{2+}. Part of this K^+ may originate from the cytoplasmic phase (Pitman, loc. cit). This assumption is further supported by the fact that apparent influx values in control treatments were also elevated during the first half hour. In the presence of Ca^{2+}, however, this K^+ exchange with the cytoplasmic phase was prevented, and together with the fact that Ca^{2+} caused a decrease of apparent efflux values over a considerable period of time, it appeared that the permeability of the plasmalemma for K^+ had decreased as a result of Ca^{2+} treatment.

The marked increase in apparent influx after the disks had aged for 10–20 hr in Ca^{2+} solutions seems difficult to explain. Recently, Higinbotham, Etherton, and Foster (1964) have shown that external Ca^{2+} caused an increase in the transmembrane electropotential of *Avena* coleoptile tissue cells. This marked increase in polarization, however, should cause a much more immediate increase in K^+ transport, unless it is offset by the Ca^{2+}-induced decrease in membrane permeability for K^+ earlier suggested. Actually, the latter might be the cause of the increased polarization.

Pitman (1964) has suggested that the Ca^{2+}-induced increase in net K^+ absorption is mediated through an increased Cl^- absorption. In this case, Ca^{2+} was thought to increase anion permeability of the membrane through the neutralization of its negative charges. Recent work by Van Steveninck (1964) has shown that fresh tissue lacks the capacity for net Cl^- absorption, the apparent Cl^- influx being almost negligible, but rising approximately 10-fold during the aging of the tissue. Although it was shown that net Cl^- absorption could take place in the absence of a suitable cation it did not disprove the contention that net K^+ absorption may be stimulated by a stimulated Cl^- absorption. Pitman's hypothesis therefore seems to be attractive, because it would explain the delayed effectiveness of Ca^{2+} in stimulating Cl^- absorption, and with it K^+ absorption, during the early stages of the lag phase.

Finally one should not overlook an entirely different aspect which could be based on Florell's (1957) work, which showed a strong correlation between the formation of mitochondria and the external Ca^{2+} concentration. Without considering further details of this effect, one would expect that the increase in mitochondria would cause a further stimulation of Cl^- absorption (cf. Robertson *et al.* 1955).

V. Acknowledgments

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VI. References

Briggs, G. E., Hope, A. B., and Robertson, R. N. (1961).—Electrolytes and plant cells. In "Botanical Monographs". (Ed. W. O. James.) (Blackwell Scientific Publications: Oxford.)

Florell, C. (1957).—Calcium, mitochondria and anion uptake. *Physiol. Plant.* **10**: 781–90.

Higinbotham, N., Etherton, B., and Foster, R. J. (1964).—Effect of external K, NH_4, Na, Ca, Mg, and H ions on the cell transmembrane electropotential of *Avena* coleoptile. *Plant Physiol.* **39**: 196–203.

Jacobson, L., Moore, D. P., and Hannapel, R. J. (1960).—Role of calcium in absorption of monovalent cations. *Plant Physiol.* **35**: 352–8.

Pitman, M. G. (1963).—The determination of the salt relations of the cytoplasmic phase in cells of beetroot tissue. *Aust. J. Biol. Sci.* **16**: 647–68.

Pitman, M. G. (1964).—The effect of divalent cations in the uptake of salt by beetroot tissue. *J. Exp. Bot.* **15**: 444–56.

Robertson, R. N. (1960).—Ion transport and respiration. *Biol. Rev.* **35**: 231–64.

Robertson, R. N., Wilkins, M. J., Hope, A. B., and Nesztel, L. (1955).—Studies in the metabolism of plant cells. X. Respiratory activity and ionic relations of plant mitochondria. *Aust. J. Biol. Sci.* **8**: 164–85.

Rothstein, A., and Hayes, A. D. (1956).—The relationship of the cell surface to metabolism. XIII. The cation-binding properties of the yeast cell surface. *Arch. Biochem. Biophys.* **63**: 87–99.

Van Steveninck, R. F. M. (1961).—The lag phase in salt uptake of storage tissue. *Nature* **190**: 1072–5.

Van Steveninck, R. F. M. (1964).—A comparison of chloride and potassium fluxes in red beet tissue. *Physiol. Plant.* **17**: 757–70.

Viets, E. G. (1944).—Calcium and other polyvalent cations as accelerators of ion accumulation by excised barley roots. *Plant Physiol.* **19**: 466–80.

Waisel, J. (1962).—The effect of Ca on the uptake of monovalent ions by excised barley roots. *Physiol. Plant.* **15**: 709–24.