Levels and Sites of Metabolically Active Calcium in Apple Fruit[1]

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Abstract. Detectable calcium accumulation of the tissue started at 10^{-3}M of the bathing solution and increased with increasing concentrations thereafter. Calcium solutions of 10^{-3} to 10^{-1}M were effective in inducing metabolic changes in tissue: they decreased respiration and increased protein synthesis. Much of the accumulated Ca (90%) could be exchanged from the tissue with Mg without changing the metabolic effect of the Ca. Although high concentration of Ca was needed to move metabolically active Ca into the site of its action, most of the Ca accumulated on exchange sites was metabolically inactive.

The role of Ca in protecting apples against metabolic disorders is well accepted. If Ca is present in sufficiently high concn in the fruit, it can decrease the occurrence of bitter pit (4), cork spot (8), internal breakdown (7), Jonathan spot (1), and watercore (2).

In mature apples, Ca decreased the overall rate of respiration, decreased the activity of malic enzyme, and stimulated a higher level of protein synthesis (5). To manifest these metabolic effects, at least 0.7 μmole of Ca was needed per g fresh wt of the fruit (5), c. $4x10^{-4}$M concn. In contrast, much higher concn of Ca were needed to protect the fruit when the fruit was infiltrated ($4x10^{-2}$M) (3) or when the fruit was dipped ($3x10^{-1}$M) (3). Because of this apparent discrepancy in the effective concn, we have investigated the need for Ca at the cellular level for regulating metabolic processes. The results of this investigation are reported here.

Materials and Methods

For all experiments, apples were harvested from a MM 104 tree. This tree was selected because it was low in Ca as evidenced by early and consistent development of cork spot in the fruit. Samples were prepared for each experiment by cutting 1x8 mm disks of flesh tissue with a cork borer and razor blade.

To determine Ca content of the tissue, 3 g of disks were incubated for 2 hours in flasks containing either water, 10^{-1}M, 10^{-2}M, 10^{-3}M, 10^{-4}M, or 10^{-5}M $CaCl_2$. Three 0.5-g samples of tissue were then taken from each flask and ashed. The ash was dissolved in HCl and diluted in $LaCl_3$. Calcium in the resulting solution was determined in a Atomic Absorption Spectrophotometer.

Respiration of the tissue was determined on a Warburg respirometer. Three ml of each solution was placed, unbuffered, in flasks with 0.5-g flesh samples and incubated at 25° for 2 hours. The results were calculated as μl O_2 uptake/g/hr.

Valine and uracil incorporation were determined by placing 0.5 g of disks in flasks containing 10 ml of varying $CaCl_2$ concn, plus 1 μc of either uracil-2-^{14}C (7.2 mc/mmole) or L-valine-1-^{14}C (34.2 mc/mmole).

After incubation for 2 hours, the tissue was ground, extracted with 80% ethanol, and filtered. Both the ethanol-soluble fraction and the ethanol-insoluble residue (EIR) were counted. The percentage of both valine and uracil incorporated into EIR was calculated, based on the total uptake of the respective compound.

To test for exchange of Ca with other divalent ions, disks were incubated for 30 min in a solution of 4 ml 0.1 M $CaCl_2$, to which 2 μc of $^{45}Ca^{++}$ with a specific activity of 14.1 mc/mg had been added. Half of the disks were then counted, and the remainder were rinsed in distilled water. After rinsing, the disks were divided into 3 groups and incubated for 30 min in either $CaCl_2$, $MgCl_2$ or $ZnCl_2$. They were then counted, and the fraction of $^{45}Ca^{++}$ that remained in the disks was determined.

To determine the importance of nonexchangeable Ca in cell metabolism, tissues were incubated in $CaCl_2$ or in $MgCl_2$ for 40 min, or in $CaCl_2$ for 20 min, and then transferred to $MgCl_2$ for 20 min. At the end of the 40-min period, all tissues were transferred to water, the labeled substrates were added, and disks were incubated for 2 hr. All groups were then counted, and the percentage of uptake and incorporation was calculated by the method described earlier.

Results and Discussion

The detectable Ca accumulation of the tissue started at 10^{-3}M of the bathing solution and increased with increasing concn thereafter. Lower concn were ineffective in inducing tissue responses (Fig. 1A). As the Ca concn in the tissue increased, respiration (O_2 uptake) of the tissue decreased (Fig. 1B), and nucleic-acid synthesis (incorporation of uracil) and protein synthesis (incorporation of valine) increased (Fig. 1C).

The increasing Ca concn gradually decreased the uptake of ^{14}C-uracil and ^{14}C-valine (Table 1). The change in uptake from water to 10^{-1}M $CaCl_2$ was 2-2.5 fold. We attribute this change in uptake to the effect of Ca on membrane permeability.

Table 1. Effect of Ca on uptake and incorporation of uracil and valine into apple fruit-tissue slices.

Incubating solution	Uracil			Valine		
	Uptake	Incorporation		Uptake	Incorporation	
	cpm	cpm	%	cpm	cpm	%
10^{-1}M $CaCl_2$	10,213[z]	112	1.1	10,020	2,505	25.0
10^{-2}M $CaCl_2$	11,425	125	1.1	11,241	1,034	9.2
10^{-3}M $CaCl_2$	16,910	101	0.6	12,930	620	4.8
10^{-4}M $CaCl_2$	20,977	63	0.3	12,843	321	2.5
10^{-5}M $CaCl_2$	24,869	99	0.4	18,910	227	1.2
H_2O	27,384	109	0.4	19,090	286	1.5

[z]Means were determined from triplicate experiments.

Uracil incorporation varied inversely with uracil uptake, so that total incorporation was the same at all concn of $CaCl_2$. Therefore nucleic acid synthesis itself was not affected by increased Ca in the tissue (Table 1). Although valine incorporation also varied inversely with valine uptake, total incorporation increased with increasing concn of $CaCl_2$. Therefore the effect of Ca on protein synthesis appears to be real.

A large fraction of Ca could be exchanged with any divalent

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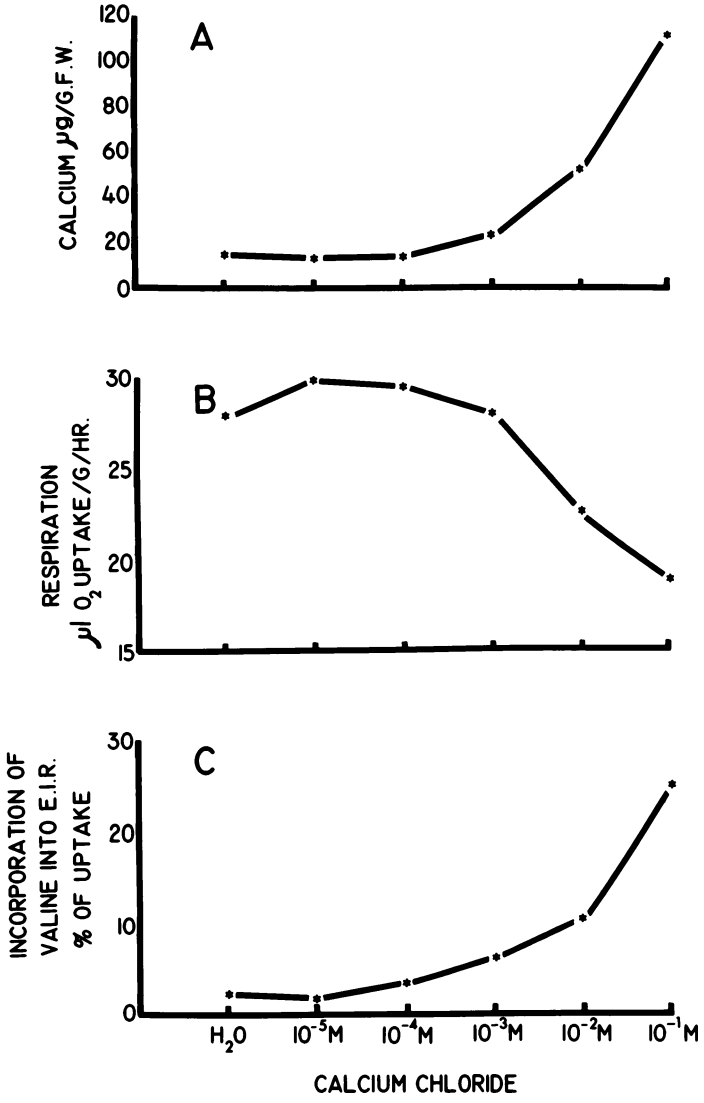

Fig. 1. Effect of Ca concn of the incubating solutions on Ca content (A), respiration (B), and protein synthesis (C) of apple fruit tissue slices.

cation such as Mg or Zn. About 10% of the Ca remained in the tissue after exchange (Table 2). This small amount of nonexchangeable Ca was biologically active, as measured by respiration or by ^{14}C-valine uptake (Table 3). This also indicates that most of the Ca does not enter into metabolic pools, probably being in an exchangeable position where it has little biological effect.

Previously, we have shown that 2 transport systems are operational for Ca in apple trees: transport through the phloem and through the xylem (6). We have also presented indirect evidence that the fruit receives its Ca through the phloem (6). We have not yet examined Ca movement within the fruit.

Calcium moves by exchange in the xylem of the tree, and lignin is the most likely exchange site (9). Xylem transport is also responsive to concn gradients (6). Data presented in this paper are consistent with xylem transport. It appears, therefore, that Ca is transported into the fruit through the phloem, but once within the fruit, it moves as if it were transported through the xylem. This finding explains the apparent discrepancy in the Ca concn discussed in the introduction. Only a small quantity of the Ca is needed for essential metabolic functions, but a much higher concn is required to move this essential active fraction to the site of its action. The lower concn of Ca in the bathing solutions were not high enough to move sufficient Ca into the tissue to manifest biological activity. Once a sufficient amount of Ca was in place, however, the Ca tied up on the exchange sites could be exchanged without reducing metabolic activities dependent on Ca.

The finding that Ca is transported within the fruit as it was transported through the xylem also explains the necessity of using 4-6% $CaCl_2$ (3-5 x 10^{-1}M) as a dip for postharvest application to avoid storage disorders (3). The realization that xylem transport of Ca operates within the fruit also applies to Ca transport through the skin of the apple. Those who in the past experienced difficulties getting Ca penetration into the apple from sprays did not consider xylem transport. In our view it is important to consider concn gradients and ion-exchange transport in attempts to get Ca into the fruit, whether Ca is applied as a postharvest dip or as a spray.

Table 2. Exchangeability of Ca in apple fruit-tissue slices.

	Total uptake	Remaining fraction	Remainder
	cpm	cpm	%
^{45}Ca exchanged with ^{40}Ca	3,286[z]	289	8.7
^{45}Ca exchanged with Mg	1,983	193	9.7
^{45}Ca exchanged with Zn	1,837	159	8.6

[z]Means were determined from 15 replications.

Table 3. Importance of nonexchangeable Ca in uracil and valine incorporation into ethanol-insoluble residue and in respiration.

	Incorporation as % of uptake		Respiration
Incubating solution	Valine	Uracil	µl O_2 uptake/g/hr
0.1 M $CaCl_2$	14.1[z]	0.83	18.0
0.1 M $CaCl_2$ transferred to 0.1 M[2] $MgCl_2$ and to water	10.7	0.56	17.6
H_2O	1.0	0.40	29.0
$MgCl_2$ concn	0.8	0.57	28.0
$ZnCl_2$ concn	0.8	0.37	28.6

[z]Means were determined from triplicate experiments.

Literature Cited

1. Bangerth, F. 1970. Die wirkung einer Immersion reifer Apfelfruchte in Ca-Losungen auf das Auftreten verschidener physiologischer Fruchtkrankungen. (Stippigkeit. Cox-Flecken, Jonathan spot). *Angew. Bot.* 44:197-207.
2. _____, and D. R. Dilley. 1971. Relationship of calcium and sorbitol to apple storage disorders. *HortScience* 6:280 (Abst.).
3. _____, _____, and D. H. Dewey. 1972. Effect of postharvest calcium treatments on internal breakdown and respiration of apple fruits. *J. Amer. Soc. Hort. Sci.* 97:679-682.
4. Faust, M., and C. B. Shear. 1968. Corking disorders of apples. A physiological and biochemical review. *Bot. Rev.* 34:441-469.
5. _____, and _____. 1972. The effect of Ca on respiration of apples. *J. Amer. Soc. Hort. Sci.* 97:437-439.
6. _____, and _____. 1973. Calcium translocation patterns in apples. Proc. 3rd symposium on accumulation and translocation of nutrients and regulators in plant organisms. Warsawa. (in press).
7. Perring, M. A. 1968. Mineral composition of apples. VII. The relationship between fruit composition and some storage disorders. *J. Sci. Food Agr.* 19:186-192.
8. Shear, C. B. 1972. Incidence of cork spot as related to calcium in the leaves and the fruit of York Imperial apples. *J. Amer. Soc. Hort. Sci.* 97:61-64.
9. _____, and M. Faust. 1970. Ca transport in apple trees. *Plant Physiol.* 45:670-674.