CHANGES IN THE ENDOPLASMIC RETICULUM OF BEETROOT SLICES DURING AGING

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Summary

Ultrastructural changes occurring in beetroot parenchyma were studied from the time of cutting into disks and throughout the following 192 hr of aerated washing. The most marked change was the reduction of the endoplasmic reticulum to small cytoplasmic vesicles immediately after cutting (when leakage of ions is greatest), followed by a reorganization into lamellae (coinciding with the onset of net ion accumulation) and subsequent extension of the lamellar system. The possible relationships between these observations and others on plant cells are discussed.

I. INTRODUCTION

Freshly cut slices of beetroot, like most other storage tissues, lack the ability to accumulate salts from dilute solutions before the completion of an initial period of aerobic washing (Stiles and Dent 1946). Thereafter, a marked increase in the capacity to both absorb and retain salts occurs. It is generally assumed that this sequence of events is related ultimately to the coincident changes in respiratory metabolism, and more particularly in oxidative phosphorylation on which many cellular activities depend directly or indirectly (Robertson 1960).

The ultrastructure of parenchyma cells of beetroot slices during aging was investigated to detect possible changes in number, form, or distribution of organelles which might be related to the progressive increase in salt uptake capacity.

II. MATERIALS AND METHODS

(*a*) *Ion Uptake*

From cylinders of beetroot (*Beta vulgaris* L.) 15 mm in diameter, disks 1 mm thick (average fresh weight 190 mg) were cut, and rinsed for 2 hr in aerated distilled water with three changes. Batches of 50 disks (three replicates) were placed in 250 ml distilled water and aerated at 24°C. Samples of the solutions were taken at intervals during the following 192 hr, and K^+ and Na^+ concentrations determined using an EEL flame-photometer.

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(*b*) *Electron Microscopy*

Samples of two disks were withdrawn after the preliminary washing period, after a further 2 hr aerated washing, and after 26, 48, 96, and 192 hr. Another sample was taken from an intact beetroot and fixed immediately. In the development of the fleshy hypocotyl root, additional cambia arise outside the normal primary and secondary vascular tissues, and these produce several concentric rings of parenchymatous xylem and phloem separated by bands of large thin-walled parenchyma (Esau 1953). In this work, care was taken to avoid, as far as possible, the tissue containing vascular elements. Strips of parenchyma 2–3 mm wide were cut from the disks, quickly cut into three strips, chopped into blocks less than 1 mm^3, and dropped into cold fixative. As difficulties were expected in achieving satisfactory fixation of these large vacuolate cells, the following fixatives were tried in preliminary tests:

(1) 2% $KMnO_4$ in veronal acetate buffer, pH 7·3, for 45 min at 0–2°C.

(2) 2% $NaMnO_4$ in veronal acetate buffer, pH 7·2, for 45 min at 0–2°C.

(3) 2% $Ca(MnO_4)_2$ in veronal acetate buffer (Afzelius 1962) for 45 min at 0–2°C.

(4) 1% OsO_4 in veronal acetate buffer and 0·4M sucrose, pH 7·3 (Caulfield 1957, modified), for 2 hr at 0–2°C.

(5) 1% OsO_4 and 1·25% $K_2Cr_2O_7$ in 0·25M sucrose, pH 7·0, adjusted with KOH (Kollman 1960) for 2 hr at 0–2°C.

(6) 4% formaldehyde in sodium phosphate buffer and 0·4M sucrose, pH 7·3, for 2 hr at 0–4°C followed by 1% OsO_4 in veronal acetate and 0·4M sucrose, pH 7·2, at 4°C (Holt and Hicks 1961).

(7) 5% glutaraldehyde in veronal acetate buffer and 0·4M sucrose, pH 7·2, for 30 min at 4°C followed by 1% OsO_4 in veronal acetate for 30 min at 4°C.

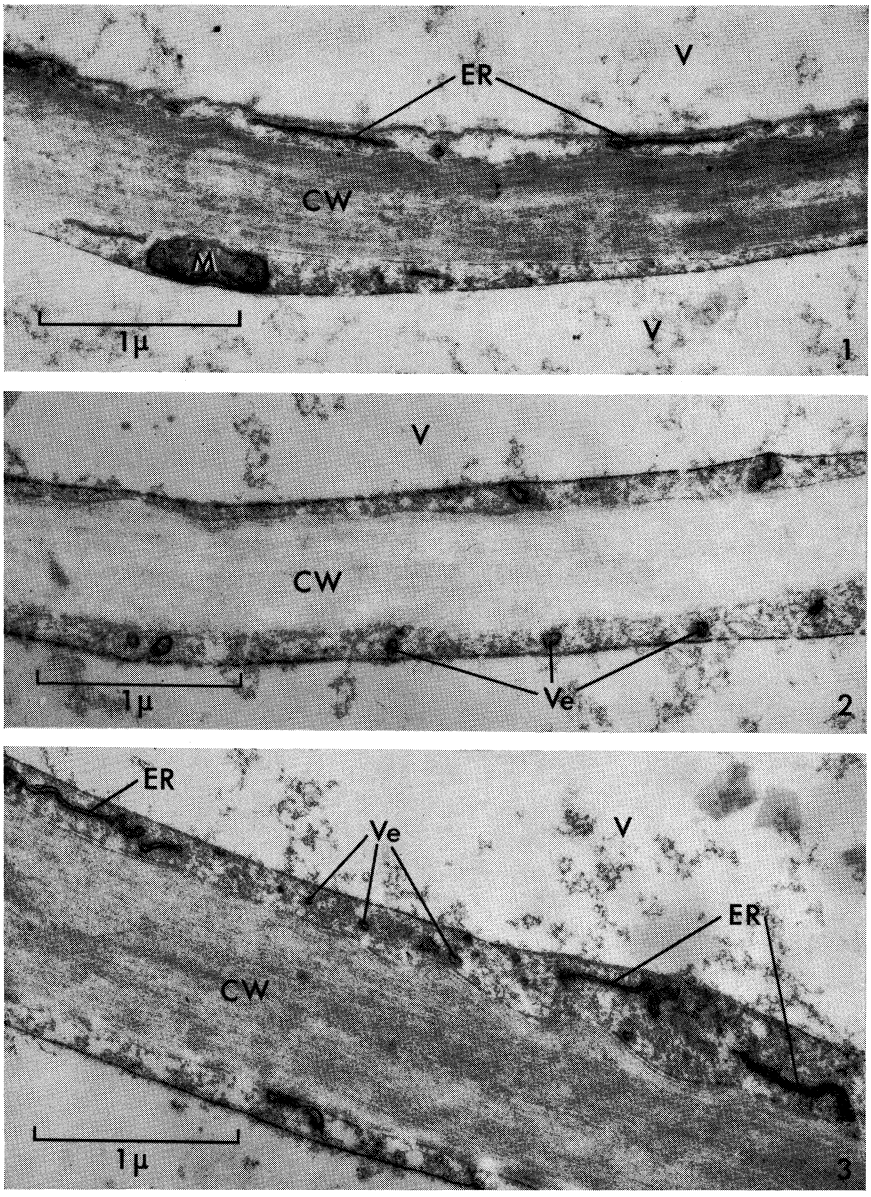
(8) 5% glutaraldehyde in sodium phosphate buffer and 0·4M sucrose, pH 7·2, for 30 min at 4°C followed by 1% OsO_4 in veronal acetate for 30 min at 4°C (Sabatini, Bensch, and Barrnett 1963).

In comparison with the other fixatives, permanganate fixation resulted in a more even precipitation of the cytoplasm, more complete preservation of the plasma membrane and tonoplast, and, in general, a smoother appearance of cell membranes. There was no appreciable difference in fixation with either potassium, sodium, or calcium permanganate. Hence, tissue blocks were fixed in 2% $KMnO_4$ buffered with veronal acetate for 45 min at 0–2°C, and, as a check, in 1% OsO_4 in veronal acetate with 0·4M sucrose for 2 hr at 0–2°C. After fixation, the blocks were rinsed twice with distilled water, dehydrated in acetone (5 min in 25% and 50%, 20 min or overnight in 75%, then 30 min in 100% with three changes), and embedded in Araldite. Thin sections cut with a Si-Ro-Flex ultramicrotome were stained in lead citrate (Reynolds 1963) for 20 min if permanganate-fixed, or in saturated 96% ethanolic uranyl acetate for 1 hr if fixed in OsO_4, and examined in a Siemens Elmiskop I electron microscope.

III. Results

The pattern of net Na^+ and K^+ movement between beetroot slices and the external solution is shown in Figure 1. Rapid leakage of Na^+ and K^+ from the tissue

CHANGES IN ENDOPLASMIC RETICULUM OF BEETROOT SLICES



All figures in Plates 1 and 2 are electron micrographs of beetroot tissue which was fixed in buffered 2% $KMnO_4$, and embedded in Araldite. Sections were stained with lead citrate. Abbreviations used on the plates are as follows: *CW*, cell wall; *D*, dictyosome; *ER*, endoplasmic reticulum; *M*, mitochondria; *V*, vacuole; *Ve*, vesicle.

Fig. 1.—Parenchyma cells from intact beetroot cut at the time of fixation. The amount of endoplasmic reticulum (*ER*) usually observed in whole tissue is shown.

Fig. 2.—Cells from beetroot disks washed for 2 hr prior to fixation. The lamellar endoplasmic reticulum (*ER*) has almost disappeared, but vesicles (*Ve*) are present in the cytoplasm.

Fig. 3.—Cells from beetroot disks washed for 26 hr prior to fixation. After 26 hr the lamellar endoplasmic reticulum (*ER*) has partially reappeared, while some vesicles (*Ve*) persist.

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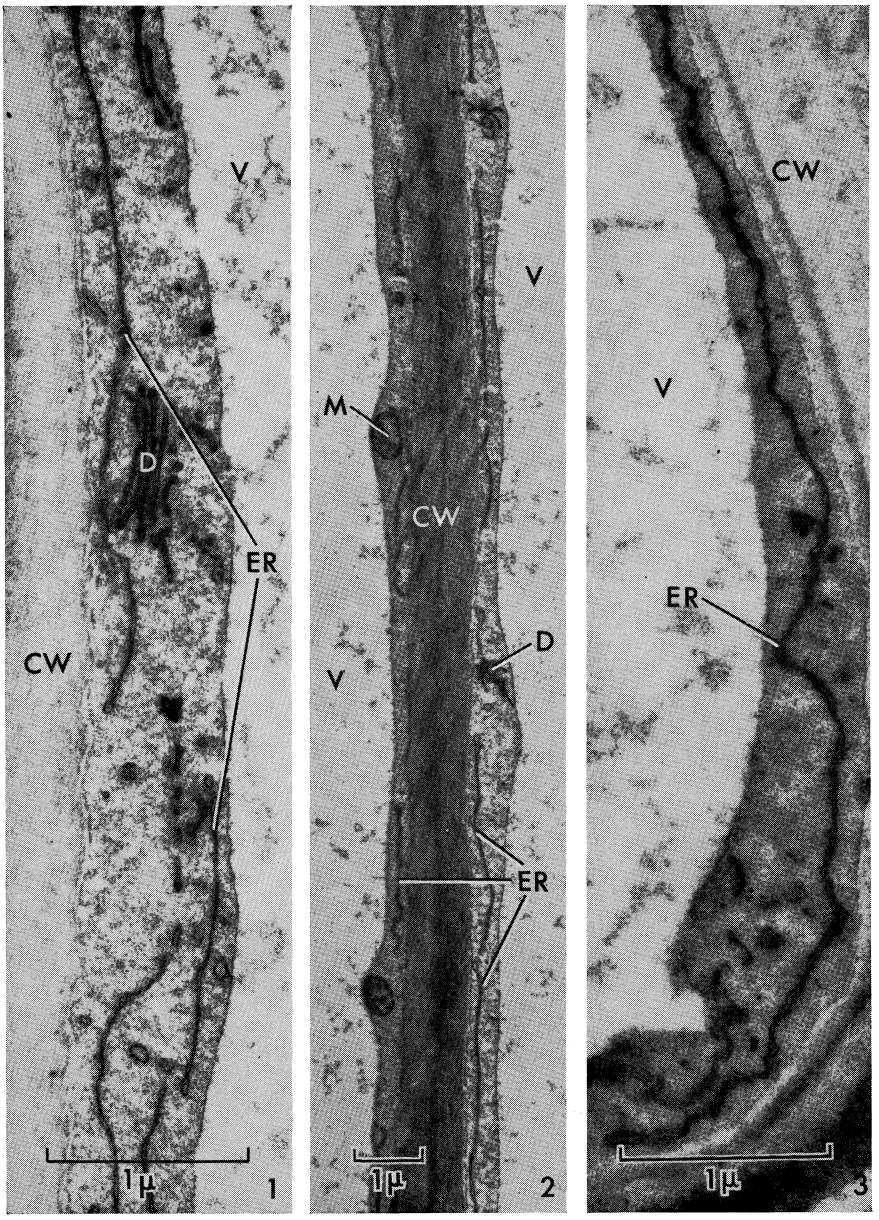

Figs. 1–3.—Cells from beetroot disks washed for 48, 96, and 192 hr respectively prior to fixation. The lamellae of the endoplasmic reticulum (*ER*) have increased in length by 48 hr and are generally longer than those found in the intact beetroot. After 48 hr the lamellae show further extension, and now form an almost continuous layer within the cytoplasm, which persists until at least 192 hr.

was followed by a phase of rapid net accumulation of Na^+ at 20 hr, while a phase of slow net accumulation of K^+ at 10 hr was followed by a rapid phase of uptake at about 50 hr. This pattern of ion movement is typical for beetroot slices (Van Steveninck 1961).

The most marked ultrastructural change in the parenchyma cells during this period of aerated washing was shown by the endoplasmic reticulum, readily observed in $KMnO_4$-fixed tissue, but also evident when fixed with OsO_4. The lamellar endoplasmic reticulum present in whole tissue (Plate 1, Fig. 1) was reduced to a number of small cytoplasmic vesicles during the cutting and preliminary washing period (Plate 1, Fig. 2). On further washing, the lamellae were reconstituted to the original extent by 26 hr (Plate 1, Fig. 3) and thereafter continued to increase in amount until 96 hr (Plate 2, Figs. 1 and 2). By this time an almost complete cell lining of endoplasmic reticulum was present, which persisted until at least 192 hr (Plate 2, Fig. 3). These changes in the extent of endoplasmic reticulum during the process of aging of beetroot disks were confirmed in several separate experiments.

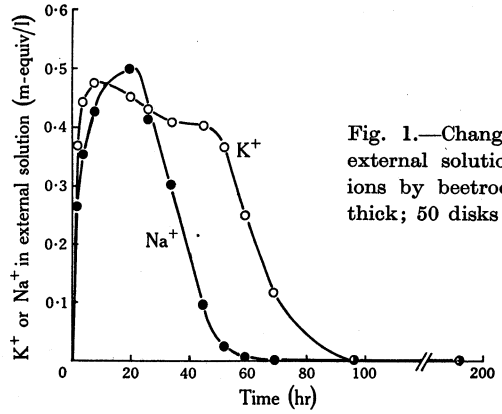

Fig. 1.—Changes in Na^+ and K^+ content of external solution due to release or uptake of ions by beetroot disks (15 mm diam.; 1 mm thick; 50 disks per 250 ml water; 24°C).

After 96 hr, and rarely after 48 hr, occasional crystal-shaped bodies were observed inside the endoplasmic reticulum. Further work on the nature of these crystals is in progress.

It should be noted that the actual slicing and washing of the tissue might alter the cellular response to fixation. The apparent absence of the lamellar endoplasmic reticulum in freshly sliced tissue then could be regarded as a secondary effect due to a lack of preservation of detail at the moment of fixation. However, no significant changes in the appearance of other organelles were found, indicating that the various treatments had not changed their response to fixation.

Observations of changes in number or nature of other cell organelles or membranes during aging were inconclusive. It is possible that some changes in size and shape of mitochondria occurred, since large irregularly shaped mitochondria were found only in the whole tissue, whereas mitochondria in parenchyma of aged disks were small and circular in section, or almost so.

IV. Discussion

It has been shown (Van Steveninck 1962) that the net accumulation of K^+ by beetroot at the completion of the lag phase is due to a decrease in apparent efflux rather than an increased influx. This observation suggests that some barrier to leakage develops during the lag phase. The highest rate of efflux, measured within 4 hr after slicing the tissue, coincides with the observed reduction of endoplasmic reticulum to small cytoplasmic vesicles, but the endoplasmic reticulum forms an almost continuous lamellar system when the efflux is greatly reduced after 48 hr. It seems likely that this extensive endoplasmic reticulum in aged beetroot parenchyma would act as a barrier to ion movement in addition to the selective resistances of the tonoplast and plasma membrane.

Numerous authors have reported marked changes in the amount of endoplasmic reticulum in plant cells. These changes can be classified into two groups:

(1) Changes caused by external factors, e.g. wilting (Schnepf 1961), mechanical injury (Mollenhauer, Whaley, and Leech 1960), increased pressure, oxygen deficiency, cyanide, high carbon dioxide or carbon monoxide tension, colchicine, and high intensity radiation (for references see Whaley, Kephart, and Mollenhauer 1964). Such treatments usually induce an almost instantaneous build-up of lamellar and often coiled reticulum. Rapid, and in some cases reversible, changes of this kind suggest the transformation of molecular or larger units already present in the cytoplasm into an organized lamellar system. This concept has been elaborated by Green and Perdue (1966) on the basis of the spontaneous reconstitution of dispersed membrane units observed by Razin, Morowitz, and Terry (1965) and Brown (1965).

In contrast to the apparent increase in the amount of endoplasmic reticulum caused by mechanical injury, increased pressure, or wilting, the cutting and initial washing of beetroot disks produced a breakdown of lamellae into small vesicles. It is possible that the elements of the lamellar membrane were still present in the cytoplasm, but the reconstitution was not instantaneous, and required several hours.

(2) Changes occurring during normal development and aging (Whaley, Mollenhauer, and Kephart 1962; Juniper 1964; Bouck and Cronshaw 1965; Bain and Mercer 1966*a*, 1966*b*). Some of these have been associated with a specific functional state of the cells, e.g. the increases observed in maize root cells at the beginning of vacuolation (Whaley, Mollenhauer, and Kephart 1962), and in pea cotyledons during germination when the tissue is temporarily accumulating soluble carbohydrate (Bain and Mercer 1966*b*). The reappearance and development of an extensive lamellar endoplasmic reticulum during aging of beetroot may be similar in nature.

Differentiation and aging may also be accompanied by morphological modifications of the endoplasmic reticulum, usually from flat cisternae or tubules to discrete vesicles, e.g. in embryonic leaf primordia of maturing seeds (Nougarede 1963), maturing and aging cotyledons (Bain and Mercer 1966*a*, 1966*b*), differentiating sieve elements (Kollmann and Schumacher 1962), and tracheids (Esau, Cheadle, and Risley 1963), root calyptra cells (Hršel 1966), and senescing leaves (Shaw and

Manocha 1965). Morphological changes of this sort are sometimes considered to be an indication of degeneration of the protoplast (Esau 1963). The vesicles apparently arising from the endoplasmic reticulum during cutting and preliminary washing of the beetroot tissue may be equivalent to spherosomes. Spherosomes are considered to originate from the ends of the endoplasmic reticulum in certain tissues (Frey-Wyssling, Grieshaber, and Mühlethaler 1963) and, when isolated, have been shown to contain hydrolases, which are thought to be responsible for the breakdown of cytoplasm in mature detached tobacco leaves (Balz 1966). Furthermore, the production of hydrolytic enzymes has been ascribed to the endoplasmic reticulum in other plant tissues (Schnepf 1963; Öpik 1966).

V. References

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