EFFECT OF MOISTURE STRESS ON SUBMICROSCOPIC STRUCTURE OF MAIZE ROOTS

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Summary

The effect of dehydration on root behaviour and its submicroscopic structure was studied. Root segments were dehydrated above sodium chloride solutions of various concentrations and the degree of dehydration was expressed as percentage loss of weight. Loss of more than 70% of initial weight proved lethal. On loss of 60-70% some of the roots preserved their ability to resume growth on rehydration. Cutting off the root tips induced structural changes at the submicroscopic level, the main change being disruption of polysomes into monosomes.

Dehydration caused changes in the fine structure of mitochondria and plastids, damage to plasma membranes, and rearrangement of chromatin in the nucleus. Severe dehydration induced parallel arrangement of long reticular elements. The extent of the various changes was proportional to the degree of dehydration.

On rehydration in water, besides restoration of the structure of various organelles to normal, formation of big extraplasmatic water "vacuoles" was observed. Such water vacuoles were also found in normal root tips that absorbed water in excess of their initial fresh weight. On rehydration in a nutrient medium restoration of the normal submicroscopic structure occurred, except for formation of polysomes.

Restoration of structure, after rehydration, apparently is not always accompanied by restoration of function, as shown by the rate of growth of the rehydrated segments. Rehydration of tissue previously dehydrated to a lethal state did not result in restoration of normal structure. On the contrary, the internal structure observed in the dehydrated tissue was completely disrupted, and only the big chromatin masses could be identified with certainty.

Fixation of dehydrated tissue with osmic acid vapour or with aqueous glutaraldehyde gave very similar results in spite of the partial rehydration occurring in aqueous fixatives during fixation.

I. INTRODUCTION

It is a well known fact that water stress induces retardation of growth and in severe cases causes death of plants. The importance of water in the life of plants is reflected in the voluminous literature on water balance. Various degrees of water stress interfere with the normal metabolic activity of various plant tissues (Todd and Basler 1965; Nir and Poljakoff-Mayber 1966, 1967). Some correlation was found between the disturbances in photochemical activity of chloroplasts induced by drought and their submicroscopic structure (Nir 1965). However, in spite of the fact that the usual hypotheses explaining drought resistance are based on cytoplasmic changes, the effect of water stress on submicroscopic structure has been investigated only to a limited extent (Schnepf 1961).

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In the work discussed here, cell fine structure was investigated in roots which were dehydrated to various degrees under strictly controlled conditions and then rehydrated. This made it possible to reach conclusions on changes occurring in the dehydrated roots, and on the reversibility of these changes after rehydration as indicated by the ability of the roots to resume growth.

II. MATERIALS AND METHODS

Seeds of maize (Zea mays), hybrid 22 Nveh-Yaar, were germinated in the dark on moist filter paper at 25°C for 72 hr. Root tips 4 mm long, cut off the main rootlet, were used in all the experiments.

The root segments were dehydrated in flasks as used for vapour equilibration in measurements of water potential of plant tissues (Slatyer and McIlroy 1961). Small glass jars (diameter 45 mm, height 75 mm) were filled with sodium chloride solutions of various concentrations so that there was a 15-mm air space between the solution and the cork. Twenty root tips were placed on a stainless steel net supported on a small tripod above the solution. The jars, closed with rubber stoppers having a glass tube for release of excess pressure, were shaken in a thermostatically controlled water-bath (25±0.01°C) for approximately 16 hr. Full equilibration was not achieved, and the degree of dehydration was evaluated by weighing the tissue, the loss of weight being expressed as percentage of initial weight.

Rehydration was carried out at 24°C. The segments were placed in Petri dishes on filter paper moistened with either distilled water or Torrey's nutrient solution (Torrey 1954). The degree of rehydration was also assessed by measuring change in weight. After rehydration, the roots were allowed to grow on filter paper moistened with Torrey's medium.

For electron microscopy root tips were fixed by one of two methods:

1. Fixation in 5% glutaraldehyde in phosphate buffer (0.1M, pH 7.5) for 60 min at 0-4°C (Sabatini, Bensch, and Barnett 1963), after which the tissue was rinsed in phosphate buffer, post-fixed in 2% osmium tetroxide dissolved in the same buffer, dehydrated in a series of graded alcohols, and embedded in an Epon mixture as described by Luft (1961). This method was used for Figures 3–13 and 15–24.

2. Fixation with osmic acid vapour. Solid osmium tetroxide was introduced into the vapour equilibration flask following dehydration of the tissue, and fixation continued for 30 min under the same conditions as the dehydration (in the 25°C water-bath). The tissue was then transferred into 50% alcohol, dehydrated, and embedded as described above. This method was used for Figure 14.

Sections were cut with a Porter–Blum Ultramicrotome, afterstained with lead citrate (Venable and Coggeshall 1965), and examined in an RCA EMU-3C electron microscope.

III. RESULTS

After dehydration above sodium chloride solutions, some of the roots were processed for electron-microscopic investigations, while others were rehydrated on wet filter paper and then allowed to grow in nutrient media. The ability of the roots to resume growth after rehydration served as evidence that the dehydration damage was reversible. The results of a typical experiment are summarized in Table 1.

Roots kept above distilled water lost 10% of their initial weight; however, when returned to water they absorbed freely and already after 45 min of rehydration weighed more than before dehydration. There was no noticeable increase in length after rehydration for 3 hr, but when placed in a nutrient solution the length of these root segments increased threefold in less than 3 days.

Root segments which on dehydration lost 67% of their fresh weight, halved this deficit during the first hour of rehydration. After 3 hr of rehydration their weight
was higher than the initial value. Their subsequent growth, however, was severely impeded.

Root segments which lost 76\% of their weight, although able to reabsorb all the water they had lost, could not resume growth. In other experiments similar results were found: loss of 74\% and above of the fresh weight was lethal to the roots. After 24 hr such roots appeared brown.

**Table 1**

DEHYDRATION AND REHYDRATION OF ROOT SEGMENTS

Twenty root segments, 4 mm long, were kept above NaCl solutions for 17 hr, transferred to water for 3 hr, and then to a nutrient medium. The root segments were weighed before and after dehydration and again after rehydration. The length of the segments was measured after incubation for 54 hr in the nutrient medium.

<table>
<thead>
<tr>
<th>Concen. of NaCl Solution (atm)</th>
<th>Loss of Weight (%)*</th>
<th>Difference from Initial Weight (%) after a Rehydration Period (min) of:</th>
<th>Mean Final Segment Length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>45 90 180</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>-10</td>
<td>+17 +27 +27</td>
<td>13.3</td>
</tr>
<tr>
<td>45</td>
<td>-67</td>
<td>-30 -5 +14</td>
<td>6.2</td>
</tr>
<tr>
<td>90</td>
<td>-76</td>
<td>-53 -23 -3</td>
<td>4.0</td>
</tr>
</tbody>
</table>

* As percentage of initial weight.

On loss of 60-70\% of the initial fresh weight some of the roots turned brown and were apparently dead, while others remained whitish and could resume growth. This degree of dehydration was therefore defined as sublethal.

**(a) Submicroscopic Cell Structure in Dehydrated Roots**

Changes in the submicroscopic cell fine structure were investigated in roots after non-lethal, sublethal, and lethal dehydration (loss of weight of less than 60\%, 60-70\%, and more than 70\% respectively).

The observations were made mainly on cells 600-800 \( \mu \) from the tip (Fig. 1). This region is usually defined as a region of differentiation. A cross section made through the root, 740 \( \mu \) from the tip, is shown in Figure 2. The cells in the stele are small and isodiametric, almost without intercellular spaces. Several metaxylem cells with transparent protoplasm, still containing nuclei, can be distinguished. The cells of the cortex are bigger and there are well-defined intercellular spaces.

Typical appearance of the organelles in the cortical and stellar cells of turgid roots is shown in Figures 3 and 4.*

* The following abbreviations are used on Figures 3-24: CH, chromatin; ER, endoplasmic reticulum; G, golgi body; IG, interchromatic granule; L, lipid droplet; M, mitochondria; N, nucleus; NL, nucleolus; NM, nuclear membrane; P, plastid; PM, plasmalemma; R, ribosomes; S, starch; V, vacuole; W, cell wall.
After fixation with glutaraldehyde and post fixation with osmic acid (method 1, Section II), the ground substance of root cells appears homogeneous. The rather numerous ribosomes (Figs. 3 and 4) are usually arranged in groups (polysomes), only some of them being attached to membranes. Elements of the endoplasmic reticulum (Figs. 3 and 4) are distributed randomly in the cytoplasm. The mitochondria usually appear elongated or ellipsoid (Figs. 3 and 4), and contain numerous cristae. Their matrix is usually more electron-dense than the ground substance of the cell, but electron-transparent spaces can be observed in most of them. Golgi elements are more numerous in epidermal cells and in the stele than in the cortex. The plastids (Figs. 3 and 4) contain only a small number of thylakoids. Their membranes, on the whole, are more electron-dense than any other membranes in the cell. Plastids containing starch grains were observed only in cortex and metaxylem cells in some of the roots. Even roots grown under exactly the same conditions differed in their starch content. When starch grains were present, their size increased with increasing distance from the root tip.

Nuclei comprise almost three-quarters of the volume of the meristematic cells and contain randomly distributed chromatin areas in the form of electron-dense flocules. The nucleoli are very electron-dense and appear granular (Fig. 4). The caryoplasm also contains granules of varying size and density. The double nuclear membrane can easily be observed (Fig. 4). Small electron-transparent vacuoles surrounded by a single membrane are usually present in the cells (Fig. 3).

Distinct changes in the submicroscopic structure could be observed following lethal dehydration, when the roots had lost 75% of their initial weight. In the cells of such root segments, folding of the cell wall occurs (Figs. 5 and 7), apparently due to mechanical stress resulting from water loss. The plasmalemma was frequently separated from the cell wall (Figs. 6 and 8). Ribosomes were still very numerous but appeared as single units, not arranged into polysomes. Their distribution in the cell was no longer uniform and they were absent from several cell areas, especially near the walls (Fig. 5). In the nuclei the chromatin aggregated into large masses around the nucleolus (Fig. 7). Between these aggregates groups of small granules were observed. (In the normal nuclei these granules were more or less evenly distributed in the caryoplasm.) Frequently the nuclear membrane is very fragmented. The plastids become rounded and most of their internal membranes have disappeared. Thin filaments can sometimes be observed (Fig. 9) in the less dense areas of the stroma. The mitochondria, too, have become globular and their volume reduced. Very few cristae, arranged on the periphery of the mitochondrion, are apparent. Thin filaments can be observed in the transparent areas of the matrix (Figs. 6 and 10). Fewer golgi bodies can be observed, and those which are found appear less compact, with swollen cisternae and a large number of vesicles (Figs. 5 and 6). In cells located 600 μ from...
Figs. 1 and 2.—Light microscope photographs of cells from control maize root tips. 1. Longitudinal section. The segment taken for electron microscopy is marked by two lines and is 0·6-0·8 mm from the tip. E, epidermis; PC, procambium; C, cortex. 2. Cross-section through maize root, 0·74 mm from the tip. X, metaxylem cells; other symbols as in Figure 1. × 55. 2. Cross-section through maize root, 0·74 mm from the tip. X, metaxylem cells; other symbols as in Figure 1. × 106.
Figs. 6–10.—Electron micrographs of roots dehydrated to loss of 74.5% of initial weight. 6, Part of a cortex cell, 0.6 mm from the tip. Arrow shows retreat of cytoplasm from cell wall. ×25,600. 7, Cell from the stele, 0.6 mm from the root tip. Arrow shows accumulation of granules
the tip, the elements of the endoplasmic reticulum are more scarce than in turgid roots (Fig. 6). In cells 800 μ from the tip, however, large numbers of units arranged in parallel were observed (Fig. 9). The plasmalemma is very fragmented and much more electron-dense than in the turgid control roots (Fig. 8). In metaxylem and the cells surrounding them large lipid droplets are found (Fig. 8 and insert in Fig. 10) grouped along the cell walls. Such lipid droplets occur also, but less frequently, in the cortical cells. More and larger vacuoles were found in the dehydrated roots than in the controls. Often these vacuoles contain “membrane knots” similar to myelin bodies (Fig. 6).

The submicroscopic structure of roots after sublethal dehydration was similar to that described above for lethal dehydration, except for certain differences in the plastid structure and in the arrangement of the endoplasmic reticulum. In the plastids long lamellae are found which are usually arranged on the periphery of the organelle (Fig. 11); they seem to have disappeared after the more severe dehydration (Fig. 9). Long units of the endoplasmic reticulum arranged in parallel are also apparent after sublethal dehydration. In addition, units studded with ribosomes were observed around vacuoles. These were found also in cases of less severe dehydration (Fig. 12).

Observations on the submicroscopic structure of cells in roots after less severe dehydration were made at two levels of water loss, 54 and 35% of initial fresh weight. After loss of 54% of initial weight, similar effects to those of sublethal dehydration were found (Fig. 12). The difference is mainly in the arrangement of the endoplasmic reticulum: the stacks of parallel reticular elements were not found, and only the elements surrounding the vacuoles could be observed.

Less pronounced changes occurred when roots lost only 35% of their fresh weight (Fig. 13). The aggregation of chromatin in the nucleus into large masses was still very apparent, as was accumulation of small granules between the chromatin masses. Mitochondria showed a tendency to become spherical and the number of cristae had diminished considerably. The plastids usually appeared the same as in normal turgid roots, but in some of them rounding and rearrangement of lamellae was apparent. The endoplasmic reticulum consists of short swollen profiles. The plasmalemma was wavy and fragmented and sometimes even formed myelin bodies. Lipid droplets began to appear; golgi bodies appeared normal.

Since in all these experiments dehydration was carried out on root segments, the structural changes could be ascribed either to their detachment from the main
root system or to dehydration or to both. An attempt was made to investigate these possibilities. Root tips 4 mm long were kept for 17 hr in a saturated atmosphere, and their submicroscopic cell structure was studied. The only evident changes observed in such roots were polysome disintegration into monosomes, and reduction of the number of the reticular and golgi elements. A very slight tendency for aggregation of chromatin was found.

If, instead of being equilibrated above distilled water, the root segments were incubated for 17 hr in the nutrient medium, the only change observed was disintegration of polysomes into monosomes. Only this change can thus be considered as due to the detachment of the root tips from the plant. The reduction in amount of endoplasmic reticulum and number of golgi bodies seems to be a result of starvation, as this did not occur if the root segments were kept in the nutrient medium. All the other changes described above appear to be true drought-induced phenomena.

In all the experiments described above, fixation was carried out in an aqueous medium, therefore rehydration of the dehydrated tissue may have occurred during fixation. Weighing the tissue before and after fixation showed that this was indeed the case; root segments which had lost 56.5% of their weight by dehydration weighed, after fixation, only 33% less than their initial weight. The partial rehydration might have induced changes in the submicroscopic cell structure. To avoid this, root segments were fixed with osmic acid vapour, as described in method 2 (Section II). No apparent change in weight occurred during this fixation process. In control experiments of roots fixed by osmic acid vapour after equilibration for 17 hr above distilled water (15% loss of weight), the structure appeared in general to be the same as after glutaraldehyde fixation.

Fixation with osmic acid vapour of more severely dehydrated roots (loss of 42% of fresh weight) showed several structural features similar to those observed after glutaraldehyde–osmium tetroxide fixation, but others differed. Some of the cell membranes appeared "negatively" stained (Fig. 14). It was difficult to recognize the mitochondria, since their internal structure could not be distinguished. Neither golgi bodies, nor vacuoles, nor large lipid droplets could be identified. In the plastids, the inner membranes were positively stained while the outer one was unrecognizable (Fig. 14).

(b) Submicroscopic Structure after Rehydration

In roots rehydrated by incubation in water (but not in nutrient medium) extensive vacuolation occurred. Some of the electron-transparent spaces surrounded by cytoplasm constituted "vacuoles", while others were "extraplasmatic water spaces".
Figs. 12–14.—Electron micrographs of cells from roots at various degrees of dehydration. 12. Parts of cortex cells, 0·8 mm from the tip of a root which had lost 54% of its initial weight. The lamellae are arranged on the periphery of the plastids. ×14,400. 13, Part of a cell from the
Figs. 15 and 16.—Electron micrographs of cells from normal roots that were kept in water for 17 hr. 15, Part of a cortex cell, 0·6 mm from the tip. The large extraplasmatic vacuole is situated
located between the plasmalemma and the cell wall (Fig. 15). A study of serial sections showed that sometimes the internal vacuoles as seen in Figure 16 are actually branches of these external water spaces. These vacuolar spaces occur already after 45 min of rehydration in the cortical cells, and somewhat later they can be observed also in the stele.

The vacuolization occurring after rehydration was independent of the degree of the preceding dehydration, except when the latter proved lethal. The changes in plasmatic fine structure, however, were correlated to the severity of the preceding dehydration. Absorption of water by tissue previously dehydrated to the lethal stage did not affect the cell structures for some time, and all the changes caused by the severe dehydration persisted; but after rehydration for 3 hr all the internal structure was destroyed. None of the organelles could then be recognized except for the nucleus which could be identified by the large aggregates of chromatin (Fig. 18). No external water spaces could be seen.

Roots dehydrated to the sublethal stage and then rehydrated in water showed a more complex picture. Those root segments that developed the brownish colour were actually dead. They showed the same structure as the root segments dehydrated to the lethal stage, and eventually after rehydration disruption of all internal structure occurred. Although the nucleus and the ribosomes could still be seen (Fig. 19), small vesicles surrounded by a single membrane and by ribosomes were also abundant (Fig. 19). No retreat of protoplasm from the cell wall was observed.

In those roots that preserved their viability, the regeneration of structure with proceeding rehydration could be observed. Already after 45 min of rehydration the large masses of chromatin, the typical structure of the nucleus in a dehydrated cell, began to disperse (Fig. 20). Large extraplasmatic water spaces were formed here, as in normal root segments kept in water. Some of the plastids have a typically dehydrated form, i.e. round with only few lamellae in the periphery, while others look normal, i.e. elongated, with lamellae in the middle.

After 90 min of rehydration the water deficit was practically replenished (Table 1) and the submicroscopic structure in general returned to normal (Fig. 21). However, in some of the cells, perhaps in those which lost viability, large lipid droplets having a strongly osmiophilic rim could be observed (Fig. 22). In other cells osmio-
Fig. 20.—Part of a cortex cell, 0.8 mm from the tip of a root which had lost 66% of its initial weight during dehydration, and was then rehydrated in water during 45 min. The large chromatic aggregates began to disperse; large lipid droplets are present as on dehydration. ×12,800.
philic precipitates were found in the vacuoles (Fig. 21). In addition, extensive vacuolization, decrease in numbers of golgi and reticular elements, and increase in the density of the mitochondrial matrix were also observed (Fig. 17).

**Table 2**

**BEHAVIOUR OF ROOT SEGMENTS DURING REHYDRATION AND GROWTH IN NUTRIENT MEDIUM**

Twenty root segments, 4 mm long, were dehydrated above NaCl solutions of different concentrations during 16 hr, and were then transferred to Petri dishes with filter paper moistened with nutrient solution. Root segments were weighed after dehydration and again after various periods of rehydration. The length of the segments was measured twice during the growth period.

<table>
<thead>
<tr>
<th>Conc. of NaCl Solution (atm)</th>
<th>Loss of Weight (%)*</th>
<th>Difference from Initial Weight (%)* after a Rehydration Period (hr) of:</th>
<th>Mean Segment Length (mm) after:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>0</td>
<td>-26</td>
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</tr>
<tr>
<td>65</td>
<td>-67</td>
<td>-9</td>
<td>+8</td>
</tr>
</tbody>
</table>

*As percentage of initial weight.

Under the conditions of these experiments, the roots remained without nutrients during the whole period of dehydration and rehydration. To avoid this starvation the root segments were allowed to rehydrate in a nutrient solution. The viability of the rehydrated roots was again judged by their ability to resume growth. Table 2 shows the results of a typical experiment with such roots.

Rehydration in nutrient solution is somewhat slower than in distilled water (Table 1) and the weight of the root segments did not exceed the initial weight even after 4 hr of rehydration. Growth was significantly inhibited only in those root tips which lost more than 60% of their fresh weight. Even so, they grew better than roots incubated in water (Table 1 cf. Table 2). After rehydration in the nutrient medium the structural damage induced by drought was repaired to a much higher degree than

Figs. 21 and 22.—Electron micrographs of cells from roots which had lost 66% of their initial weight, and were rehydrated during 90 min in water. 21, Part of a cortex cell, 0·8 mm from the tip. The vacuole contains osmiophilic precipitate. Plastids and mitochondria returned to normal. ×22,000. 22, Cells from transition area between the cortex and the stele, 0·8 mm from the tip. In the lower cell the ground substance is much more dense than in the upper one, and also contains numerous lipid droplets. The regeneration in the nucleus of both cells seems to be complete. ×7,150.

Figs. 23 and 24.—Electron micrographs of cells from roots dehydrated to loss of 66% of initial weight, then rehydrated during 19 hr in nutrient medium. 23, Part of a cortex cell, 0·8 mm from tip. Regeneration of plastids seems to be complete and they contain starch grains. ×13,400. 24, Part of a cortex cell, 0·8 mm from the tip. Plastids do not contain starch grains. ×13,400.
in roots rehydrated in water. Whenever the dehydration was not too severe the structure was completely restored and, except for the presence of monosomes instead of polysomes, the cells resembled those in tips fixed immediately after removal from the seedling. Root segments incubated in nutrient solution after having been dehydrated to the sublethal stage also showed a high degree of structural regeneration. In no case were extraplasmatic water spaces observed.

It seems that sublethal dehydration affected to some extent the function of the organelles, e.g. plastids — when the growth of the segments was severely diminished, no starch was found in the plastids (Fig. 24), whereas in roots that resumed growth accumulation of starch occurred (Fig. 23) in cells in the same position in the root.

**IV. DISCUSSION**

The cells in the region of the root chosen for this investigation are meristematic in nature. They are still thin-walled, and have relatively big nuclei. The big central vacuole has not yet developed and their cytoplasm is very rich in organelles. If moisture stress affects the structure of organelles, it was expected to detect such changes in these cells.

The method for dehydration chosen in this work allows control of the rate of water loss from the tissue and thus differs from the method used by Schnepf (1961) who exposed the roots to the external atmosphere and allowed them to dry relatively quickly.

In addition to the usual method of fixation in aqueous glutaraldehyde, osmic acid vapour fixation was also used. This gave results inferior to those obtained with glutaraldehyde and post-fixation in aqueous osmic acid. The results obtained with both types of fixation of dehydrated tissues were, however, very similar. It appears that cell structure in the root segments is preserved during fixation by glutaraldehyde in the typical dehydrated form, in spite of the partial rehydration occurring at the same time.

The detachment of the root tips causes disintegration of polysomes into monosomes. This may indicate certain changes in rate of RNA and protein metabolism of the detached root tips. Certain reservation in use of detached root segments should therefore be exercised.

One of the earliest and most striking changes due to dehydration is the distribution of chromatin in the nucleus. Dehydration of the tissue results in the aggregation of chromatin into large masses surrounding the nucleolus. Again, the first signs of rehydration in tissue still viable is the dispersal of these masses, whereas in dead tissue the dispersal does not occur. A similar aggregation of chromatin was described by Trump and Erriesson (1965) as the first signs of damage caused to animal tissue by anoxia, poisons, infection, radiation, etc. They also described concentrations of small granules (interchromatic granules) between the big masses of chromatin similar to those described in this work. In the nucleus of the normal cell these dense particles are more or less evenly distributed in the caryoplasm (Lafontaine 1965). What the changes in the internal conditions of the cell that may bring about the aggregation of chromatin are is not yet known; increase in acidity and increase in concentration of solutes have been mentioned as possible causes (Caufield and Klionsky 1959; Swift
Typical for more severe dehydration is the fragmentation of the nuclear membrane. Sometimes considerable parts of the nuclear membrane are missing. The higher the degree of dehydration the more fragmented becomes the nuclear membrane. Similar changes occurred as a result of senescence, mineral deficiency, and mechanical damage to plants (Mollenhauer, Whaley, and Leech 1960; Marinos 1962; Shaw and Manchoa 1965).

On increasing dehydration the amounts of the normal reticular elements in the cell decreases, but whenever dehydration approaches 50% loss of weight a parallel arrangement of the reticular membranes becomes evident. Similar structures were described by Frey-Wyssling and Mühlethaler (1965) as ergastoplasm. There is no correlation between appearance of these structures and loss of viability of the tissue, although the ergastoplasm usually appears under unfavourable conditions (Schnepf 1961; Bouck 1963; Wrischer 1965). However, ergastoplasm has also been described in normal tissue (Esau 1965; Klein and Pollock 1968) and may therefore have a definite function (Hršel 1966).

When tissues dehydrated to a relatively high degree were fixed with osmic acid vapour, some of the membranes in the cell appeared light on a dark background ("negatively stained"), while others appeared darker than the background. A similar effect of osmic acid vapour fixation was reported by Perner (1965) in dry seeds. This different behaviour of the various membranes in the same cell suggests that not all the cellular membranes are affected in the same way by water loss.

On increased dehydration plastids and mitochondria tend to become more and more rounded, and their internal membranes (cristae or lamellae) become less visible. The matrix of the organelle becomes less electron-dense and thin threads may be observed. These may be DNA threads which have been described by several workers in plastids and mitochondria (Nass and Nass 1963; Kislev, Swift, and Bogorad 1965). Similar findings in ripening seeds were described by Klein and Pollock (1968).

On rehydration of still viable tissue, the structure of plastids is restored to normal, but functional damage may still persist. This is illustrated by the fact that in such root segments the plastids do not resume starch synthesis. In mitochondria, on rehydration, matrix density increases. These changes in matrix density on dehydration and on rehydration may be related to changes in activity. This, however, has still to be proved experimentally. Similar changes in matrix density were described by Hackenbrook (1966) in animal mitochondria which showed differences in matrix density in different states of activity.

In preliminary work, when tissue was fixed with permanganate, it was found that in dehydrated tissue all the cytomembranes appeared completely destroyed. This finding, together with the finding of the negative staining of membranes on fixation with osmic acid vapour, may serve as indirect evidence for change of structure occurring in cell membranes due to water loss. Some supporting evidence may be gained also from Finean's work (1960), who found distinct changes in the organization of the various layers of the myelin sheath due to dehydration. It is also worthwhile noting that these "changes in structure" and the simultaneous fragmentation of membranes are accompanied by accumulation of many rather big droplets with strongly osmiophilic rims. Similar structures were defined by Frey-Wyssling and Mühlethaler (1965) as lipid droplets. Whenever regeneration of membrane structure
occurred, these droplets disappeared. In dead tissue, however, rehydration occurred without regeneration of structure and the presence of these lipid droplets persisted.

Extraplasmatic water spaces were found in tissue rehydrated in distilled water only after dehydration to the sublethal and not to the lethal stage. Such water spaces were also found in normal turgid root tips after soaking in distilled water. It is possible, therefore, that the ability to form such water spaces may serve as an indication of the viability of the tissue.

Regeneration of structure, however, is not necessarily indicative of regeneration of function. Damage to function caused by dehydration, and its reversibility, are apparently proportional to the degree of dehydration. This is because even after rehydration and regeneration of structure, inhibition of growth is still proportional to previous degree of dehydration. Root tips seem to be very resistant to dehydration damage, as already stressed by Milthorpe (1950) and Schnepf (1961). This can be attributed, to some extent, to the meristematic nature of the cells. It follows from this work that water stress does induce structural changes. How these changes are related to the various functions of the tissue is now under investigation.

V. Acknowledgment

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