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The Effect of Water Stress on Mitochondria of Root Cells

A BIOCHEMICAL AND CYTOCHEMICAL STUDY[1]

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ABSTRACT

Water loss from root cells of *Zea mays* induced by exposure to severe water stress, caused a reduction in the respiratory rate (O_2 consumption) and at the same time an increase in activity of the mitochondrial cytochrome oxidase (histochemical oxidation of diaminobenzidine). The connections between these changes and the changes in the fine structure of the mitochondria occuring in the stressed roots are discussed.

In previous reports (5, 6) the effect of dehydration on mitochondrial fine structure was described. A change in shape, a decrease in matrix density, and a decrease in the visibility of the cristae were reported.

In order to correlate these structural changes with biochemical function, experiments were carried out in which cytochrome oxidase activity was tested *in vitro*, in isolated mitochondria, using biochemical methods and *in situ* by cytochemical methods. In addition, the rate of O_2 uptake by entire root tips was investigated.

MATERIALS AND METHODS

Seeds of *Zea mays* (var. Neve Yaar 22) were germinated on wet filter paper for 15 hr in the dark at 25°. Root tips, 5 mm long, were cut off the main rootlets and served as the experimental material. Groups of such root tips were weighed and exposed to water stress above concentrated solutions of NaCl as previously described (6). Water loss was determined by weighing and expressed as a percentage of initial weight.

The cytochrome oxidase activity in the cell was localized in prefixed root tips by reaction with diaminobenzidine, as described by Seligman *et al.* (7). Root tips 2 mm long were prefixed for 60 min in 4% formaldehyde at 2°. The formaldehyde was prepared by depolimerization of *p*-formaldehyde (1).

Sections 100 μ thick were cut from the fixed root tips with a freezing microtome. The sections were incubated for 30 min at 30° with a reaction mixture containing 10 ml of phosphate buffer (0.05 M, pH 7.5), 0.5 mg of diaminobenzidine, 10 mg of cytochrome *c*, and 50 mg of sucrose. Then the sections were washed for 15 min with the phosphate buffer containing 5% sucrose and exposed for 60 min at 35° to 2% OsO_4 (dissolved in phosphate buffer). The sections were dehydrated in graded ethanol and embedded in Epon (4). Thin sections were cut with a Porter-Blum ultramicrotome and examined with an RCA EMU-3G electron microscope.

For isolation of mitochondria, 150 root tips (5 mm long) were ground in a prechilled mortar with 3.0 ml of phosphate buffer (0.05 M, pH 7.5) containing 0.4 M sucrose and 3×10^{-3} M $MgSO_4$. The homogenate was centrifuged for 5 min at 2,000 *g*; the mitochondria were sedimented from the supernatant by centrifugation at 20,000*g* for 30 min and resuspended in 1.0 ml of the isolation medium. The cytochrome oxidase activity was determined by measuring spectrophotometrically the oxidation of reduced cytochrome *c* as described by Smith (10). The results are given as specific activity which was obtained by relating the calculated first order reaction constant to milligrams of protein. Oxygen uptake was measured in a Warburg respirometer.

RESULTS

Mitochondria were isolated from turgid and dehydrated root tips, and their cytochrome oxidase activity was assayed. Enzyme activity was assayed also in the supernatant after sedimentation of the mitochondria. The results are summarized in Table I. Dehydration (group c) induced a considerable increase in cytochrome oxidase activity in the mitochondrial fraction and caused the appearance of some activity in the supernatant. These changes were not dependent in any way on the detachment of the root tips from the root system, since detached root tips which were not exposed to water stress but were kept for 16 hr in a saturated atmosphere above distilled water (Table I, group b) did not show such changes.

Cytochemical investigations of the control roots showed that the electron-dense reaction products are connected exclusively to the mitochondrial membranes (Figs. 1 and 2); however, not all the mitochondria in the cell of the control roots show cytochrome oxidase activity (see arrows in Fig. 2).

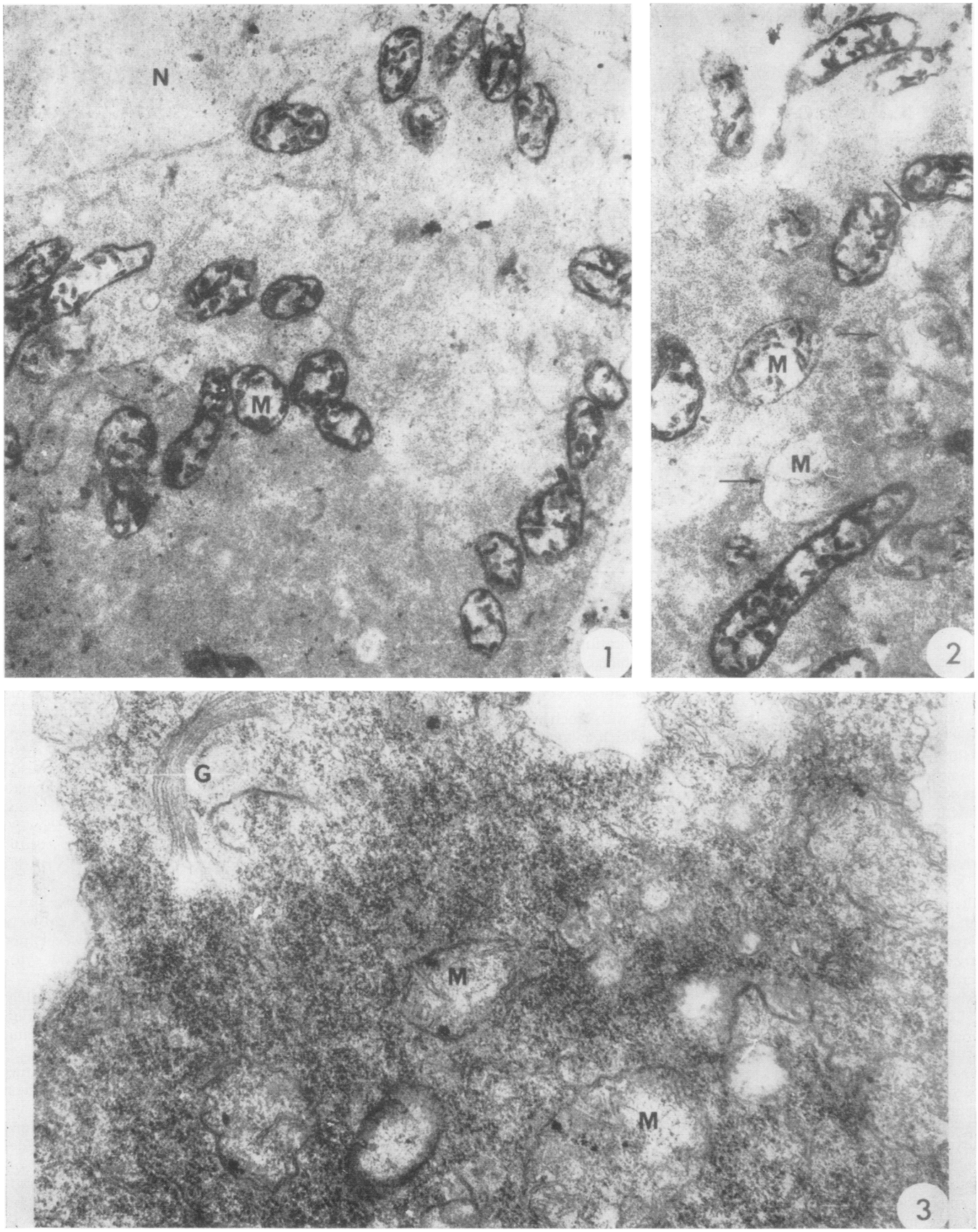
The addition of 10^{-3} M KCN to the reaction mixture completely prevents the accumulation of the electron-dense products (Fig. 3), showing that this product is a result of an iron-containing enzyme action.

Cytochemical examination was also carried out in root tips that were exposed to severe water stress and lost 70% of their initial weight (Figs. 4 and 5). Electron dense cristae and membranes were clearly distinguished, contrary to the observations made on cells fixed in glutaraldehyde and osmium tetraoxide, which showed mitochondria almost empty of cristae (6).

Since, cytochemically, cytochrome oxidase activity was demonstrated only on the mitochondrial membranes and nowhere else in the cell, the activity appearing in the supernatant (Table I) could be attributed to fragmentation of the mitochondria and dispersion of the enzyme-containing fragments during the isolation of the particles.

Oxygen uptake was studied in root tips after exposure to water stress and after complete rehydration in Torrey's nutrient me-

[1] This work is a part of the Ph.D. thesis of I. Nir.

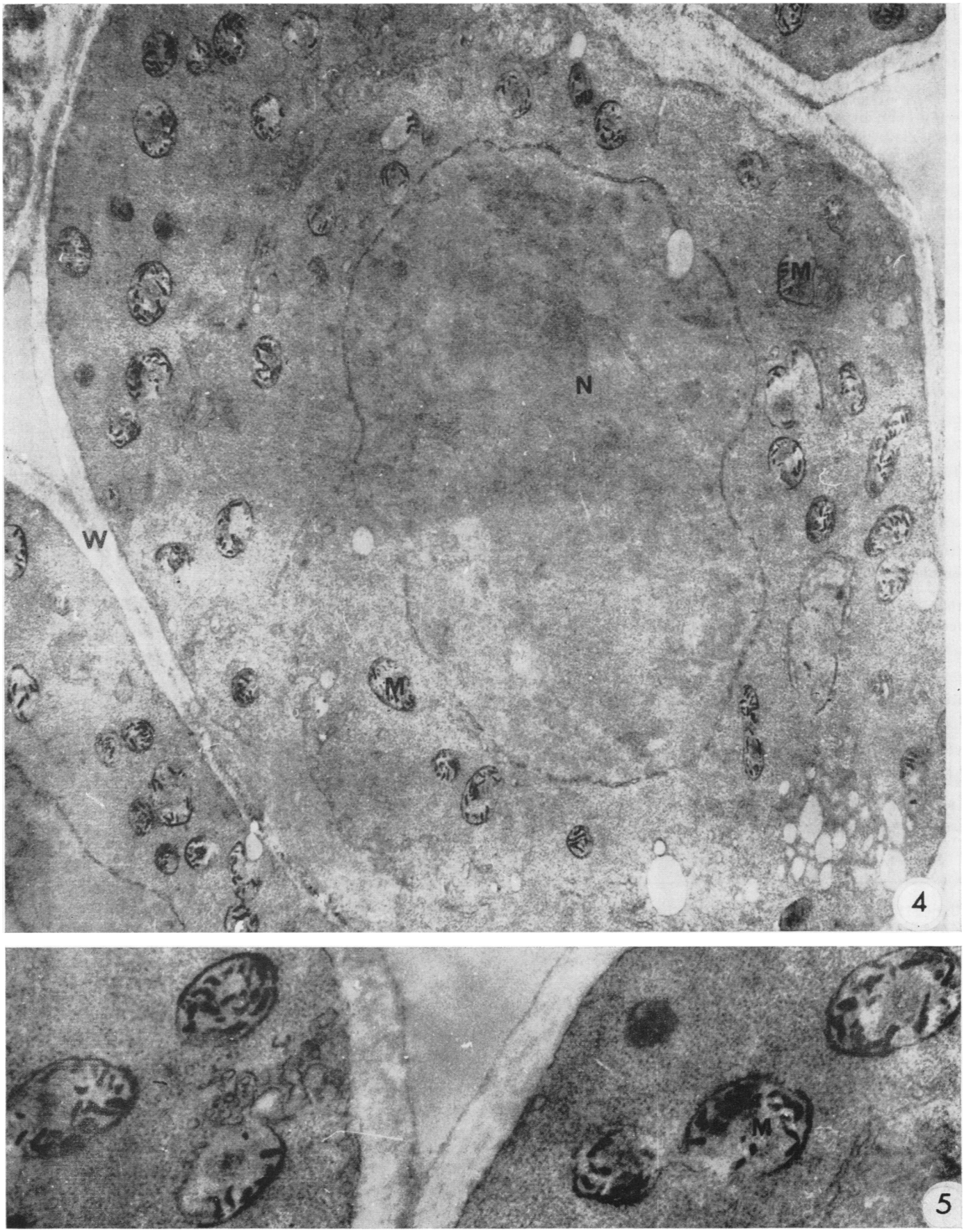


FIGS. 1 to 3. Cytochrome oxidase activity in cells of turgid roots. Roots were prefixed in formaldehyde. The sections were stained with lead citrate. Reactant for the enzyme activity was diaminobenzidine. M: Mitochondria; N: nucleus; G: golgy body.

FIG. 1. A part of a stelar cell. × 16,500

FIG. 2. A part of a cortical cell. Arrow points to inactive mitochondria. × 22,000.

FIG. 3. A part of a cortical cell in which cytochrome oxidase activity was inhibited by 10^{-3} M K cyanide.



FIGS. 4 and 5. Cytochrome oxidase activity in root cells after exposure to severe water stress—loss of 70% of the initial weight (see "Materials and Methods"). Reactant for enzyme activity was diaminobenzidine.

FIG. 4. A cortical cell. × 15,000.

FIG. 5. Mitochondria in a cortical cell. × 37,000.

Table I. *Cytochrome Oxidase Activity of Mitochondria Isolated from Dehydrated Roots*

Three groups of 90 root tips, 5 mm long, were prepared. Group a was ground immediately, and mitochondria were isolated. Group b was placed for 16 hr in air-tight containers above distilled water, and group c above a solution of 22 M NaCl. The roots of group b lost 8% of their initial weight and the roots of group c 78%. Cytochrome oxidase activity was measured in isolated mitochondria as described in "Materials and Methods." The results are given as specific radioactivity (see "Materials and Methods").

Group	Treatment	Fraction	Specific Activity	Percentage of Activity
a	Fresh control	Mitochondria	0.047	100
		Supernatant	0	
b	Kept above distilled water	Mitochondria	0.032	68
		Supernatant	0	
c	Kept above 22 M NaCl solution	Mitochondria	0.213	450
		Supernatant	0.012	

Table II. *Oxygen Uptake by Root Tips That Were First Dehydrated and Then Rehydrated*

Groups of 20 root tips, 5 mm long, were exposed to atmospheres of various relative humidities above solutions of NaCl. The control root tips were kept during the same time in a saturated atmosphere above distilled water. Degree of dehydration was defined as water loss expressed as percentage of initial weight. After the period of exposure to water stress the root tips were transferred to Petri dishes containing Torrey's nutrient medium with 2% glucose, for 3 hr. During this time the water deficit was abolished and the initial weight was regained. Oxygen uptake was measured in phosphate buffer (0.1 M, pH 6.5) with 0.02% glucose. Results are expressed as μl of oxygen absorbed by 1 mg of turgid tissue during 1 hr.

	Equilibrated above Water		Equilibrated above NaCl			
Degree of dehydration	−1%	−3%	−53%	−50%	−66%	−72%
Oxygen uptake after rehydration	0.75	0.71	0.46	0.46	0	0

dium (11) containing 2% glucose. In the first case, to prevent rehydration during the respiration measurements, a few experiments were run in a rather unconventional way in Warburg flasks without any extra fluid. These experiments showed that root tips which had lost approximately 50% of their water content consumed less than 50% O_2, as compared with turgid control roots which were kept for a comparable length of time above distilled water. Root tips which had lost approximately 70% of their initial water content (lethal dehydration) consumed only 10% of the amount of O_2 absorbed by the controls.

The ability to consume O_2 was not fully restored on rehydration (Table II). Moreover, the roots that before rehydration had been exposed to lethal dehydration were not able to consume any O_2, although during rehydration their initial (turgid) weight was restored. This inability to consume O_2 is probably connected with the complete disruption of the internal cellular structure which was observed after rehydration (6).

DISCUSSION

Cells of roots which were exposed to water stress and then fixed by conventional methods with glutaraldehyde and OsO_4, contained mitochondria which were almost devoid of cristae (6), in contrast to controls. However, if the prefixed sections were incubated with diaminobenzidine, according to Seligman *et al.* (7), the cristae were clearly stained, because of the activity of cytochrome oxidase and the accumulation of the electron-dense reaction product. No difference could be detected between these mitochondria and those in the cells of the fully turgid control roots. It seems, therefore, that on dehydration the cristae are not destroyed, but some change occurs in their structure. Since demonstration of mitochondrial membranes in the electron microscopic preparations is due to their reaction with the OsO_4, it is suggested that water loss, due to drought, changes the ability of the membranes to react with osmium tetroxide. The cristae are considered to be made up of structural proteins, enzymes, and lipids (3). Water loss, under conditions of water stress, apparently induces changes in the arrangement of these compounds. Numerous lipid droplets were found in the cytoplasm of the dehydrated cells (6), and it is possible that the lipid components were displaced from their position in the mitochondrial and other cellular membranes. On rehydration of the tissue these lipid droplets disappear again. If these droplets are indeed formed from lipids displaced from the various membranes, including the mitochondrial cristae, the decrease in the contrast of the cristae in the mitochondria of the dehydrated cells may be explained by an inability to bind osmium.

A loss of the lipids which are assumed to serve in the electron transport particle as a hydrophobic medium in which electrons flow from one enzyme to another (2), may also explain the low O_2 uptake of the tissue. The enzymic proteins, apparently, are not drastically affected; their activity may be even enhanced because they are liberated from the control imposed on them by the highly organized multienzyme complex (chain of electron transport)

Some support for this assumption may be found in reports about the effect of various chemicals on mitochondrial activity. For example, treatment with digitonin and other detergents was reported to increase the cytochrome oxidase activity while the activity of the multienzyme complex (succinoxidase system) decreased (9). This phenomenon is explained on the basis of solubilization of the lipids by the detergent and release of the enzyme from the structural complex in which it is normally bound.

The existence of active and nonactive mitochondria in the same cell was described by Seligman *et al.* (8) for animal tissues and was also found in cells of normal root tips (Fig. 2). According to Seligman *et al.* (7), the activity of cytochrome oxidase in the mitochondria may be better demonstrated when membrane permeability to substrate is increased. In the stressed root cells nonactive mitochondria were practically absent. It is possible that as a result of water loss the permeability of the previously inactive mitochondria increases and they become active.

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