THE EFFECT OF SODIUM CHLORIDE ON SOME METABOLIC AND FINE STRUCTURAL CHANGES DURING THE GREENING OF ETIOLATED LEAVES

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INTRODUCTION

Plants respond to a saline environment by metabolic and morphological changes (16, 17, 20). Most of the information on salt effects stems from work on plants adapted to saline conditions, the halophytes, or from mesophytes which were grown under conditions of increased salinity for prolonged periods. In the present work some short-term effects of saline conditions on greening leaves of an unadapted mesophytic plant were studied. Exposure of the leaves to salt-containing media introduced changes in chlorophyll content, rate of respiratory O_2 uptake, chloroplast fine structure, and number of mitochondria. These changes may be of interest in relation to problems of salt tolerance and adaptation.

MATERIAL AND METHODS

Bean seedlings were grown from seed (variety French Dwarf) for 14 days on vermiculite in complete darkness at 22 ± 2°C. Then the first pairs of leaves were detached from the etiolated plants and cut along the midrib; the halves were floated in Petri dishes on distilled water or on various salt-containing solutions under fluorescent light of 400 ft-c at 27°C. Gas exchange by the halved leaves was measured in the dark in a Warburg apparatus after the leaves had been transferred from the Petri dixhes into Warburg vessels containing 1.6 ml distilled water or the respective salt solutions with or without 0.2 ml KOH in the center well. Chlorophyll content of the leaves was determined spectrophotometrically in acetone extracts by using the constants of Mackinney (13).

For electron microscopy 1 mm^2 pieces of tissue were

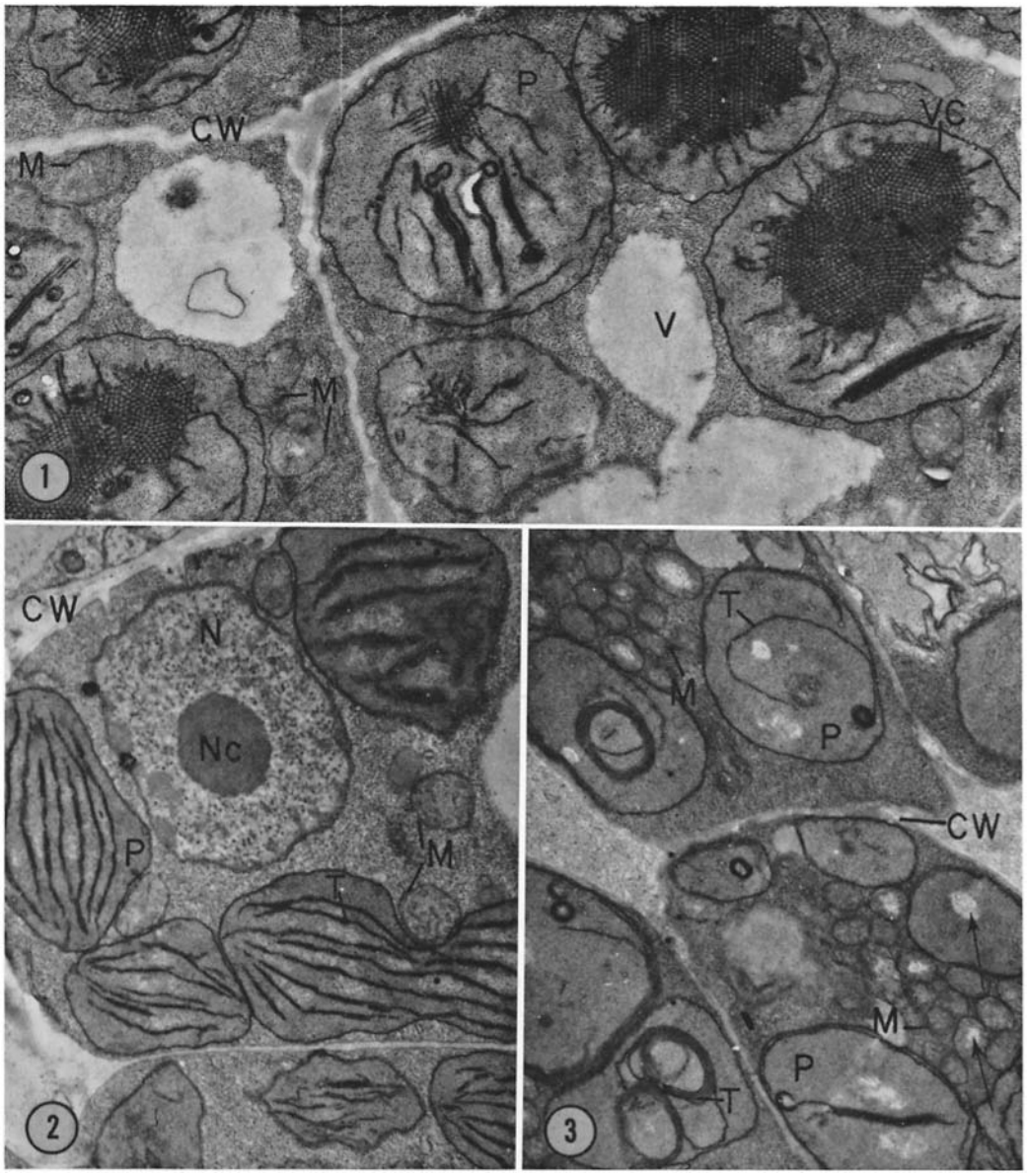

FIGURE 1 Section through cells of an etiolated leaf. Plastid, *P*; vesicular center (prolamellar body), *VC*; vacuole, *V*; mitochondria, *M*; cell wall, *CW*. × 13,000.

FIGURE 2 Section through a cell of an etiolated leaf, after 24 hr illumination. Incubation medium: water. Thylakoids, *T*; nucleus, *N*; nucleolus, *Nc*. × 11,000.

FIGURE 3 Section through cells of an etiolated leaf, after light exposure for 24 hr, incubated in 0.2 M sodium chloride. Note the atypical development of the thylakoids (*T*), the increase in number of mitochondria (*M*), and the electron optically "transparent" regions in the plastic (*P*) stroma and mitochondrial matrix (arrows). × 8000.

TABLE I

The Effect of 0.2 M NaCl on Number of Plastids and Mitochondria and on Size of Mitochondria in Etiolated Leaves Exposed for 24 hr to Light

	Plastids per cell section	Mitochondria per cell section	Mean diameter of mitochondria
			μ
Etiolated leaves before light exposure (data are from sections through 20 different cells from four leaves)	6 ± 0.9*	5 ± 0.7	0.3 ± 0.04
Leaves after 24 hr in light, water (43 cells from six leaves)	5 ± 0.3	4 ± 0.3	0.6 ± 0.02
Leaves after 24 hr in light, 0.2 M NaCl (46 cells from 10 leaves)	6 ± 0.3	24 ± 1.5	0.4 ± 0.06

* mean value and standard error

cut from the leaves and fixed for 2 hr in 5% glutaraldehyde at pH 7.5 in a phosphate buffer, the osmotic value of which was equal to that of the solution upon which the leaves had been floated. The fixative of the controls always contained 0.1 M phosphate buffer pH 7.5. The tissues were postfixed in 2% OsO_4 in a 0.1 M phosphate buffer at pH 7.5, dehydrated in graded alcohols, put through propylene oxide, and then embedded in an Epon mixture. Sections were cut on a Porter-Blum or LKB ultramicrotome, poststained with lead citrate (23), and examined in an RCA 3G electron microscope.

RESULTS

Fig. 1 shows the fine structure of plastids in the etiolated leaves at the beginning of the experiments prior to light exposure. The rounded plastids contain the crystalline centers, typical for dark-grown plants (5, 6, 10, 24), together with a number of single thylakoids. Plastids in cells from leaves floated as controls on distilled water for 24 hr in light are shown in Fig. 2. They are larger, somewhat elongated, and contain a number of stacks of two to three thylakoids representing the newly formed grana, and interconnecting "stroma lamellae"; the crystalline centers are absent. The number of plastids per cell section did not change (Table I). These light-induced structural changes are in agreement with previous findings (10, 24). The plastids in leaves floated for 24 hr on a 0.2 M NaCl solution in light (Figs. 3, 4) retained their original number, shape, and size (Table I), but their internal organization differed from that found in the etiolated leaves and the illuminated controls. The crystalline centers disappeared, but only a small number of elongated pairs of membranes, occasionally arranged in concentric rings, could be recognized. The latter resemble thylakoids and are probably formed from material originally present in the crystalline centers. Well-defined, electron optically empty areas occur in the dense stroma (Figs. 3, 4) and these contain thin strands resembling the DNA threads found previously in plastids (1). Plastids in leaves incubated for 24 hr with 0.3 M NaCl appeared to have been destroyed and were not recognizable.

Only a small number of mitochondria, usually not more than five, could be found in any section through an etiolated leaf-cell (Table I, Fig. 1). These mitochondria had an approximate diameter of 0.3 μ. After 24 hr in light the diameters of the mitochondria in the control leaves had approximately doubled, but no apparent changes had occurred in their number or structure (Fig. 2).

In the leaves floated on 0.2 M NaCl in light, the number of mitochondria was greatly increased, and up to 30–40 mitochondria (Figs. 3–5) per cell section could be counted (Table I, Figs. 3–5). The mitochondria were somewhat smaller than those of the illuminated control leaves, but they were somewhat larger than those in the etiolated material. In the matrix of some of these mitochondria appear electron optically empty spaces which contain, as in the plastids, thin threads. The occurrence of this phenomenon, however, is less frequent than in the plastids. The mitochondria in the treated leaves occur usually in clusters, and a number of these clusters may be found in a given section. Frequently, parallel arranged units of the endoplasmic reticulum, studded with ribosomes, were found in the vicinity of these clusters.

An increase in number of mitochondria could

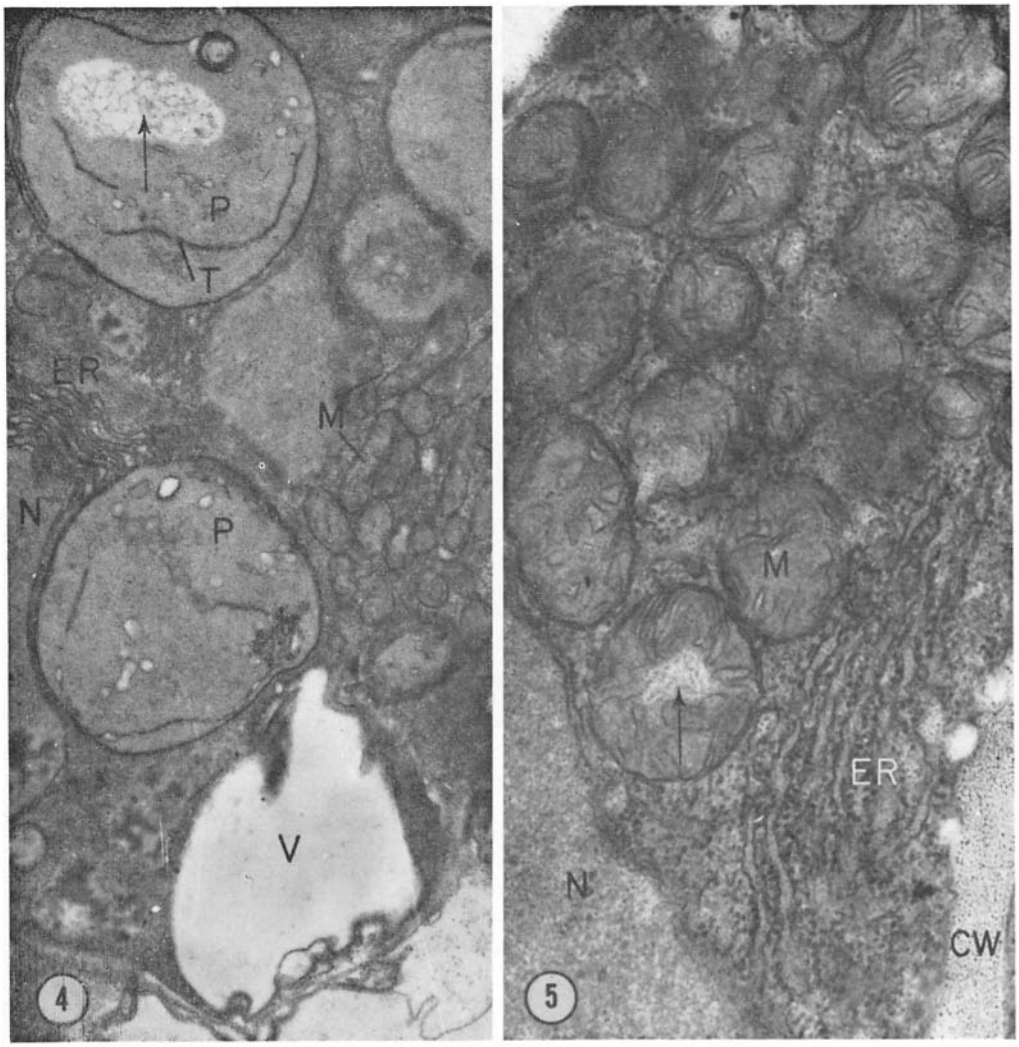

FIGURE 4 Same treatment as for Fig. 3. Note the threadlike content (arrow) of the transparent regions in the stroma and the ergastoplasma-like orientation of the endoplasmic reticulum (*ER*). Plastid, *P*; thylakoids, *T*; mitochondria, *M*; nucleus, *N*; vacuole, *V*. × 19,000.

FIGURE 5 A group of mitochondria (*M*) in the illuminated etiolated leaves treated with sodium chloride. The parallel arranged units of the endoplasmic reticulum (*ER*) occur frequently close to the clusters of mitochondria. Note thin threads in electron-transparent space in mitochondrial matrix (arrow). Cell wall, *CW*. × 47,000.

also be observed in leaves treated with 0.3 M NaCl, although fine structure was badly preserved under these conditions.

The changes in the structure of the plastids and in the number of mitochondria are paralleled to a certain degree by differences in chlorophyll content and O_2 uptake by the treated and nontreated leaves.

The chlorophyll contents of the leaves floated for 24 hr on various concentrations of NaCl are given in Fig. 6. Chlorophyll accumulation decreased with increasing salt concentrations, and in 0.2 M NaCl solution leaves contained only 10% of the chlorophyll in the controls. Chlorophyll content was similarly reduced when mixtures of inorganic salts were used and also when the leaves

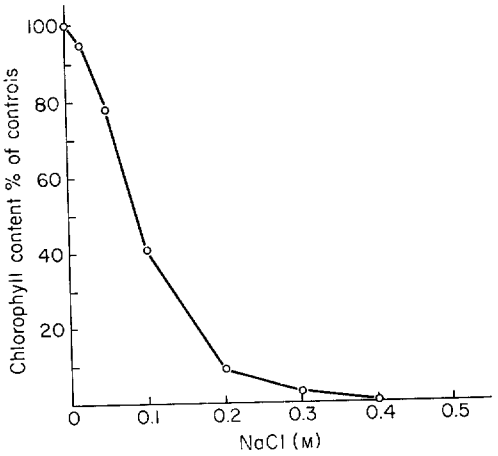

FIGURE 6 Chlorophyll content in etiolated bean leaves which were incubated with various concentrations of NaCl and exposed to white fluorescent light of 400 ft-c for 24 hr. Chlorophyll concentration is given as per cent of that in the control leaves which were incubated with distilled water.

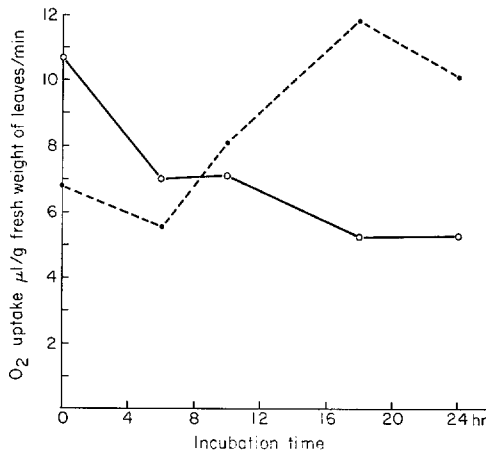

FIGURE 7 Changes in rate of O_2 uptake by water-treated and NaCl-treated leaves during exposure to light. The leaves were exposed to white fluorescent light of 400 ft-c while incubated in distilled water (solid line) or in a 0.2 M NaCl solution (broken line). The leaves were transferred to Warburg vessels after various periods, and O_2 uptake was measured manometrically in the dark.

were floated on balanced nutrient solutions (Hoagland and Snyder, reference 8) of higher concentrations. Phosphate buffer at equivalent concentrations had a similar effect. In agreement with previous work, it was found that incubation of leaves with sucrose increased the amount of chlorophyll present after 24 hr (11, 26), but addition of sucrose to the salt solutions did not remove the inhibition caused by the latter, and the leaves behaved as if they had been exposed to NaCl alone.

The time curves for the rate of O_2 uptake by the NaCl-treated leaves and by the controls are given in Fig. 7. In the control leaves, respiration rate decreases during the first 2–3 hr and reaches a steady state until the 24th hour. Leaves floated on an 0.2 M NaCl solution start to take up O_2 at a lower rate, and a drop in rate similar to that in the control is apparent. Then, respiration rate rises, and after 18 hr a maximum is reached which is significantly higher than the rate of the controls at any time during the experiments. The possibility suggested itself that the drop in rate of O_2 uptake by the controls could be due to depletion of substrate, while in the leaves treated with NaCl initial slower depletion of substrates could allow for the later rise. That this was not the case was shown when leaves were transferred, after 24 hr, from water to 0.2 M NaCl, and then O_2 uptake was measured after an additional 24 hr. The uptake was found to be higher than in the water controls after 24 or 48 hr. This indicates that substrate level is not the limiting factor in these experiments. The salt-induced increase in O_2 uptake is not easily reversible. Leaves incubated for 24 hr in 0.2 M NaCl, then washed thoroughly and kept for 1–2 hr on distilled water, still retained a higher respiration rate.

DISCUSSION

In the experiments described here, leaves from a mesophyte unadapted to a saline environment were transferred to solutions containing relatively high concentrations of ions. The choice of etiolated material made it possible to study the effect of this change in environment on developmental processes, since both structural and biochemical changes occur as a result of exposing etiolated leaves to light.

The results indicate that the salt concentrations inhibit developmental processes in the chloroplasts and increase the number of mitochondria. The latter changes may constitute an emergency regulatory mechanism in order to overcome the detrimental effect of the saline solution.

In animals, organs active in osmoregulation are frequently rich in mitochondria which may be grouped to form "mitochondrial pumps" (2, 3).

Among plants, halophytes are frequently equipped with cells specialized for salt excretion (7) and which are rich in well-developed mitochondria (21). In mesophytes exposed to saline conditions, respiration rises (16), but it has not yet been shown that these conditions affect the number of the mitochondria themselves.

The large number of clustered mitochondria visible in the thin sections through the leaves floated on salt media could be caused by a local concentration and clumping of already existing ones. Systrophe, a concentration of cytoplasm and/or plastids around the nucleus, may be caused when high concentrations of plasmolyticum are used (19). However, the distribution of the clusters of mitochondria and especially the unchanged number and location of plastids in the sections of the NaCl-treated leaves make this possibility unlikely. The electron micrographs indicate, therefore, a real increase in mitochondria per cell; the mechanism and the time curve of this multiplication are now under investigation.

In the leaves treated with sodium chloride, the plastid stroma and the mitochondrial matrix showed areas with low electron opacity, which contained thin strands, this indicates localization of DNA. Similar "empty" spaces occur in mitochondria of the excretory cells of *Tamarix aphylla* (21) and can also be found in plastids and mitochondria of partially desiccated root tips (Y. Nir. Data unpublished).

Chloroplast development and chlorophyll accumulation can be inhibited by various means (9, 14, 15, 18, 22, 25). Pertinent to the findings described here may be the finding that water stress, induced by floating leaves on saccharose or on mannitol solutions, causes reduction in chlorophyll accumulation by inhibiting protochlorophyll synthesis (25).

The suppression of chlorophyll accumulation in illuminated etiolated leaves is always coupled with absence of normal chloroplast development (4, 12, 14, 22). Depending on the agents used, either development of chloroplasts stops at intermediate stages or aberrant structures are formed. The latter appears to be the case when the greening leaves are exposed to NaCl solutions, since the features of the leaves exposed for 24 hr to light on the salt medium do not fit any of the stages in chloroplast development described by Virgin et al. (24) and Klein and Bogorad (10). Structurally deficient chloroplasts have also been described in the secretory cells of *Tamarix* (21).

In regard to control mechanisms in halophytes, it is not possible to draw direct conclusions from the behavior of the isolated mesophyte leaves when they are transferred abruptly to a saline medium. Nevertheless, the fact that salt solutions can affect the development of cell organelles and especially that the number of mitochondria can be increased experimentally in developing leaves by this treatment makes it possible that such a system, as described here, may be useful in the elucidation of adaptive mechanisms resulting in salt tolerance.

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