LIGHT-INDUCED EFFECTS OF TRIS ON THE ULTRASTRUCTURE OF LEAF TISSUE

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Abstract

The effects on ultrastructure of tris(hydroxymethyl)aminomethane (Tris), pH 8·0, were examined in leaf segments of wheat, barley, and maize in both light and dark. Tris in the light induces drastic changes in chloroplast ultrastructure which are not apparent in the dark except in mesophyll cells of maize. Both grana and stroma lamellae are affected and structural changes are apparent after treatment with Tris for 3 hr. Vacuoles appear in the chloroplasts and both grana and stroma lamellae frequently undergo coiling. At later stages, disruption of the tonoplast occurs. The action of Tris appears to be specific for chloroplast membranes and the tonoplast, there being no apparent effect on the plasmalemma, or the membranes of mitochondria or microbodies.

It seems likely that the well-known Tris inhibition of the Hill reaction and photosynthetic electron transport could be linked to this disruption of chloroplast lamellae.

I. INTRODUCTION

Tris(hydroxymethyl)aminomethane (Tris) is widely used as a buffer although it has been known for many years that it cannot be regarded as inert (Mahler 1961). It has been shown to affect many biological processes including ion transport (Van Steveninck 1961, 1966*a*, 1966*b*; Kholdebarin and Oertli 1970), the Hill reaction (Jacobi 1961), uncoupling of photosynthetic phosphorylation (Good 1962), isolation of mitochondria (Stinson and Spencer 1968), sucrose storage (Humphreys and Garrard 1969), and amino acid incorporation (Bewley and Marcus 1970). Ikehara and Sugahara (1969) suggested that Tris at high pH may alter the structure of chloroplast lamellae. A possible effect of Tris on membrane structure was suggested also by Humphreys and Garrard (1969) and by Yamashita, Tsuji, and Tomita (1971). Light is involved with Tris effects on the Hill reaction and photoreduction (Ikehara and Sugahara 1969), salt uptake in barley leaves including reduction of the rate of Cl^- uptake (Kholdebarin and Oertli 1969), and absorbance changes in chloroplast fragments (Inoué, Wakamatsu, and Nishimura 1971). Hence it was considered important to examine the ultrastructural effects of Tris on leaf tissue in both light and dark.

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II. Materials and Methods

(a) *Plant Material and Incubation Methods*

Seeds of barley (*Hordeum vulgare* cv. Manchuria), wheat (*Triticum aestivuum* cv. Mendos), and maize (*Zea mays*) were sown in moist sand and placed in a Sherer growth cabinet (16 hr photoperiod, 8500 lux, 26±2°C day, 18±2°C night). After germination, the plants were supplied with nutrient solution (James 1963) twice a week.

Uniform leaves were selected, the tip 1·5 cm discarded, and the next 4-cm region sliced transversely into 2-mm segments and aerated in 0·5 mM $CaSO_4$ for 1 hr at room temperature under laboratory light conditions. The segments then were transferred to flasks containing solutions of either 20 mM or 200 mM Tris.sulphate, pH 8·0, 20 mM *N*-tris(hydroxymethyl)-methyl glycine (Tricine), pH 8·0, or 20 mM sodium phosphate buffer, pH 8·0. The controls and all treatment solutions contained 10 mM NaCl and 0·5 mM $CaSO_4$. The flasks were shaken at 26±1°C under a mercury vapour lamp supplying 26,000 lux to the leaf tissue. For dark treatments, the flasks were wrapped in aluminium foil.

(b) *Electron Microscopy*

Samples of leaf tissue were quickly blotted dry and cut into 1-mm^2 segments in a drop of fixative. Two different methods of fixation were used:

(1) 4% glutaraldehyde in 50 mM sodium phosphate buffer, pH 8·0, for 2–3 hr at room temperature followed by washing in the buffer for 1 hr (six changes) and post-fixation with 1% osmium tetroxide in the above buffer for 1·5 hr at room temperature.

(2) 1% osmium tetroxide in 50 mM sodium phosphate buffer, pH 8·0, for 2 hr at room temperature.

After washing with deionized water, the fixed tissues were dehydrated through an ethanol series and embedded (Spurr 1969). Thin sections cut with an LKB Ultrotome were stained with uranyl acetate and lead citrate (Reynolds 1963) and examined in a Siemens 1 or 1A electron microscope operated at 60 kV.

III. Results

(a) *Barley Leaves Treated with* 20 mM *Tris*

The ultrastructure of mesophyll cells of primary barley leaves detached 11 days after sowing and fixed in glutaraldehyde followed by osmium tetroxide is shown in Figure 1. After incubation of the leaf segments in the control solution for 24 hr in the light, there is no apparent change in structure (Fig. 2). However, treatment

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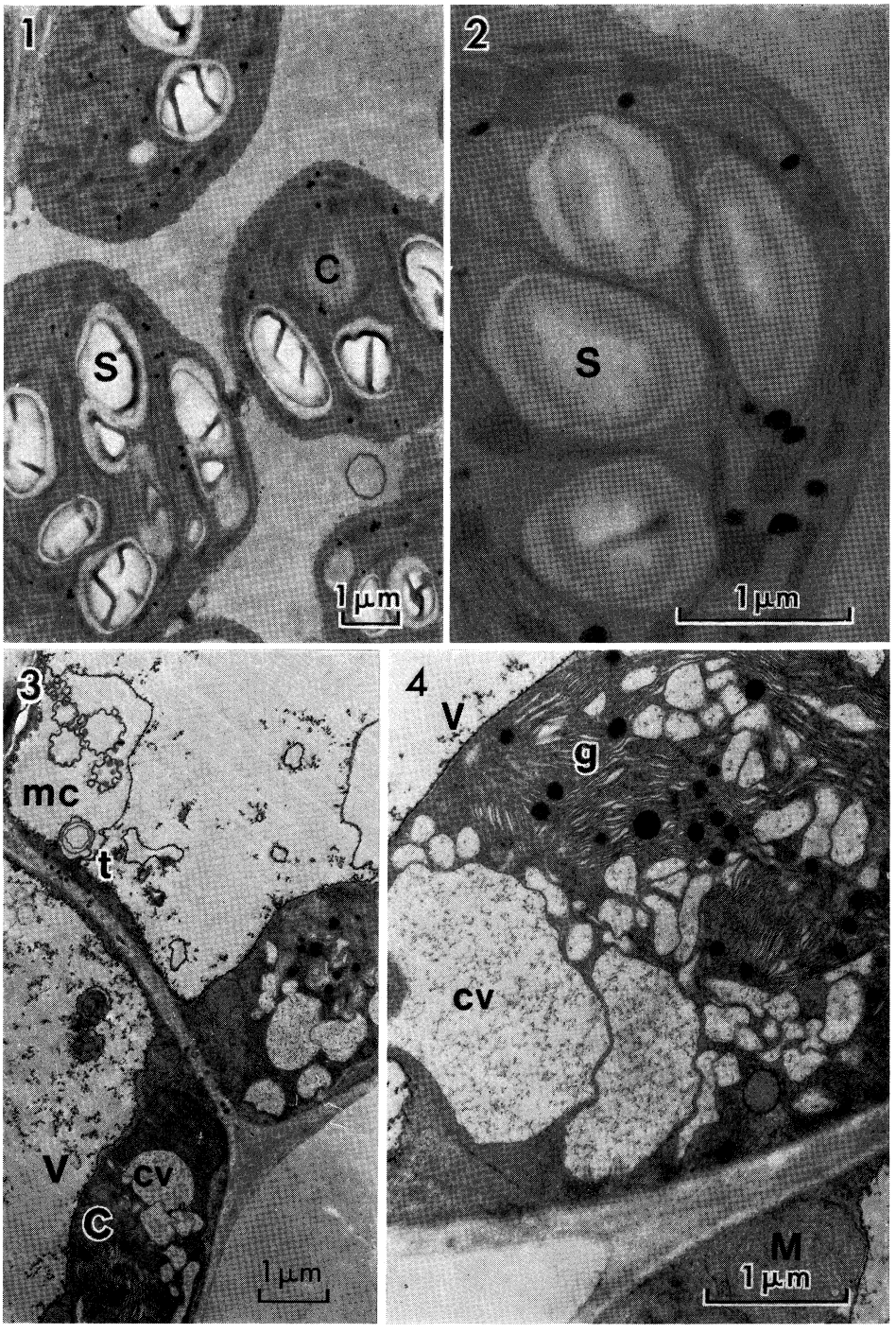
Fig. 1.—General structure of primary leaf cells of 11-day-old barley showing chloroplasts (*C*) with starch grains (*S*). (Unless otherwise stated, fixations in all figures were in glutaraldehyde followed by osmium tetroxide.)

Fig. 2.—Barley chloroplast after incubation of leaf segments in the control medium for 24 hr in the light showing starch grains (*S*).

Fig. 3.—Part of two cells of barley after incubation in 20 mM Tris for 24 hr in the light. Chloroplasts (*C*) contain vacuoles (*cv*). Deposits are present in the vacuoles (*V*) which also contain coils of membranes (*mc*). The tonoplast (*t*) has partially separated from the cytoplasm.

Fig. 4.—Detail of chloroplasts after incubation of barley leaf segments in 20 mM Tris for 24 hr in the light. Grana lamellae (*g*) have separated and the chloroplast vacuoles (*cv*) contain finely dispersed deposits. There are deposits in the vacuoles (*V*). Mitochondria (*M*) are intact.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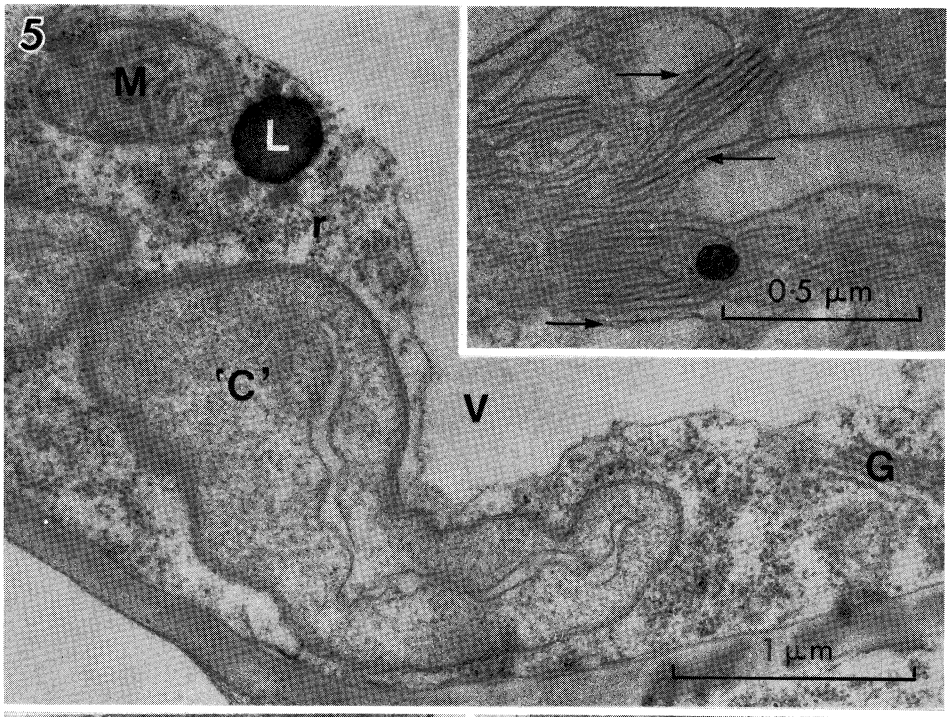
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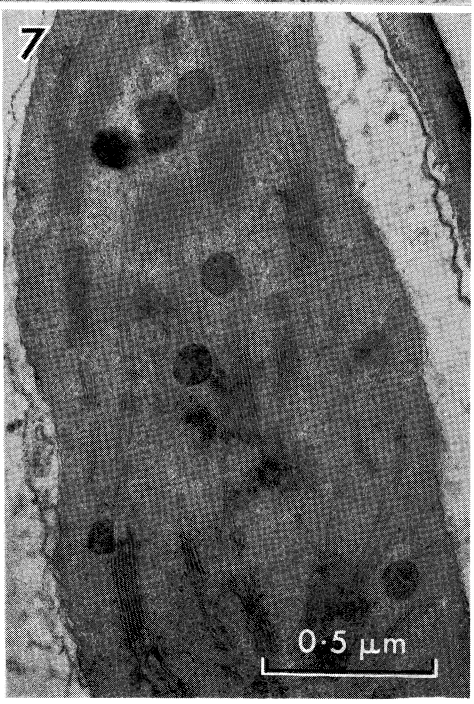

7
0·5 μm

with 20 mM Tris under these conditions results in the separation of chloroplast lamellae and the development of deposit-containing vacuoles within the chloroplasts (Figs. 3 and 4). Starch grains disappear almost entirely. An amount of deposit is present also in the cell vacuoles which contain coils of membranes, some appearing to have arisen from the tonoplast. Mitochondrial structure does not appear to be affected by Tris (Fig. 4).

Fixation with osmium tetroxide was carried out because of the possibility of a reaction between glutaraldehyde and Tris. Tris is known to react with acetaldehyde (Hauptmann and Gabler 1968). However, Tris-treated leaf segments fixed in osmium tetroxide show similar ultrastructural changes to those seen in tissue pre-fixed with glutaraldehyde (Fig. 5). Some chloroplast remnants appear to possess few membranes but when grana are present, separation of the lamellae is observed (Fig. 5, inset).

In contrast, no change in chloroplast membrane structure is observed in leaf tissue treated with either 20 mM Tricine, pH 8·0 (Fig. 6), or 20 mM sodium phosphate buffer, pH 8·0 (Fig. 7), but, in general, very little starch is present. No changes are apparent in the ultrastructure of chloroplasts treated with Tris in the dark (Fig. 8). However, as with incubation in the light, deposits are present in the vacuoles of Tris-treated leaves in the dark and, again, membrane coils are frequently observed in the vacuoles and cytoplasm.

(*b*) *Maize Leaves Treated with* 200 mM *Tris*

Part of a mesophyll cell from segments of the third leaf of maize following incubation in the light in the control medium for 24 hr is shown in Figure 9. Figures 10 and 11 show the result of 200 mM Tris in the incubation solution under similar conditions. Little granal structure remains and grana and stroma lamellae can no longer be differentiated. The outer membrane of the chloroplasts frequently cannot be detected. Peripheral reticulum, when present, does not appear to be altered by Tris (Fig. 11, inset). Disruption of lamellae is apparent in chloroplasts from both bundle sheath cells and mesophyll cells. Tris has no apparent effect on the membranes of mitochondria or microbodies, the plasmalemma, or the nuclear envelope (Fig. 10).

Tris treatment of maize leaf segments in the dark for 24 hr produces ultrastructural effects similar to those obtained with incubation in the light (Fig. 12). The cells are transformed into a mass of membrane coils and it is impossible to recognize the tonoplast.

Fig. 5.—Chloroplast remnant ('*C*') after incubation in 20 mM Tris for 24 hr in the light. Note the intact mitochondrion (*M*), Golgi apparatus (*G*), lipid body (*L*), and cytoplasmic ribosomes (*r*). The vacuole (*V*) is free of deposits. The inset shows granal separation (arrows). Fixation was in osmium tetroxide.

Fig. 6.—Chloroplast of barley leaf segments after incubation in 20 mM Tricine for 24 hr in the light.

Fig. 7.—Chloroplast of barley leaf segments after incubation in 20 mM sodium phosphate buffer for 24 hr in the light.

(c) *Wheat Leaves Treated with* 20 mM *Tris*

The structure of cells of 11-day-old primary wheat leaves is shown in Figure 13. The result of treatment with 20 mM Tris in the light for 24 hr is shown in Figures 14 and 15. Chloroplast vacuolation is apparent and is particularly marked in some cases and starch is rarely seen. Membrane coils are present in the cytoplasm and vacuoles which also possess an amount of deposit. Mitochondria and ribosomes of both cytoplasm and chloroplasts are not affected.

Dark incubation of wheat leaves with 20 mM Tris has no effect on ultrastructure.

(d) *Wheat Leaves Treated with* 200 mM *Tris*

The effect of treatment of segments of 10-day-old primary wheat leaves with 200 mM Tris for 3 hr in the light is shown in Figure 16. Some separation of grana lamellae and vacuolation is present.

By 6 hr, drastic ultrastructural changes are induced by Tris [compare Figures 17 (control) and 18 (Tris)]. The outer membrane of the chloroplasts in Tris-treated leaf tissue can rarely be detected. There is little evidence of granal structure, the chloroplasts being reduced to a number of concentric double membranes.

Fig. 8.—Cells from segments of barley leaves after incubation in 20 mM Tris for 24 hr in the dark. Chloroplasts (*C*) are intact but membrane coils (*mc*) are present in the cytoplasm and vacuoles (*V*) which contain deposits. The tonoplast (*t*) is absent in some regions (arrows).

Fig. 9.—Mesophyll chloroplasts of maize leaf segments after incubation in the control medium for 24 hr in the light showing starch grains (*S*), grana lamellae (*g*), stroma lamellae (*sl*), and peripheral reticulum (arrow).

Fig. 10.—Part of two cells of maize after incubation in 200 mM Tris for 24 hr in the light. Only groups of membranes ('*C*') remain of the original chloroplasts. Mitochondria (*M*), microbodies (*m*), the nuclear envelope (*n*), and plasmalemma (arrows) are intact. Some lipid bodies (*L*) are present in the cytoplasm.

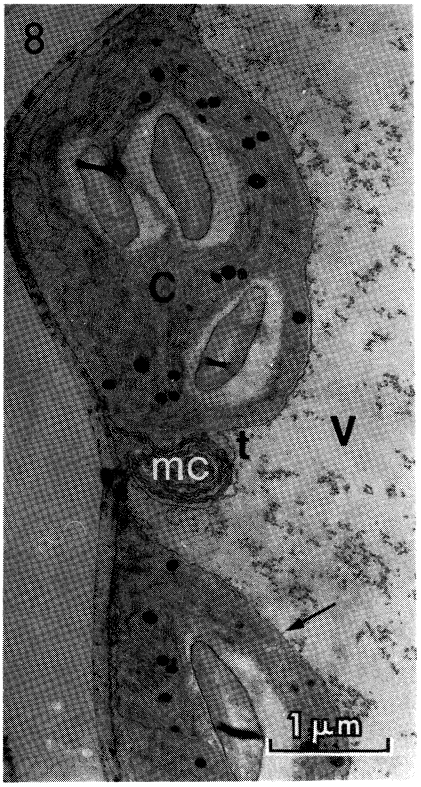
Fig. 11.—Mesophyll chloroplast of maize leaf segments after incubation in 200 mM Tris for 24 hr in the light. Only a few small regions of grana lamellae (*g*) remain. The inset shows a region of peripheral reticulum (arrow).

Fig. 12.—Part of a mesophyll cell of maize leaf segments after incubation in 200 mM Tris for 24 hr in the dark. The tonoplast has disappeared and the cell is a mass of membrane coils (*mc*). The presence of plastoglobuli (*p*) suggests that some of the membrane coils have arisen from chloroplasts. The plasmalemma (arrow) and mitochondria (*M*) are intact.

Fig. 13.—Part of two mesophyll cells of 11-day-old wheat leaves.

Fig. 14.—Part of two cells of wheat leaf segments after incubation in 20 mM Tris for 24 hr in the light. Large deposit-containing vacuoles are present in the chloroplasts (*C*) and grana lamellae have separated. In some "chloroplasts" ('*C*') there is no evidence of lamellae. Deposits are present in the vacuoles (*V*).

Fig. 15.—Detail of chloroplasts from wheat leaf segments after incubation in 20 mM Tris for 24 hr in the light. A small starch grain (*S*) is present and chloroplast ribosomes as well as cytoplasmic ribosomes (arrows) can be seen. Separation of grana lamellae (*g*) is apparent and deposit-containing chloroplast vacuoles (*cv*) are present. An intact mitochondrion (*M*) and microbody (*m*) can be observed. Membrane coils (*mc*) are present.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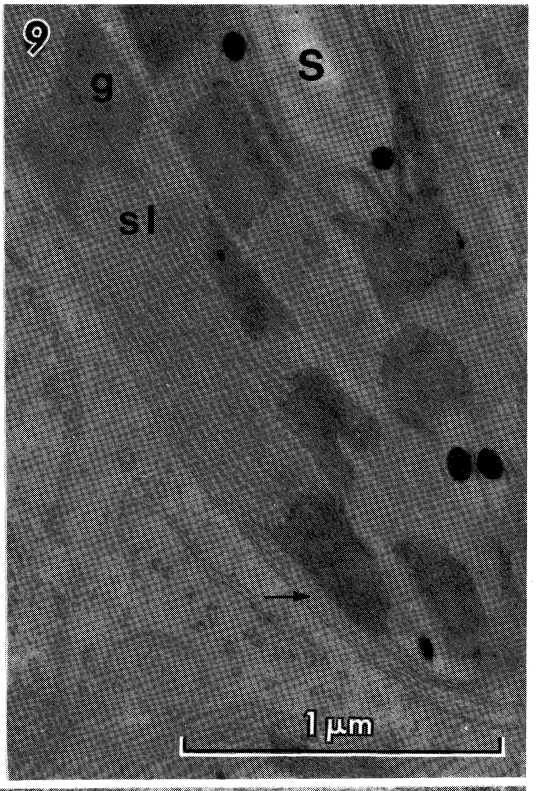
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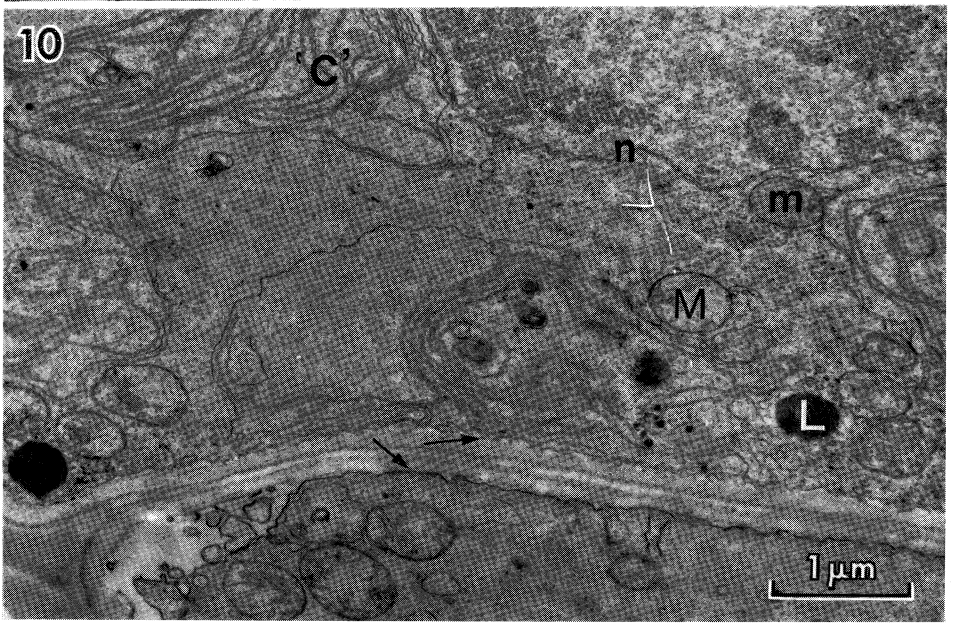
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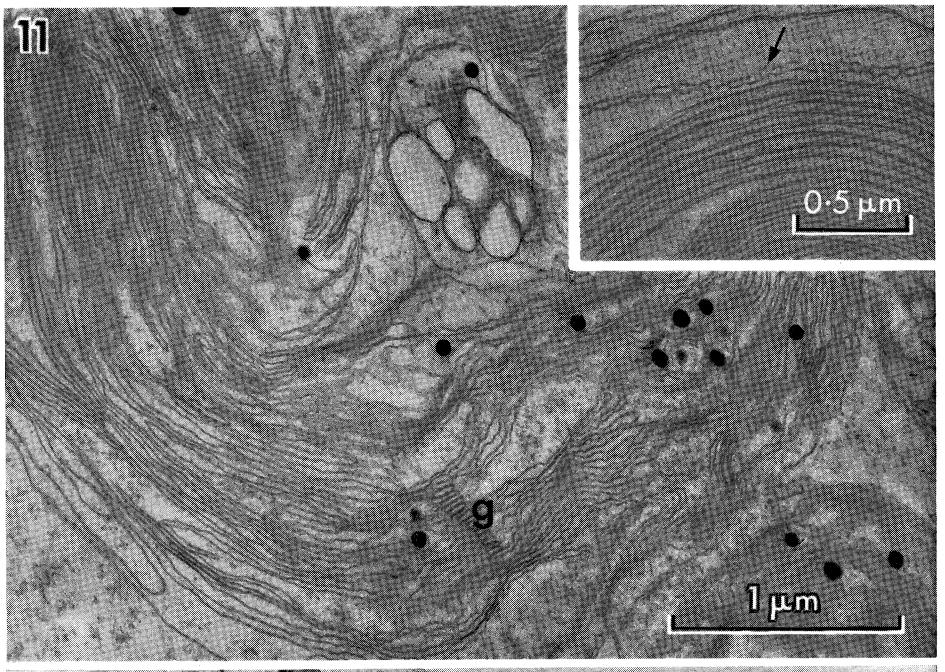
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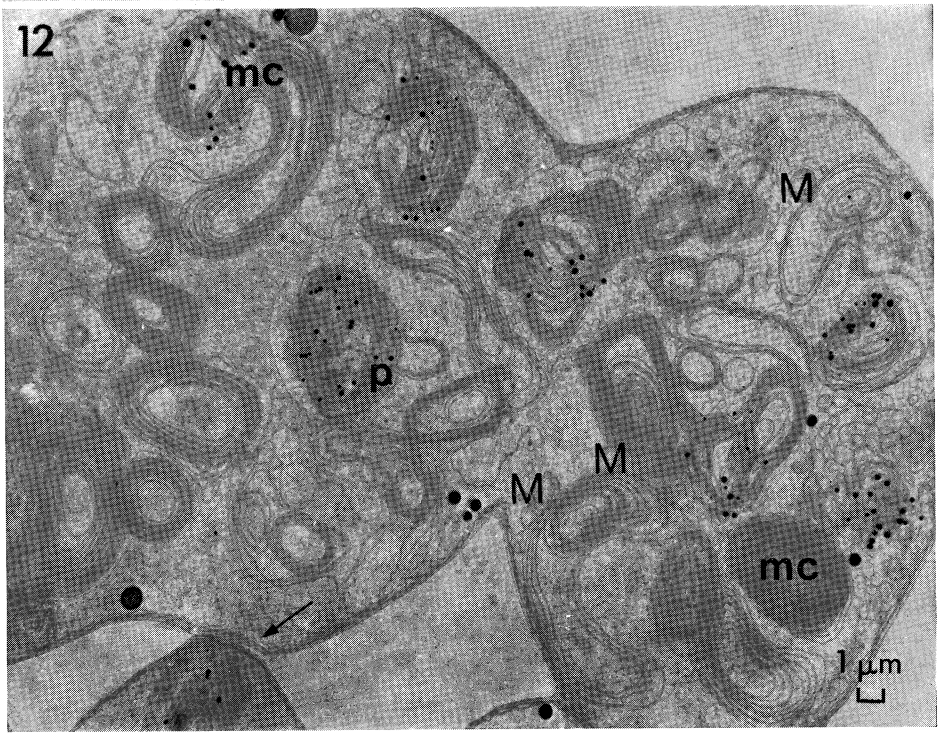
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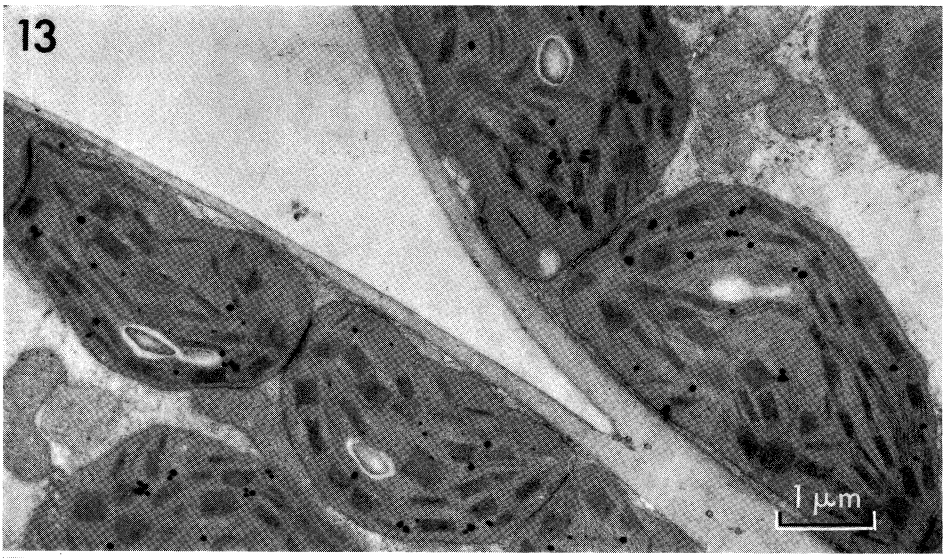
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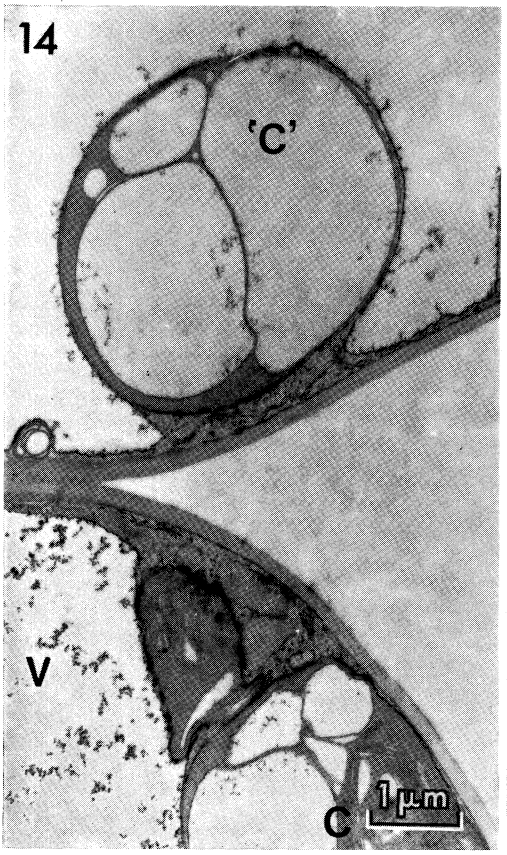
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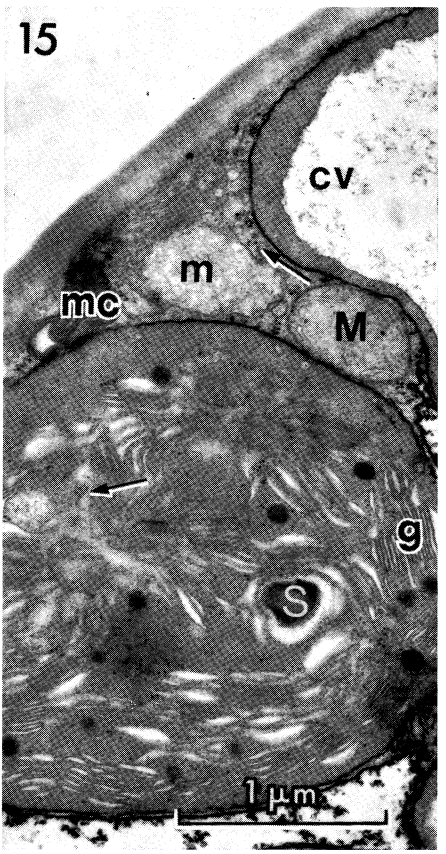
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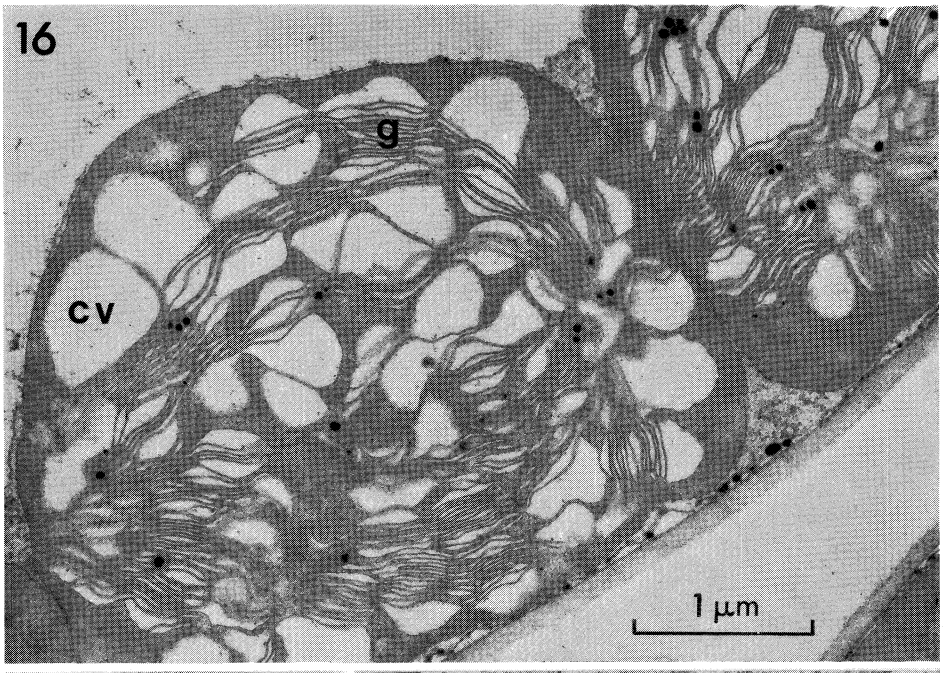
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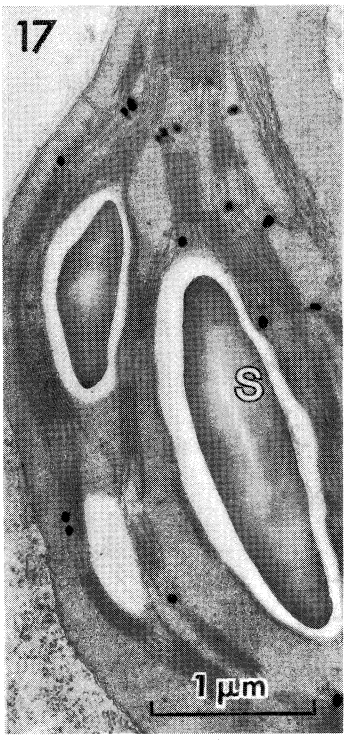
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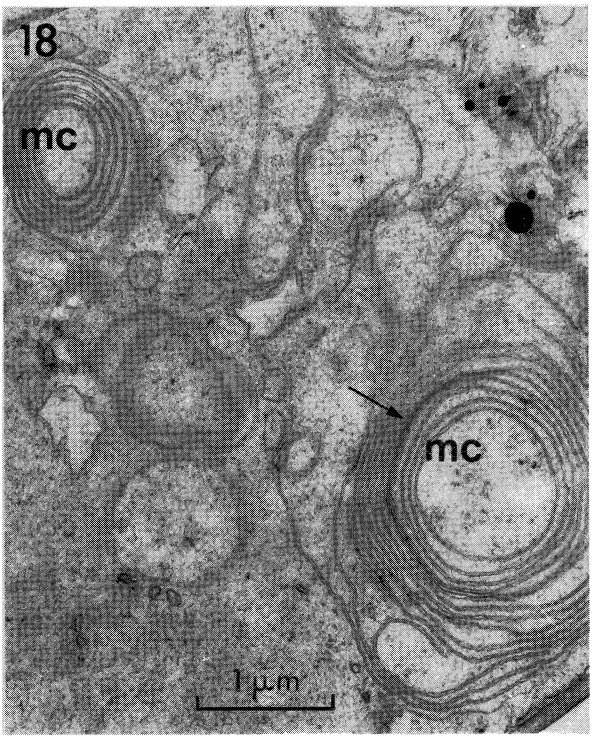
18
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mc
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However, with dark incubation for 6 hr, the only difference observed between the control leaf tissue (Fig. 19) and the Tris-treated leaf tissue (Fig. 20) is the presence of vacuolar deposits in the latter.

Little remains of the original chloroplast structure in Tris-treated leaf tissue after light incubation for 24 hr (Fig. 21). Only a few granal remnants can be detected. Although the tonoplast is broken the plasmalemma and mitochondria appear intact.

IV. Discussion

It is of particular interest that Tris-induced changes in chloroplast structure are associated with light incubation of the leaf tissue. Only with maize leaf segments treated with 200 mM Tris were ultrastructural changes induced in the dark. Ikehara and Sugahara (1969) found that pre-incubation of isolated spinach chloroplasts with 0·8M Tris, pH 8·0, in the light completely inactivated oxygen evolution whereas only a slight loss of activity was observed with dark pre-incubation. At pH values above 8·0, oxygen evolution was inactivated by Tris in both light and dark. Our experimental evidence suggests that maize leaf tissue may be particularly sensitive to treatment with Tris.

The Tris effect on ultrastructure was more pronounced at 200 mM than at 20 mM. Chloroplast structure of wheat leaves was affected by 200 mM Tris after incubation in the light for only 3 hr and by 6 hr little granal structure remained. Park and Sane (1971) consider that oxygen evolution and photosystem II are situated within the grana lamellae. Chloride is known to be involved in the water-splitting steps of photosynthesis (Bové *et al.* 1963). We have found that the uptake of chloride in wheat leaf segments is inhibited by Tris within 3 hr in both light and dark (Mittelheuser and Van Steveninck, unpublished results). Thus, it seems reasonable to consider that light-induced Tris inhibition of the Hill reaction and photosynthetic electron transport might be associated with the drastic effect of Tris on chloroplast membranes described here.

The absence of starch in Tris-treated leaf segments may be a pH effect as little starch was observed in tissue treated with either Tricine or sodium phosphate buffer at pH 8·0.

The nature of the vacuolar deposits in Tris-treated leaf tissue with both light and dark incubation is unknown. These deposits were not always apparent after dark incubation, nor were they observed in Tris-treated tissue fixed in osmium tetroxide. It is possible that the deposits may represent a reaction product of glutaraldehyde and Tris, or, alternatively, they could be the result of precipitation of cytoplasm and vacuolar material following rupture of the tonoplast.

Fig. 16.—Chloroplasts of wheat leaf segments after incubation in 200 mM Tris for 3 hr in the light. Note chloroplast vacuoles (*cv*) and separation of grana lamellae (*g*).

Fig. 17.—Chloroplast of wheat leaf segments after incubation in the control medium for 6 hr in the light showing starch grains (*S*).

Fig. 18.—Membrane coils (*mc*) in cell from wheat leaf segments after incubation in 200 mM Tris for 6 hr in the light. One of the membrane coils may have arisen from a chloroplast as a region (arrow) resembles a remnant of grana lamellae.

It is difficult to account for the Tris-induced coiling of membranes. Tris appeared to attack preferentially chloroplast membranes and the tonoplast rather than mitochondrial membranes, the membrane of microbodies, the plasmalemma, Golgi apparatus, or nuclear envelope. Some cytoplasmic membrane coils appeared to have arisen from the endoplasmic reticulum, but there were no attached ribosomes. It has been suggested that the stimulatory effect of Tris on cation uptake in sliced beetroot tissue may depend partly on the capacity of Tris base to act as a proton acceptor (Van Steveninck 1966*a*, 1966*b*). Taking into account the absence of changes with the zwitterion Tricine, it seems possible that the effect on chloroplasts of Tris at relatively high pH may involve a mechanism by which Tris base readily penetrates into the chloroplast and then interacts with the membranes in a capacity as a proton acceptor.

In considering the results of this ultrastructural investigation and other known adverse effects of Tris described earlier, it is suggested that Tris at high pH values be used with caution as a buffer for reactions involving green plant tissue.

V. Acknowledgments

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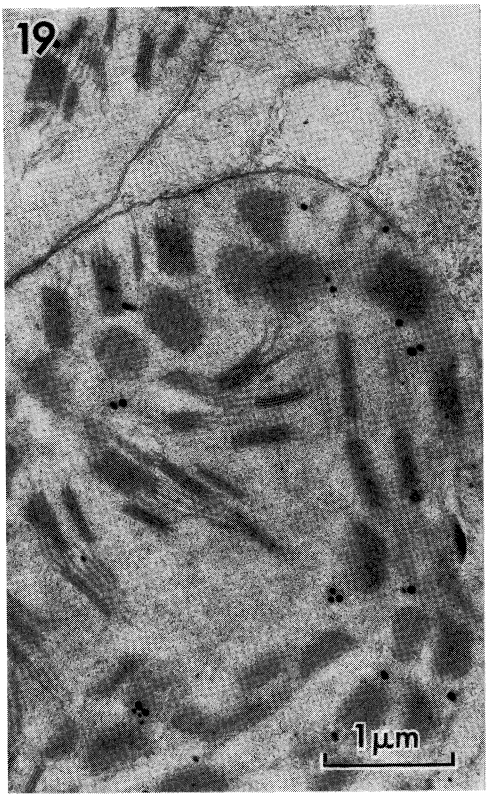
Fig. 19.—Chloroplast of wheat leaf segments after incubation in the control medium for 6 hr in the dark.

Fig. 20.—Chloroplast of wheat leaf segments after incubation in 200 mM Tris for 6 hr in the dark. Grana lamellae (*g*) are intact and small starch grains (*S*) are present. Deposits are present in the vacuole (*V*).

Fig. 21.—Part of a cell from wheat leaf segments after incubation in 200 mM Tris for 24 hr in the light. The presence of plastoglobuli (*p*) and possible remnants of grana lamellae (arrows) indicate that the regions of disordered membranes may have been chloroplasts. The tonoplast cannot be detected but the plasmalemma (*Pl*) can be observed.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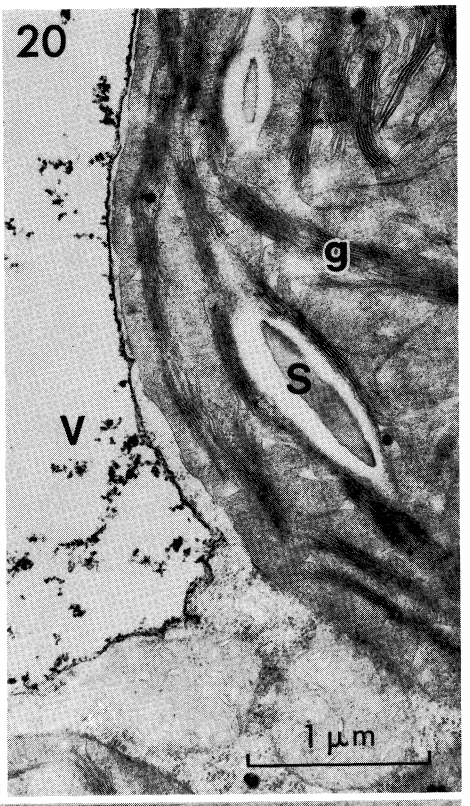
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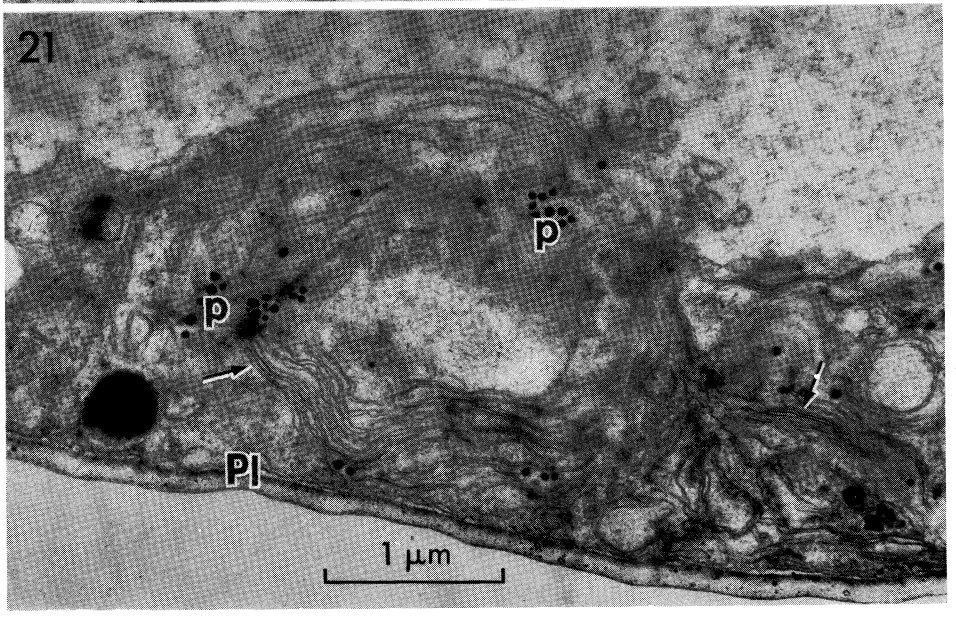
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