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SUBMICROSCOPIC STRUCTURE OF SOME CHLOROPLASTS

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INTRODUCTION

With regard to a study on chlorophyll concentrations in plastids from various species (THOMAS, MINNAERT and ELBERS (1956)) ultrathin sections of a number of these organelles were prepared and examined under the electron microscope. The technique used in this investigation is described in the present paper while some of the electron micrographs are shown and discussed.

METHODS

Freshly collected leaves were cut into pieces of about 0.5 mm thickness. Unicellular algae and bacteria were harvested by centrifugation.

Fixation was carried out in a 1 % osmium tetroxide solution, phosphate-buffered at p_H 7.2. The specimens were fixed at room temperature for 4 hours. Afterwards, they were briefly washed with distilled water, transferred into 70 % ethanol and left there overnight. After dehydration with 96 % ethanol for 2 hours and, next, with freshly prepared 100 % ethanol for 3 hours -3 changes- the specimens were transferred into a mixture of 90 % n-butyl- and 10 % methylmethacrylate containing 1 % Luperco CDB (2, 4 dichlorobenzoylperoxide) as a catalyzer. During the following 3 hours the methacrylate was changed twice. Next, the specimens were embedded in a pre-polymerised methacrylate mixture. Complete polymerisation was obtained at 45° C after 36 hours.

Cutting was done with the aid of a glass edge. The microtome we used was constructed according to the following design, *cf.* Fig. 1.

The specimen block is radially inserted in a steel cylinder (1) with a diameter of 4.5 cm and rotating with a specially devised axis in a V-shaped bearing (2). The axis was ground, lapped and honed to the highest possible degree of roundness.

The knife is adjusted in its holder between two rigid spring blades

(3) which are vertically mounted on a support with micrometer screw for gross adjustment. The top ends of the springs together with the knife holder move towards the rotating specimen by thermal expansion of a small brass bar (4) which is mounted between the knife holder support and a rigid stop block. This brass bar is heat-insulated from the microtome by means of two glass beads. It is heated electrically from room temperature to about 60° C. This meets the preparation of 2500 sections of 200 Å thickness.

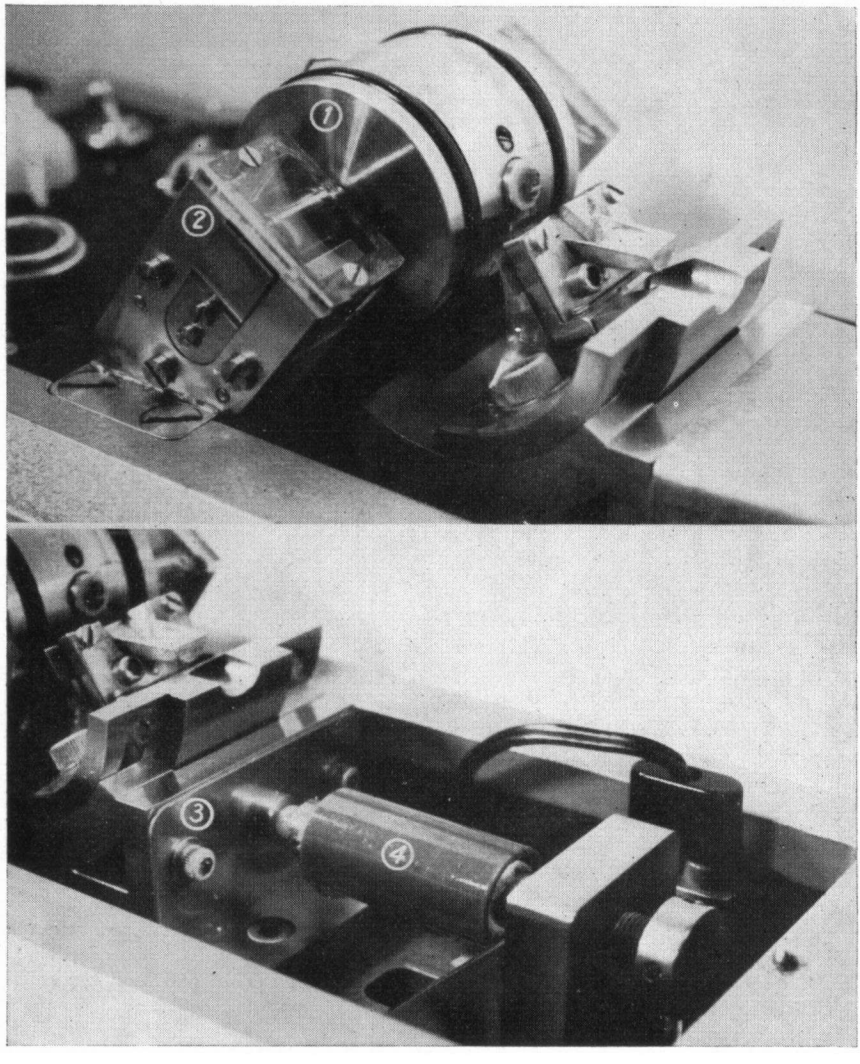
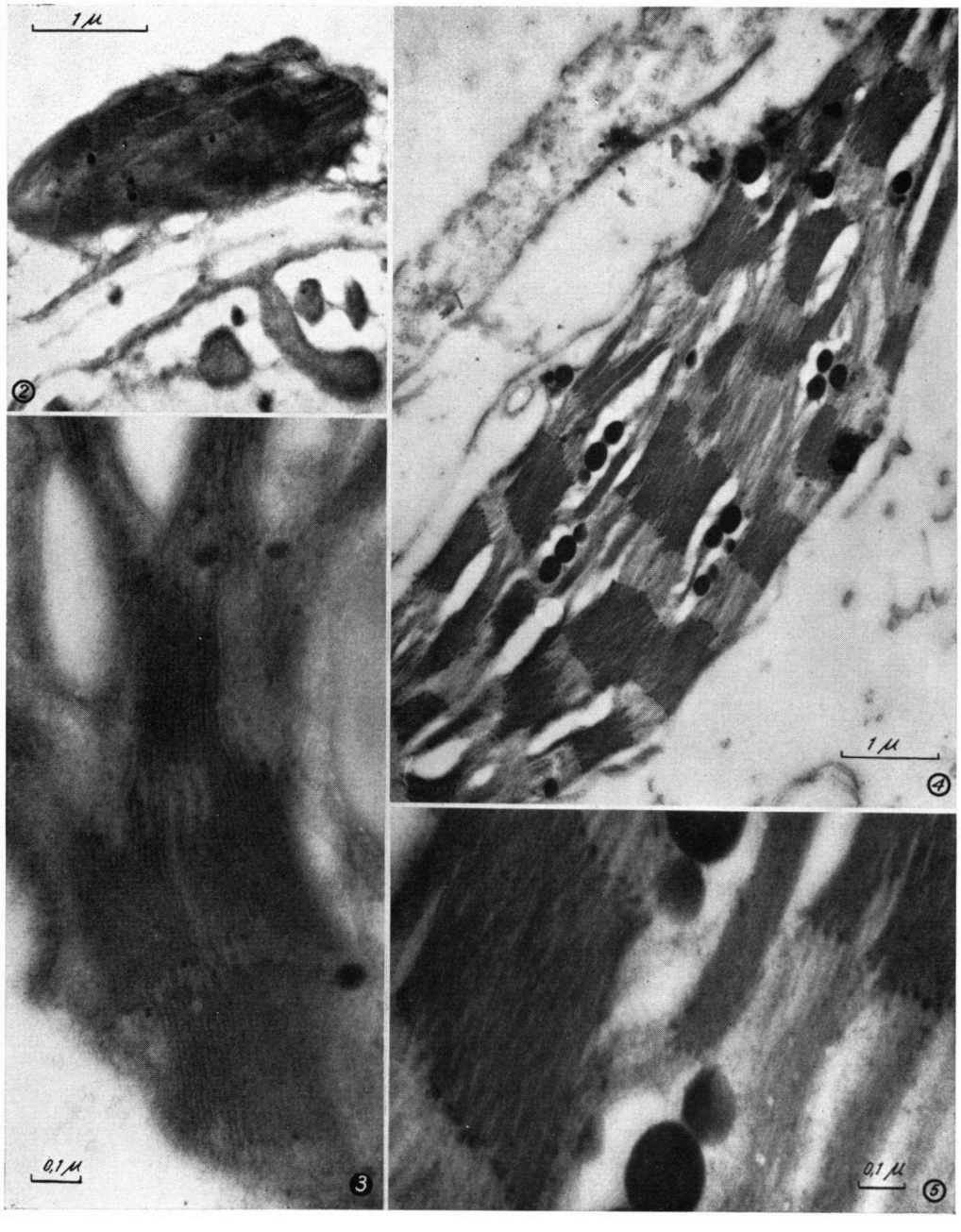
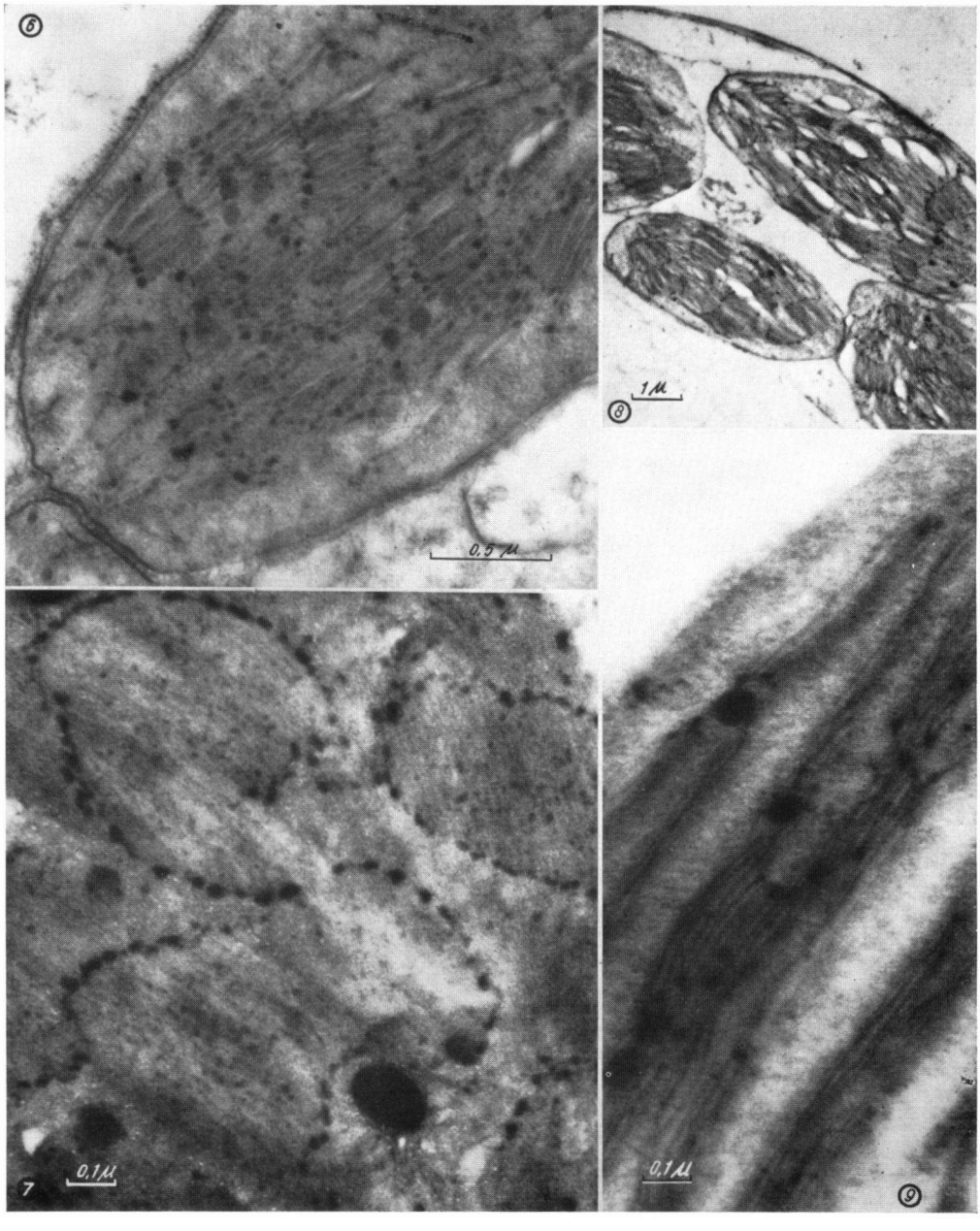


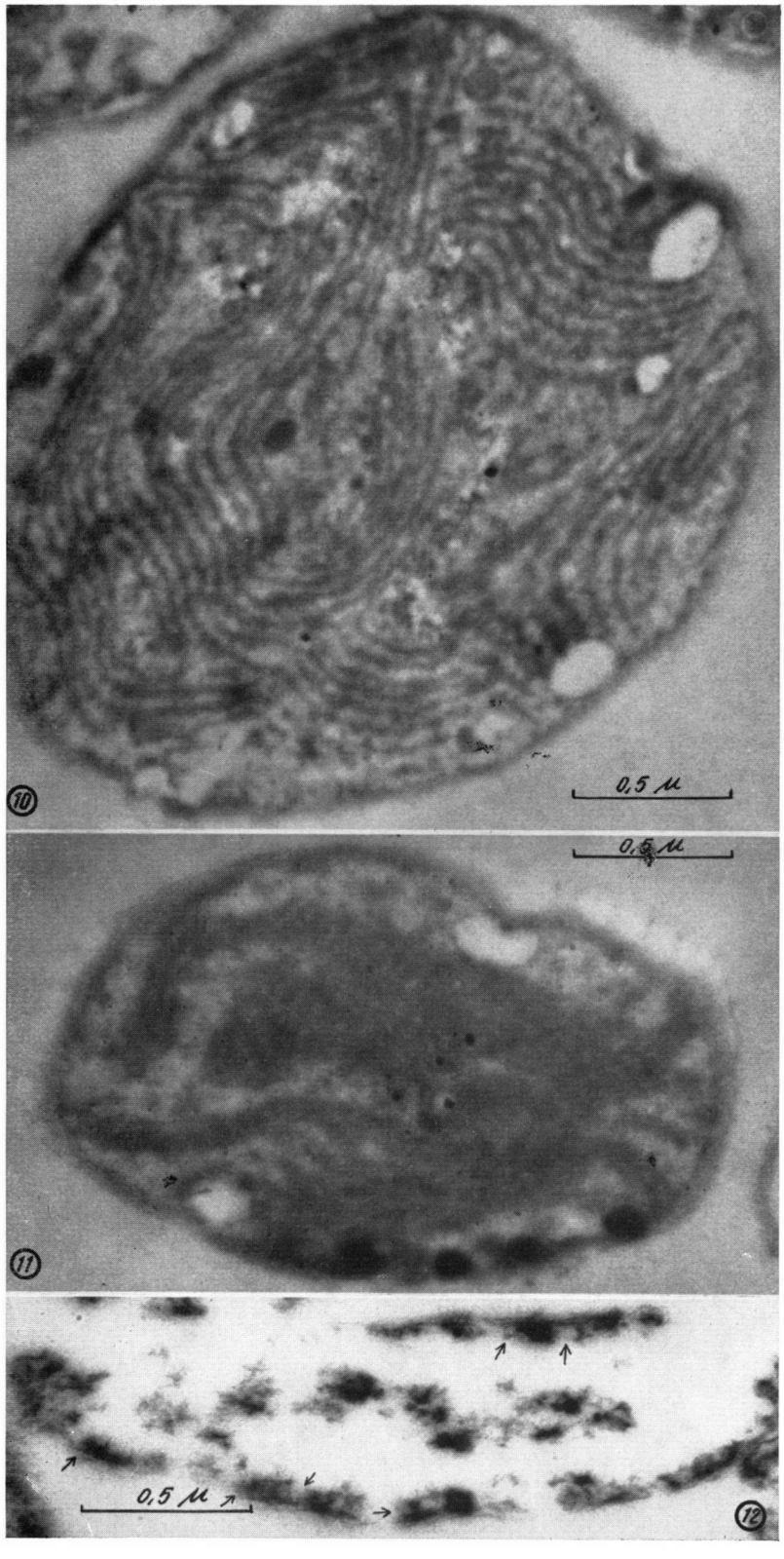
Fig. 1. The microtome. Explanation see text.



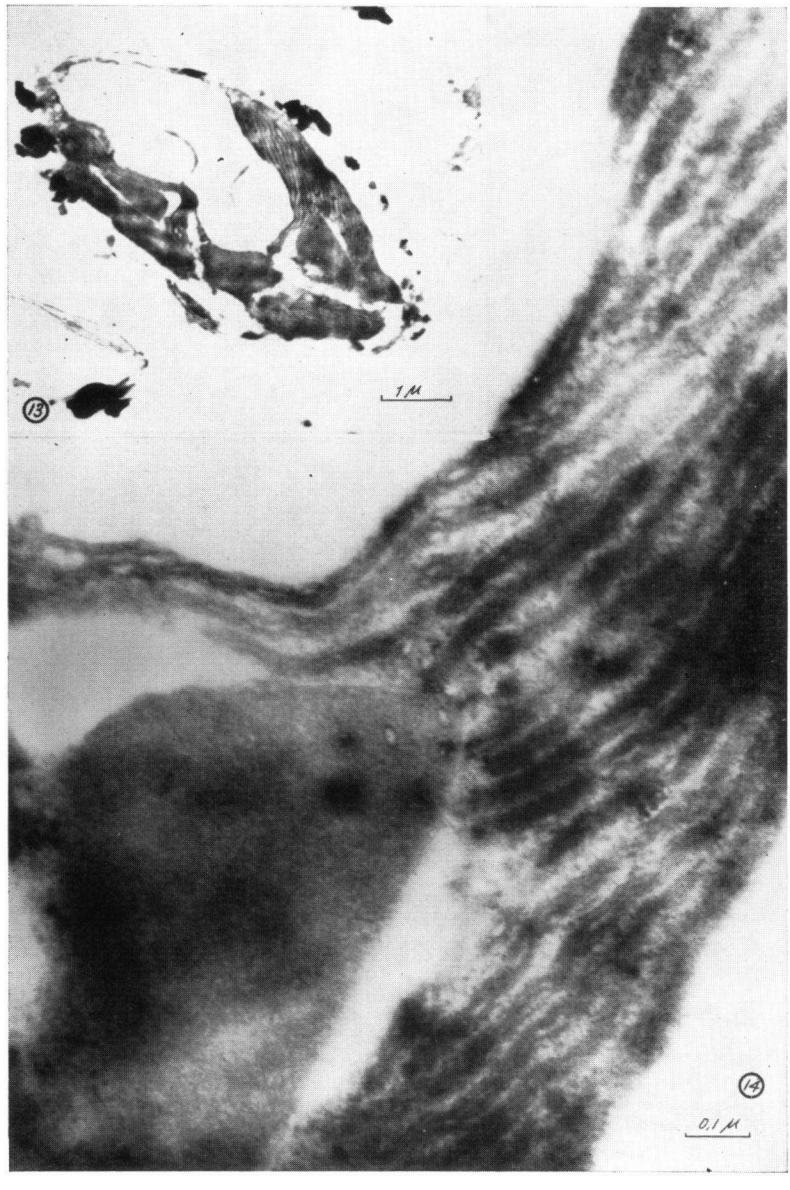
Figs. 2–5. 2: Chloroplast of *Elodea densa*. 3: Detail of fig. 2. 4: Chloroplast of *Aspidistra elatior*. 5: Detail of fig. 4.



Figs. 6–9. 6: Tulip chloroplast. 7: Detail of a chloroplast of *Hibiscus rosa sinensis*. 8: Chloroplasts of *Hibiscus rosa sinensis*. 9: Chloroplast of the sugar beet.



Figs. 10–12. 10 and 11: Sections of whole cells of *Synechococcus cedrorum*. 12: Section of a cell of *Rhodospirillum rubrum*.



Figs. 13–14. 13: Section of a whole cell of *Nitzschia dissipata*. 14: Detail of the chloroplast from fig. 13.

The heating current can be controled with a potentiometer and a transformer. A section thickness of 200 Å is obtained with a heating current of 80 mA at 4.5 V. The steel cylinder carrying the specimen is rotated by a vibration-free coupled asynchronous electromotor with a high torque providing a cutting period of about one second.

The actual cutting speed is regulated as follows. The rotating steel cylinder operates an electronic relay which reverses the electric connections to the motor when the specimen reaches a point at about 60° before the cutting position is reached. This yields a turning force in the opposite direction for the motor. The braking effect thus obtained is regulated with a variable resistance in the proper electric connections to the motor. Just after cutting, the electric connections are reversed again by the electronic relay, thus allowing the steel cylinder to rotate once more at the original speed.

In this way a very low actual cutting speed can be obtained, the cutting period remaining short and practically constant. Such a low cutting speed is essential for avoiding vibrations of the block and the knife edge during the cutting of the object.

The microtome has now worked satisfactorily for a period of $2\frac{1}{2}$ years.

The sections were studied with a Philips microscope type EM 100 at 60 kV. The minimum astigmatism of the 25 Å non-compensated objective lens was about 1.5 μ. The photographs were made at initial magnifications of 2700 ×, 6500 ×, and 13000 × on Kodak Fine Grain Positive film and processed in Kodak D 11 or D 19 b developer. The prints were made on extra hard paper.

Results

Fig. 2 represents a section of a chloroplast of *Elodea densa*. Between the grana, stroma lamellae are visible. The stroma, however, also consists of a granular component. Some details are shown in Fig. 3. The stroma lamellae seem to show a forking as they enter the grana. In this way double lamellae are formed. At the boundary of the grana osmiophilic substance is present. The same is shown most clearly in sections of chloroplasts of *Aspidistra elatior*, *cf.* Figs. 4 and 5, tulip, Fig. 6, and *Hibiscus rosa sinensis*, Figs. 7 and 8. Apart from these osmiophilic regions at the granum-stroma boundary, relatively large osmiophilic globules occur in the stroma. They can be seen very clearly in Figs. 4 and 5.

The sections mentioned so far were prepared from full-grown chloroplasts. It is obvious that the ratio of the quantities of both lamellar and granular stroma varies for chloroplasts from various species.

Fig. 9 shows developing grana in a young chloroplast of *Beta saccharifera*. At the lamellar furcation points concentration of osmiophilic substance is discernable.

Figs. 10 and 11 represent sections of the blue-green alga *Synechococcus cedrorum*. Instead of free grana, as was anticipated, a more or less cup-shaped chloroplast can be observed in Fig. 11. Most probably,

Fig. 10 shows a cross-section through the "bottom" of the chloroplast "cup". This figure also shows that the lamellae occur in bundles.

Fig. 12 shows free grana in the purple bacterium *Rhodospirillum rubrum*. The spots at which lamellae are discernable are indicated by arrows.

Figs. 13 and 14 refer to a chloroplast of the diatom *Nitzschia dissipata* containing a large deposit of photosynthesates (×). The lamellae occur in bundles of 4 to 6 throughout the chloroplast.

Discussion

Since, due to the presence of lipids, in dried chloroplast preparations precipitation membranes were observed under the electron microscope, it was argued by *e.g.* Frey–Wyssling and Steinmann (1953) that layers which were interpreted to represent chloroplast membranes may well be such precipitation membranes. Though this possibility should not be neglected, results obtained with improved sectioning techniques seem to prove that chloroplast membranes do occur. This evidence was procured by Leyon (1954) who also noticed that this membrane consists of two layers, and by Mercer *et al.* (1955) who, moreover, found indications for the view that the chloroplast membrane is differentially permeable. The present electron micrographs, Figs. 2, 4, 6, and 8, support the evidence for the existence of an outer chloroplast membrane. At × in Fig. 6 an indication of its two-layered structure occurs.

Electron micrographs of dried chloroplast suspensions suggest that grana are disc-like structures. From experiments on mild protease and lipase digestion of spinach grana Thomas, Bustraan and Paris (1952) concluded that, most probably, these grana are surrounded by a membrane consisting of an inner protein layer and an outer lipid one. However, in electron micrographs of ultra-thin sections grana membranes were not observed and, thus, the membrane-like structures described by these authors may well be artifacts. Moreover, the sectioning technique reveals that the way in which the intragranar lamellae are piled up is not as perfect as is suggested by pictures of dried grana suspensions.

What happens to stroma lamellae as they enter grana, is a question on which opinions diverge. Leyon (1954, 1956) suggested that, in the *Aspidistra* chloroplast, "some substances" are deposited at distinct circular spots of the stroma lamellae which become thickened in this way. A pile of such thickened lamellae may constitute a granum. Thus, the number of grana lamellae is the same as that of stroma lamellae. However, Leyon mentioned that, in the micrographs, the boundary between grana and stroma lamellae often is somewhat obscured because of the occurrence of osmiophilic substance in this region. The occurrence of such a substance was also observed in the present study.

Hodge, McLean and Mercer (1955), working with chloroplasts of *Zea mays* and *Hydrangea*, observed a forking of the stroma lamellae

as they enter the grana. In this way, the number of grana lamellae is twice that of stroma lamellae.

STEINMANN and SJÖSTRAND (1955) presented a schematic picture of *Aspidistra* grana in which they agreed with LEYON with regard to the ratio of both kinds of lamellae. However, they assumed that LEYON's "deposited substance", apart from covering the opposite surfaces of a pair of lamellae, also connects these layers at the peripheral zone of the granum. In this way, a granum lamella was suggested to consist of two layers which are linked together at the periphery of the granum. Fine electron micrographs support this conception. However, these pictures do not seem to exclude the possibility that forking of the lamellae, when entering the grana, also occur in *Aspidistra* chloroplasts at some spots.

STUBBE and VON WETTSTEIN (1955) presented a schematic picture for *Oenothera* chloroplasts, which, in a sense, is intermediary between those of LEYON (1956) and STEINMANN and SJÖSTRAND (1955).

As far as can be concluded from the present micrographs, both forking of stroma lamellae when entering the grana, and a linking in pairs of these lamellae by the aid of "some substance" in this region, might occur in chloroplasts of *Elodea*, *Aspidistra*, tulip and sugar beet. However, to the authors' opinion some uncertainty about this conception remains. In any case, the grana lamellae of *Aspidistra* must consist of two circular layers which are connected to each other at their circumference. Otherwise, swelling phenomena such as those obtained by FREY-WYSSLING and STEINMANN (1953), see their Figs. 10 and 11, could never occur.

In contrast with the widely accepted conception, no free grana were observed in the blue-green alga *Synechococcus cedrorum*. When an aqueous extract of this alga is studied under the electron microscope, grana-like structures are observed (CALVIN and LYNCH, (1952), THOMAS (1952)). Apparently, these structures are artifacts. Fig. 11 shows the occurrence of a cup-shaped chloroplast in which the lamellae occur in bundles of 4–6 throughout the plastid. This chloroplast more or less resembles the *Chlorella* chloroplast (ALBERTSSON and LEYON, (1954)). In *Phormidium uncinatum*, also a blue-green alga, NIKLOWITZ and DREWS (1955*a*) observed a lamellate "Chromatoplasma". At distinct spots the lamellae are thickened in a grana-like manner. The authors are of opinion that here too a single "chromatophor" per cell occurs.

NIKLOWITZ and DREWS (1955*b*) also prepared ultra-thin sections of the Athiorhodacea *Rhodospirillum rubrum*. The present Fig. 12 is in agreement with their results.

The structure of the diatom chloroplast, Figs. 13 and 14, much resembles that of the *Fucus* chloroplast (LEYON and VON WETTSTEIN, (1954)). This figure may demonstrate that the chlorophyll-bearing plastid of the diatom *Nitzschia dissipata*, *cf.* THOMAS, MINNAERT and ELBERS (1956), should be considered as a lamellate chloroplast. The lamellae occur in bundles of 4–6 also here.

SUMMARY

A microtome for preparing ultra-thin sections is described.

Some electron micrographs of chloroplasts from different species and of free grana in a purple bacterium are presented and discussed.

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