# SOME DATA ON THE STRUCTURE OF THE CHLOROPLAST, OBTAINED BY ELECTRON MICROSCOPY

by

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## INTRODUCTION

In 1883 A. MEYER wrote: "In der Tat lässt sich an dem lebenden Autoplasten (i.e., the chloroplast), wenn er völlig unverletzt ist, eine eigentümliche Struktur erkennen, die den Eindruck macht, als seien in eine mehr oder weniger farblose Grundmasse und von dieser überall umschlossen dunkelgrüne Körner oder Kugeln eingelagert."

SCHIMPER (1885) was of the same opinion; green grana embedded in a colourless stroma should occur in the chloroplast.

This theory, however, after a period of controversy, passed into oblivion till 1935.

In that year MISS J. DOUTRELIGNE published the results of her investigations on the structure of chloroplasts. She motivates the choice of the material, thin leaves of submersed aquatic plants, in these sentences: "Les divergences de vue montrent que la question méritait bien d'être reprise, mais il était essentiel que les recherches auxquelles nous nous proposions de nous livrer fussent exécutées sur du matériel en parfait état de conservation, observé autant que possible dans son milieu naturel, de façon à éviter toute cause d'erreur due à une altération des chloroplastes."

DOUTRELIGNE gives photographs in her publication showing a very distinct granular structure in the chloroplast. Her results are a firm base for further investigation.

In 1936 and 1937 HEITZ published the results of his important investigations on the structure of the chloroplast. He was able to prove that the grana are not grana in the strict sense of the word, but are small discs of a diameter of 0.35–1.7 $\mu$ situated parallel to the outer surface of the chloroplast.

Another point of interest is the observation that even by means of photography with ultraviolet light no differentiation in the internal structure of the grana could be discovered.

An increasing number of investigators described the granular structure of the chloroplast, not only, in higher plants but in mosses and algae too. For particulars we refer to the summarizing literature (RABINOWITCH, 1945).

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Worth mentioning are the observations of MENKE and KOYDL (1939) and MENKE (1940). These investigators prepared sections of the large chloroplasts of *Anthoceros*. The sections, photographed in ultraviolet light, give the impression to be composed of a number of lamellae. The reproductions of the photographs in their publications, however, are not very convincing. MENKE is of the opinion that the grana might be parts of the lamellae in which certain compounds are accumulated.

KAUSCHE and RUSKA (1940 a) published an electron-micrograph of a broken tobacco chloroplast, showing a.o. a number of grana. It is remarkable that the grana are very divergent in diameter. By measuring the grana reproduced in the publication of these authors we come to the conclusion that the diameter varies from 0.1 to 0.4 $\mu$.

MISS ROBERTS (1940) published a number of microphotographs from which she concluded that the chloroplast is composed of smaller units, so-called "plastidules". These plastidules would contain the grana.

In 1942 the same author published some photographs, a.o. of chloroplasts, taken with an electron microscope. She gives as her opinion that "chloroplasts are composed of units 1 micron in size and these are termed plastid granules . . . The granule bodies are themselves composed of smaller units — about 0.5 microns in size — termed primary granules, and these, in turn, are composed of still smaller units — about 0.25 microns in size — termed secondary granules. The secondary granules are composed of units about 0.1 microns in diameter — called tertiary granules. These units are made up of quaternary granules — 0.04 microns in diameter, and again these are formed of 0.02 micron units or quintary granules."

The reproductions of her photographs, however, are not sufficiently clear to give convincing evidence of this very complicated structure.

Apart from the grana, another point of interest in relation to the structure of the chloroplast is the occurrence of the discs as described by KAUSCHE and RUSKA (1940 b).

Chloroplasts of *Nicotiana tabacum* occasionally show, as has been elucidated by electron microscopy, discs of a diameter varying from 0.4 to 2.5 $\mu$ with a thickness of 10 to 20 m$\mu$. The authors are of the opinion that these discs are of the utmost importance in relation to the lamellar structure of the chloroplast. They do not mention the way by which they measured the thickness of the discs nor the method by which they obtained them.

As a matter of fact the discs obtained by KAUSCHE and RUSKA seem very similar to the bladders described by HARTKAMP (1936) and by BUNGENBERG DE JONG and HARTKAMP (1939) in *Paramecium caudatum*. The authors summarize the literature on the occurence of similar bladders and compare them with those obtained in coacervates of phosphatides (BUNGENBERG DE JONG and WESTERKAMP , 1932).

It seemed sufficiently important to us to investigate by means of the electron microscope the possible occurrence of well-defined grana and the alleged internal structure of them. Besides we succeeded in obtaining some data on the nature of the discs of KAUSCHE and RUSKA.

*Material and methods*

Most plants store considerable amounts of amylum in their chloroplasts which may give trouble in photographing. Therefore the tulip was chosen because the chloroplasts of this plant do not synthesize amylum except those in the guard-cells of the stomates.

Leaves of the Darwin tulip William Pitt were grinded in a mincer. During this treatment a

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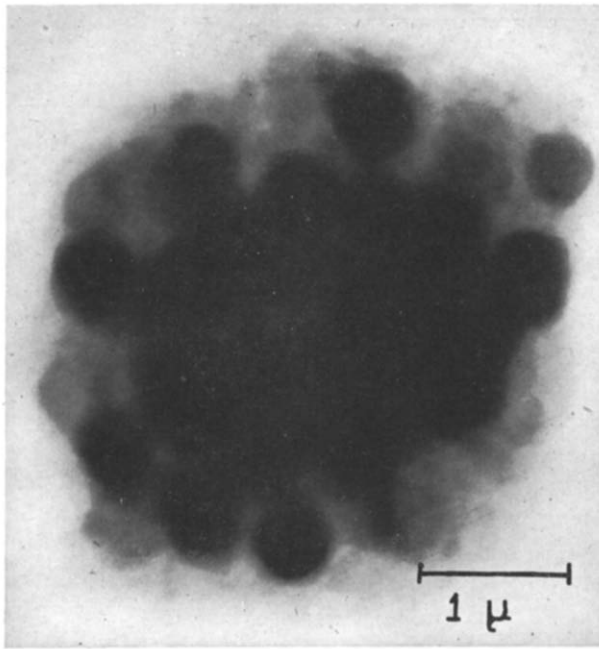


Fig. 1. Chloroplast of the tulip, showing grana. 20 000 ×. 80 Kv

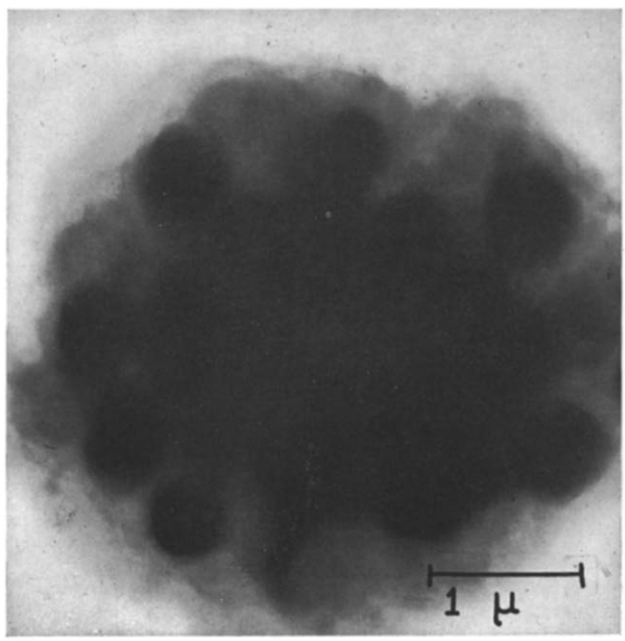


Fig. 2. Chloroplast of the tulip, showing grana. 20 000 ×. 80 Kv

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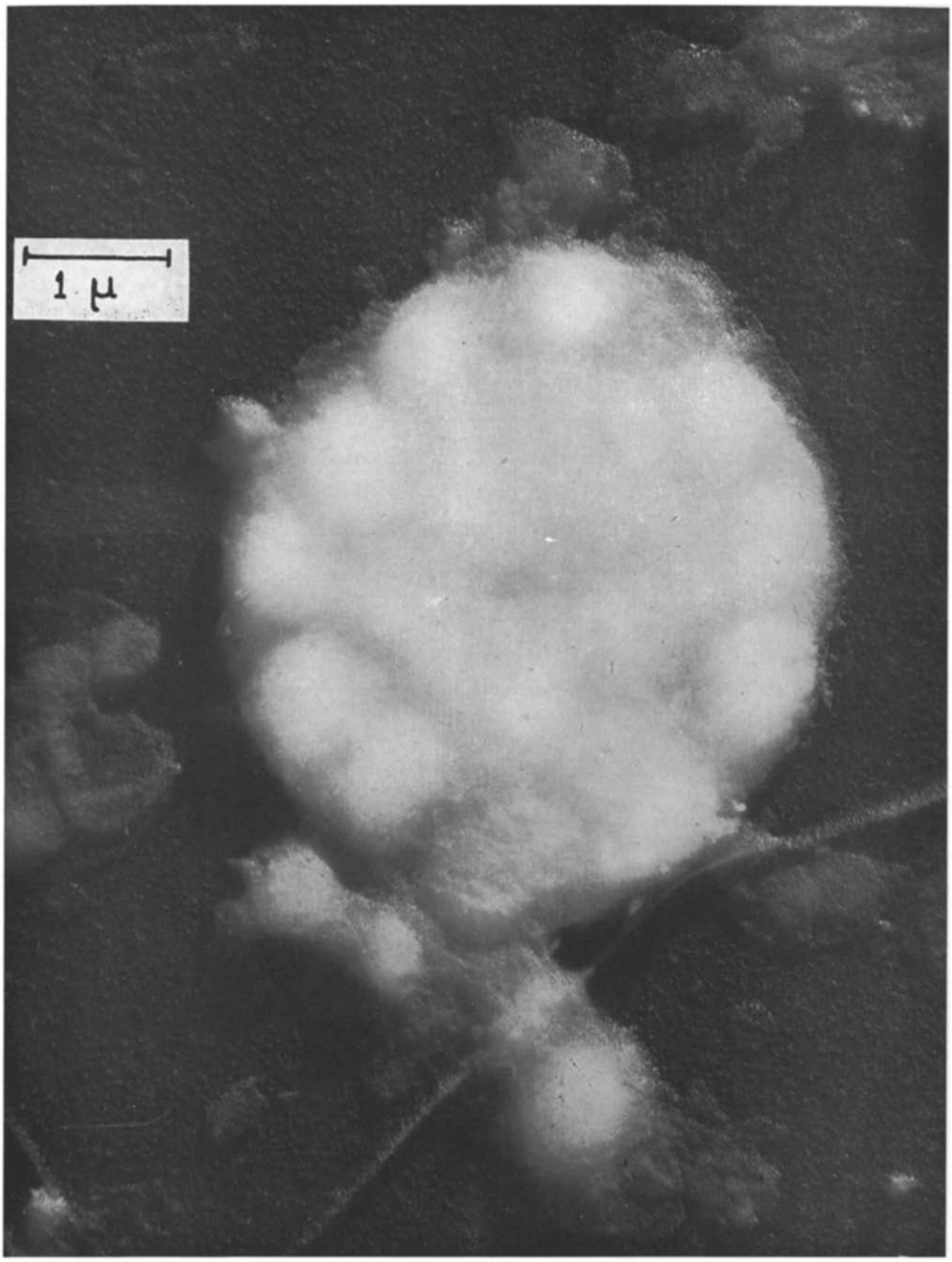


Fig. 3. Chloroplast of the tulip, showing grana. 20 000 X. 80 Kv. Shadowcast

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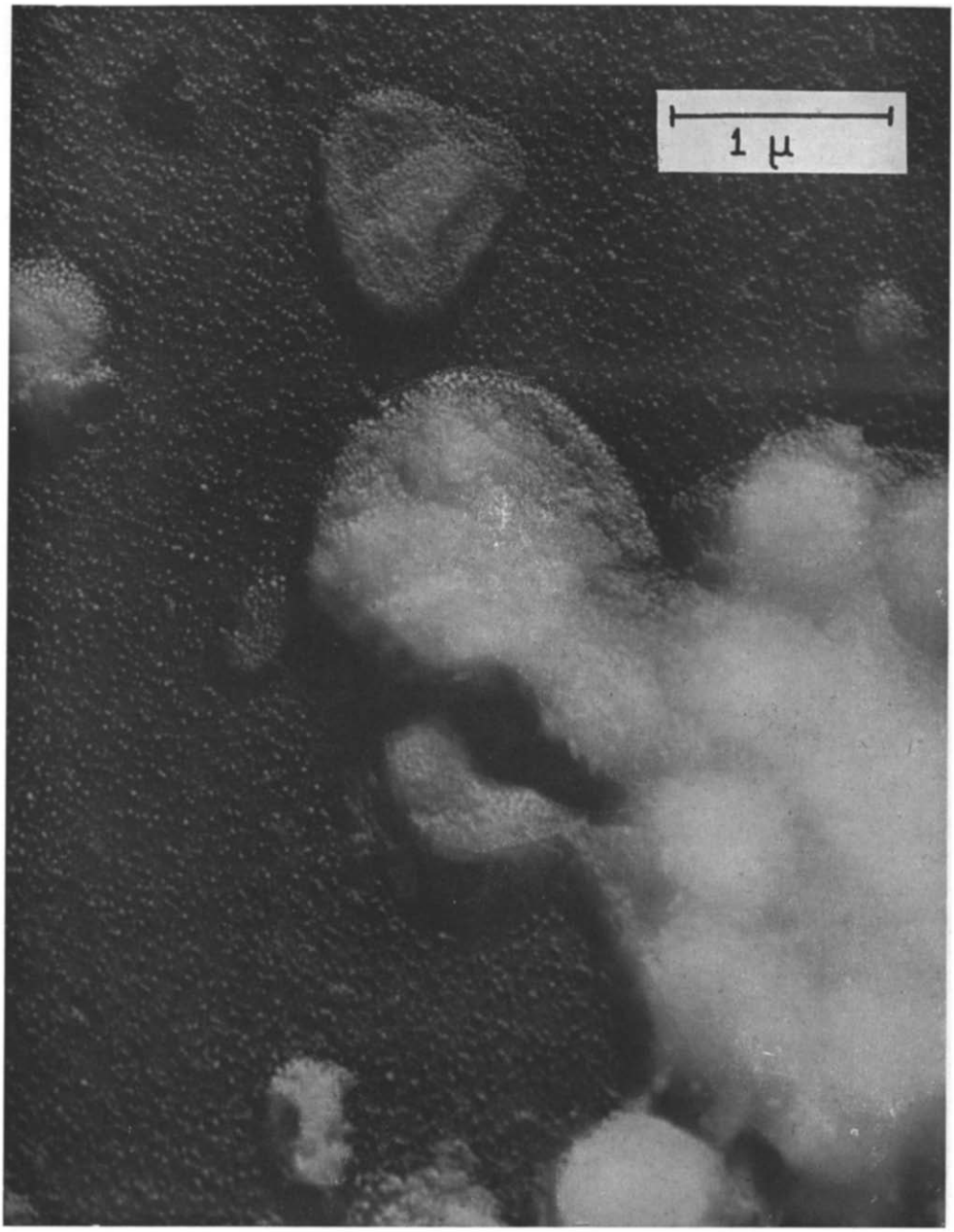


Fig. 4. Broken chloroplast of the tulip, showing the structure of the stroma. 30000 ×. 80 Kv. Shadowcast

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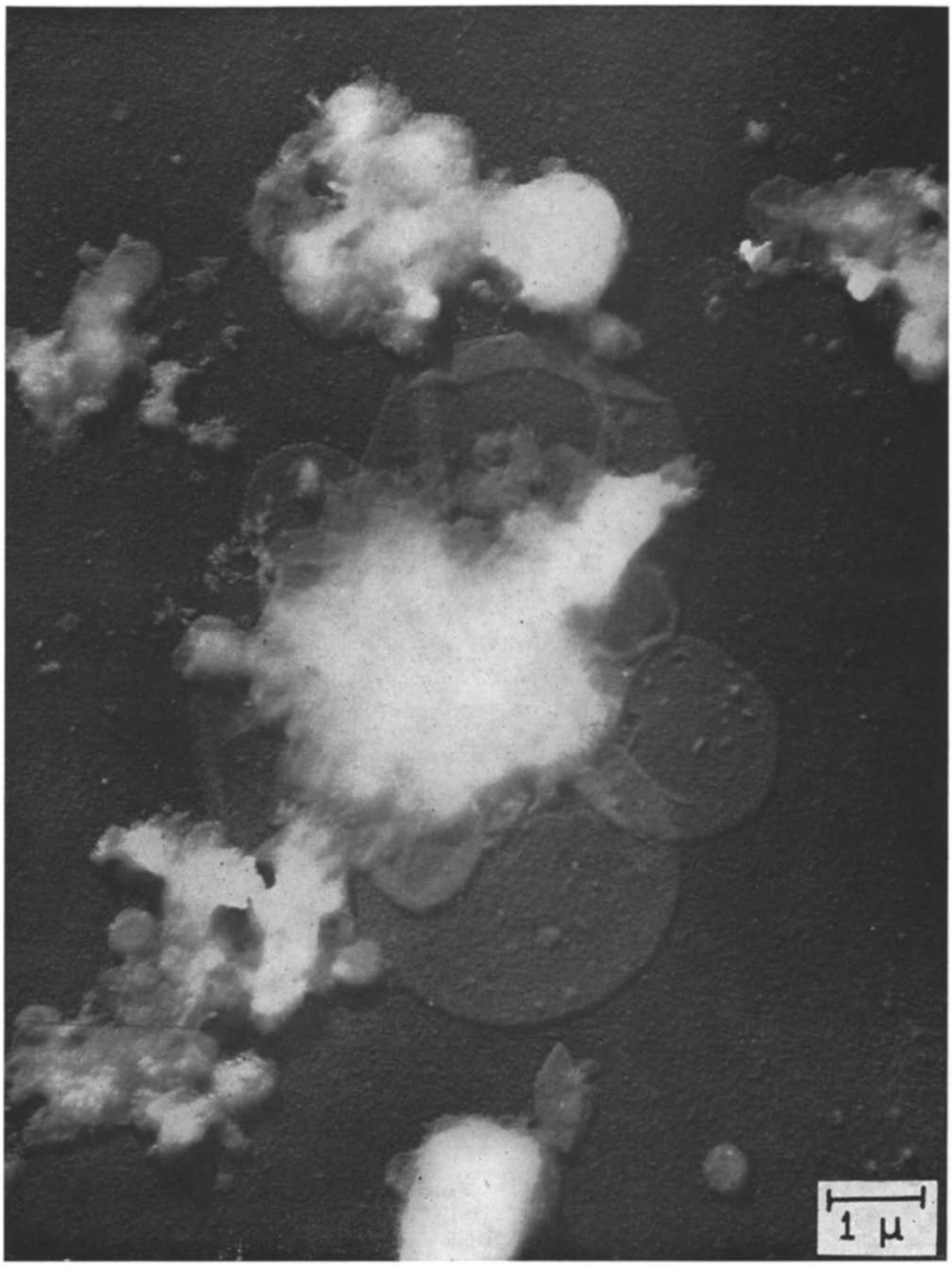


Fig. 5. Chloroplast of the tulip, showing bladders, some of them collapsed. 13 000 ×. 80 Kv. Shadowcast

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Fig. 6. Collapsed and heavily folded bladder. A piece of the membrane lying isolated. 20000 ×. 80 Kv. Shadowcast

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0.1 mol $Na_2CO_3$-solution was added drop by drop until the pH was about 6.0. After filtering through cheese-cloth the dark green opalescent suspension was centrifuged during 5 minutes at a speed of 3000 rpm. The dark green sediment was gently rubbed and resuspended in a 10 % saccharose solution. By centrifuging at a very low speed and resuspending in a 10 % saccharose solution several times a pure suspension of intact chloroplasts could be obtained. The grana were equally well visible — by means of the light microscope — in the chloroplasts of intact cells as in isolated chloroplasts.

For particulars of the electron microscope used — as well as of the further preparation of the objects — we may refer to the publication of one of us (L. H. BRETSCHNEIDER and WOUTERA VAN ITERSON, 1947).

As may be seen from the explanation of the photographs in some cases the shadow-casting method of WILLIAMS and WYCKOFF (1946) has been used.

To obtain a suitable contrast between the chloroplast grana and the surrounding stroma the shadow-casting method proved to be unnecessary (see Figs 1 and 2). It is, however, very useful in obtaining an impression of the three dimensial structure of the objects (see Figs 3–6).

*Internal structure of the chloroplast*

From the photographs Nos 1–3 it becomes clear that the chloroplast consists of a stroma with embedded grana. No evidence is brought forward with respect to the plastidules of MISS ROBERTS.

The grana appear almost perfectly circular. From a number of quite distinct grana of the reproduced photographs the diameter has been measured. The diameter amounts to about 0.6 $\mu$.

We did not try to fix the chloroplast in such a position as to prove the discoidal shape of the chloroplast-grana (HEITZ, 1936, 1937).

The perfect circular shape and the constancy in diameter are points strongly in favour of the theory of the existence of clearly circumscript units in the interior of the chloroplast, the grana.

As to the internal structure of the grana, we cannot subscribe to the views of ROBERTS. As far as we can see from our photographs the grana do not show any internal structure.

As to the structure of the stroma micrograph No 4 shows a heterogeneity. The shadow-cast technique reveals the presence of a large number of small granules with a rather uniform diameter of about 25 m$\mu$ both on the stroma and scattered all over the object film. The nature and the origin of these granules are at present quite unknown.

*Discs or bladders*

In the introduction we mentioned the discs or lamellae found by KAUSCHE and RUSKA along the border of chloroplasts of *Nicotiana tabacum* photographed with the electron microscope.

We drew the attention to the work of BUNGENBERG DE JONG and WESTERKAMP (1932) and especially of HARTKAMP (1936) and BUNGENBERG DE JONG and HARTKAMP (1939) who described phosphatide bladders on the surface of *Paramecium caudatum*. These bladders could be obtained, according to these authors, by the action of a large number of external factors.

It is our conviction that the phenomena observed at the chloroplasts of *Nicotiana tabacum* and of the Tulip are identical with those observed and investigated by BUNGENBERG DE JONG and his co-workers.

From our photographs it becomes clear at once that the shape of the objects along the border of the chloroplasts are no discs but small bladders which have originated in the final preparation of the object.

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Photograph No 5 shows a chloroplast from whose surface a number of bladders originates. The wall of some of the bladders is folded and still the circumference remains circular. This proves the impossibility of the discshaped structures of KAUSCHE and RUSKA.

Still better is photograph No. 6. Here the object is heavily folded, keeps its circular outline and shows a small piece broken out of the wall and lying apart. The shape of the isolated piece of wall and the way it is broken out of the wall already clearly shows that the formations arising from the surface of the maltreated chloroplasts are of the nature of true bladders.

Finally the diameter of the bladders is often larger than the diameter of the chloroplasts or fragments of chloroplasts themselves, a fact that is not in agreement with the alleged occurrence of discs coming out from the chloroplasts.

It has been possible to undertake this investigation by the courtesy and under the auspices of the Laboratory for Flowerbulb-research at Lisse, Holland (Director Prof. Dr E. VAN SLOGTEREN).

## SUMMARY

It has been shown:

1. That in the chloroplast of the tulip leaf no plastidules can be detected by means of the electron microscope.
2. That the granular structure of the chloroplast, with difficulty to be seen with the aid of the light microscope, is confirmed by making use of the electron microscope.
3. That the shape and size of the grana of the chloroplast of the tulip are singularly constant. In surface-view the grana are circular in shape, the diameter is about 0.6 $\mu$.
4. That an internal structure of the grana could not be observed.
5. That the stroma possibly shows some special structure.
6. That by the influence of some external factor bladders, probably phosphatide bladders, arise from the surface of the chloroplasts.

## RÉSUMÉ

Il a été montré que:

1. Dans les chloroplastes de la feuille de tulipe, le microscope électronique ne permet de détecter aucun plastidule.
2. La structure granulaire du chloroplaste, difficile à voir avec le microscope ordinaire, est bien mise en évidence par le microscope électronique.
3. La forme et la taille des grains du chloroplaste de la tulipe sont remarquablement constants. En projection plane les grains apparaissent comme des cercles de 0.6 $\mu$ environ de diamètre.
4. Une structure interne des grains n'a pu être observée.
5. Il est possible que le stroma présente quelque structure spéciale.
6. Sous l'influence d'un facteur externe, des vésicules, constituées probablement par des phosphatides, se forment à la surface des chloroplastes.

## ZUSAMMENFASSUNG

Es wurde gezeigt:

1. dass im Chloroplast des Tulpenblattes keine Plastidula mit Hilfe des Elektronenmikroskops entdeckt werden können;
2. dass die körnige Struktur des Chloroplasts, die mit Hilfe des Lichtmikroskops mit Mühe zu sehen ist, durch Benutzung des Elektronenmikroskops bestätigt wird;
3. dass die Form und Grösse der Körner des Tulpenchloroplasts besonders konstant sind. In der Oberflächenansicht sind die Körner von runder Form, und der Durchmesser ist ungefähr 0.6 $\mu$;
4. dass eine innere Struktur der Körner nicht wahrgenommen werden konnte;
5. dass das Stroma möglicherweise eine Spezialstruktur zeigt;
6. dass unter dem Einfluss irgendeines äusseren Faktors Blasen, wahrscheinlich Phosphatidblasen, aus der Oberfläche der Chloroplasten entstehen.

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