länge entspricht etwa 50 Einheiten. Desazetylierungsversuche wurden mit Lithiumhydroxyd, Bariumhydroxyd und Ammoniak angestellt. Polyserin (VI) wurde als harter Film erhalten. Es ist hygroskopisch, wasserlöslich und zeigt positive Biuret- und Ninhydrinreaktion.

Contribution to the Structure of Granular Chloroplasts

The ultrastructure of chloroplasts is of importance for an understanding of the complicated reactions connected with photosynthesis. Although the grana in the chloroplasts of the higher plants are visible using the light as well as the electron microscope[1], there are still some unsolved problems which concern the structure of these important cell constituents. Three of these may be mentioned:

(a) What is the connection between chloroplast ultrastructure and the characteristic swelling in unphysiological media?

(b) How are the grana which are arranged in layers parallel to the surface of the chloroplasts[2] connected with each other? Is the chloroplast enclosed by a definite membrane?

(c) Is it possible to prove the proposed lamellar structure of the grana?

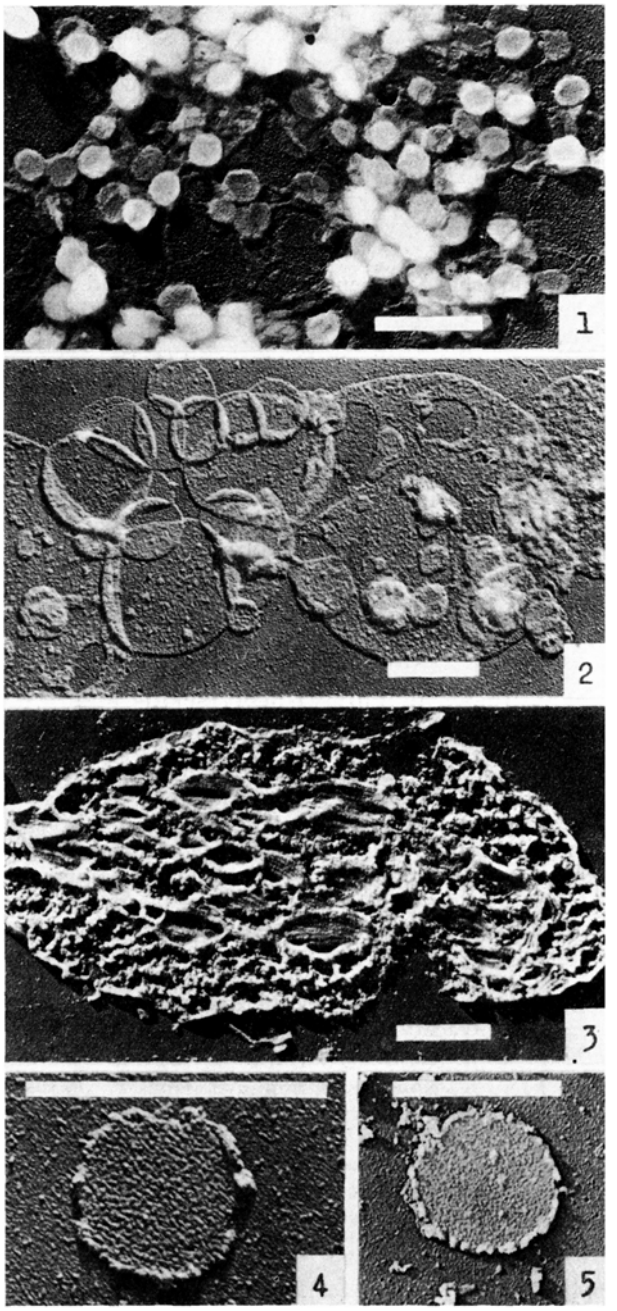
Modern methods of electron microscopy provide a mean to solve certain of these problems.

Swelling of Chloroplasts: The fact that different plant species show different patterns of swelling complicates the problem of the ultrastructural basis of swelling.

Sometimes the mere contact of chloroplasts with distilled water causes their swelling to structureless blebs of a foamy appearance. Thereby the grana, easily visible in material isolated with phosphate buffer pH 6·4 or sucrose solution (Fig. 1), disappear almost completely (Fig. 2). Spinach chloroplasts are very suitable for studying this phenomenon. Only exceptionally can intact grana be seen in preparations of swollen chloroplasts. We presume from the granular appearance of the surface of the formed blebs that the structural units responsible for the rapid swelling are globular particles. To consider these blebs, even when they are folded, as the remainder of a membrane surrounding the chloroplast, is highly questionable. As yet the existence of such a well defined membrane, suggested by indirect methods, has not been proved.

It will be an important task to investigate the connection between the characteristic swelling figures of certain chloroplasts and their ultrastructure. A valuable effort in this direction has been made by STRUGGER[3] using the phase microscope.

The Ultrastructure of the Grana: The same methods used to investigate the arrangement of the lamellae in the big algal chloroplasts[4] are suitable to inform us about the ultrastructure of the grana whose lamellar composition has already been suggested by experiments with the polarizing microscope[1] and the electron microscope[2].



The white mark on the photographs corresponds to one micron. All preparations are shadowed with chromium.

Fig. 1.–Spinach grana, isolated in phosphate buffer pH 6·4. 14,000:1.
Fig. 2.–Spinach chloroplast, isolated in distilled water. 12,000:1.
Fig. 3.–Section through a tulip chloroplast. Fixation 15 min with 1% OsO_4. 12,000:1.
Fig. 4.–Single circular layer obtained from a tulip chloroplast treated with sonic oscillations. Fixation 25 min with 1% OsO_4. 39,000:1.
Fig. 5.–Single circular layer obtained from an *Aspidistra* chloroplast treated with sonic oscillations. Fixation 17·5 h with 1% OsO_4. 22,000:1.

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Figure 3 shows a thin section through an OsO_4-fixed chloroplast of tulip prepared by standard methods (Minot rotary microtome, glass knife, *n*-butyl-methacrylate embedding). Many lens-shaped, regularly laminated areas corresponding to the grana can be observed. The structure of the section agrees well with a scheme proposed by FREY-WYSSLING[1]. However it is not yet possible to decide whether or not the so-called "carrier lamellae" are present by which the grana are supposed to be attached[2]. Further improvements in sectioning technique are necessary to settle this question.

By treating OsO_4-fixed granular chloroplasts with sonic oscillation (10 kc.), a suspension of circular lamellae of the diameter of a granum is obtained (Fig. 4 and 5). The thickness of these lamellae, about 70 Å, is in good agreement with the thickness of layers of similarly prepared algal chloroplasts.

We consider the circular lamellae as the structural units of a granum. A stack of such layers forming a cylinder composes the granum.

I wish to express my deep gratitude for the opportunity to carry out this work under the guidance and in the laboratory of Professor FRANCIS O. SCHMITT.

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Zusammenfassung

Es wird darauf hingewiesen, dass die an Chloroplasten zu beobachtenden Quellungserscheinungen auf keine wohldefinierte Chloroplastenmembran schliessen lassen. Durch Herstellung dünner Schnitte und durch Ultraschallbehandlung OsO_4-fixierter Chloroplasten kann gezeigt werden, dass die Granen aus Schichten aufgebaut sind.

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[3] Present address: Pflanzenbiologisches Institut der ETH., Zürich, Switzerland.

The Occurrence of Melanin and Phenolases in *Holothuria forskali* Delle Chiaje

Recent investigations by the author[1] have shown that the echinoderms *Diadema antillarum* Philippi, and *Thyone briareus* Le Sueur, contain an amorphous granular pigment characterized by insolubility in a wide range of solvents, bleaching when subjected to oxidizing agents, and by the capacity to reduce ammoniacal silver nitrate under certain conditions (see LISON[2]).

The abundant, readily obtained black pigment in the body wall of *Holothuria forskali* also shows these properties, being but slightly soluble in water, pyridine, 1·0 *N* hydrochloric acid and 0·5 *N* sodium hydroxide, and insoluble in ethanol, acetone, benzene, ether, petrol ether, chloroform and carbon disulphide at room temperatures. It is bleached by chlorine, bromine, hydrogen peroxide, chromic acid, potassium permanganate and oxalic acid, and will reduce directly ammoniacal silver nitrate. Such properties are characteristic of melanin[3], and these findings strengthen earlier indications of its occurrence in this and other echinoderms[1].

As in *Diadema* and *Thyone*[2], coelomic fluid exposed to air *in vitro* forms a clot which darkens due to the formation within it of a black or brown pigment. In both *Holothuria* and *Diadema*, under microscopic examination, this can be seen to be due to the development of a yellow to dark brown colour in spheroidal cytoplasmic inclusions of the coelomic amoebocytes. Black or brown pigment granules also frequently appear at the periphery of the spheroids, and *in vitro* the pigment is eventually liberated by disintegration of the amoebocytes, with the formation (in *Holothuria*) of a dense reticulum of chocolate-brown pigment. This at once recalls the appearance and apparent mode of origin, of the skin pigment[3].

Since it has been shown in *Diadema*[4], that the colouring of the clot involves the action of phenolases which are present in the coelomic amoebocytes, it is highly significant that the existence of such enzymes can be clearly shown in *Holothuria*. Coelomic fluid, exposed to air and incubated at 24°C with buffered substrates (pH 6·5–7·3) such as "dopa", cresol (mixed isomers) and glycine, pyrocatechol and pyrogallol, readily yielded coloured oxidation products, provided that a detergent such as saponin or "cetavlon" was added to the mixture. L-tyrosine is blackened within 3 h, but oxidation of this and pyrocatechol, is inhibited completely by boiling, and strongly by 0·0002 *M* potassium cyanide at pH 7·3. The oxidation appeared unaffected by acetone, and 0·002 *M* sodium azide at pH 7·3. Thus the oxidations involve both mono- and poly-phenolases and are not attributable to cytochrome-oxidase[5]. It must be noted, however, that they appear unaffected by 0·001 *M* thiourea at pH 6·5 and but little by ultra-violet irradiation.

The enzyme system is associated with the coelomic amoebocytes and is not present in the fluid per se, a fact which is most suggestive when it is borne in mind that it is in the amoebocytes that granular brown or black pigment appears in relation with the spheroids. It is also noteworthy that, whereas in *Diadema* a heat-labile factor inhibiting pigment production exists in the coelomic fluid[4], I have found no evidence of such a factor in *Holothuria*.

The precise relation between the phenolases and colouring of the spheroidal inclusions of the amoebocytes awaits further investigation, but it may be noted that in *Diadema*[4], pigment produced in the clot has been shown to possess the characteristics of melanin, and its production is influenced by the same factors as those which affect phenolases.

A full account of the work will be published elsewhere. My thanks are due to the Director and staff of the Plymouth Station of the Marine Biological Association of the United Kingdom where the work was carried out.

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