OBSERVATIONS ON THE STRUCTURE OF GRANA-CONTAINING CHLOROPLASTS AND A PROPOSED MODEL OF CHLOROPLAST STRUCTURE

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Summary

Observations have been made by phase contrast and fluorescence microscopy on chloroplasts both in living cells of higher plants and in an isolated state.

In the living cell the chloroplast is often surrounded by a mobile jacket of material which resembles mitochondrial substance. Many chloroplasts retain this outer jacket after isolation.

The behaviour of isolated chloroplasts in hypotonic sucrose solutions indicates that this jacket of mitochondria-like material is the only all-encompassing structure around the chloroplast. The jacket swells and ruptures in 0·1–0·2M sucrose solution. At still lower sucrose concentrations the remaining chloroplast behaves as though composed of numerous individual osmotic units. Many small blebs appear around the margin of the chloroplast and with increasing dilution these swell further, often to a larger size than the original chloroplast. The inner surfaces of these swollen blebs can fuse, but the outer surfaces do not have this property.

Grana are fairly resistant to swelling in hypotonic solutions and many are well preserved even in distilled water.

A model is proposed for the structure of a typical grana-containing chloroplast, and the swelling patterns which have been observed are interpreted in terms of this model. The chloroplast is depicted as a stack of flat sealed bags, each bag corresponding to a pair of stroma lamellae. The many blebs which form in sucrose concentrations below 0·1M are envisaged as individual swollen stroma bags. It is suggested that the chloroplast jacket is commonly represented as a double-layered outer membrane in electron-micrographs.

I. INTRODUCTION

This paper reports observations made by phase contrast and fluorescence microscopy on higher plant chloroplasts. These observations were made in an effort to learn something of the structure of chloroplasts as revealed by their appearance both in the living cell and under a range of osmotic conditions in the isolated state. By combining these observations with information derived from electron microscopy, a model has been constructed to depict the probable structure of a grana-containing chloroplast. The model appears to be consistent with available knowledge of the details of chloroplast structure, and serves to bridge the gap which sometimes appears to exist between observations of light- and electron-microscopy.

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II. Methods

Observations were performed with a Zeiss research model microscope equipped with "Neofluar" phase contrast objectives or, for fluorescence microscopy, with Plan-achromatic objectives. For phase contrast, illumination from a tungsten lamp was used; for fluorescence, the source was a high-pressure mercury vapour lamp. Most observations required magnifications of 1250 diameters.

In order to observe chloroplasts in living cells, free-hand sections of leaf tissue from a variety of plants were examined during the course of this investigation. Small pieces of leaf tissue were vacuum-infiltrated with water, supported between pieces of compressed "Styrofoam", and sections of the tissue cut with a razor. With practice, sections can be cut that consist of a single layer of cells in thickness, and hundreds of living cells can be exposed for microscopic examination. There is a remarkable degree of uniformity in the organization of the protoplasm of such cells and, for chloroplast studies, cells of both the spongy mesophyll and the palisade parenchyma were equally suitable. For the most part, observations have been made on spinach leaf cells because of the relatively large size and openess of those cells, and because the chloroplasts are larger than those contained in some other leaves.

In a free-hand section, the continuance of protoplasmic streaming readily distinguishes living cells from damaged cells. Streaming has been observed to continue in a section for as long as 5 days. During subsequent prolonged illumination of the section, the chloroplasts became gorged with starch, indicating that photosynthetic capacity had been retained.

Isolated chloroplasts were prepared by the conventional method of grinding leaves in a mortar in a buffered solution containing 0·4M sucrose, 0·05M Tris (tris-(hydroxymethyl)aminomethane), pH 7·8, and 0·01M NaCl. The brei was then filtered through cloth and chloroplasts were sedimented from the filtrate by centrifugation at either 500 or 1000 *g* for 5 min.

The effect of reduced osmotic pressures on chloroplast structure was observed in two ways. Isolated chloroplasts were resuspended in the original grinding solution, and this was then diluted to give a range of sucrose concentrations. Alternatively, water or dilute sucrose solution was introduced under the cover-slip into a suspension of isolated chloroplasts mounted on a microscope slide in the above sucrose–Tris–NaCl solution.

Any suspension of chloroplasts represents a heterogeneous population. The observations reported on the appearance of chloroplasts at a particular sucrose concentration applies to the majority, but not all, of the chloroplasts present.

While most of the observations were made on spinach (*Spinacia oleracea*) chloroplasts, investigation of tobacco (*Nicotiana tabacum*), tomato (*Lycopersicon esculentum*), and *Nicotiana glutinosa* chloroplasts has presented the same general picture of behaviour of isolated chloroplasts.

III. Observations

(*a*) *Appearance of Chloroplasts and Surrounding Cytoplasm in the Living Cell*

As previously observed and recorded on cinematographic film by Honda, Hongladarom, and Wildman (1961), chloroplasts in living cells are surrounded by

jackets of a non-chlorophyll-containing material. The jackets continually change shape, in contrast to the fixed shape of the chloroplast proper. The jackets frequently extend into protuberances, and the latter appear to segment into cytoplasmic particles which move in the protoplasmic stream and are indistinguishable from mitochondria. These observations suggest that the jacket may be closely related to, if not the same as, the material out of which mitochondria are formed. Plate 1, Figure 1, is a photomicrograph of a spinach leaf cell showing chloroplasts encased in jackets. Plate 1, Figure 2, shows the same condition but in addition reveals the numerous protuberances that may extend from the jackets. The prominence of the chloroplast jackets varies from section to section, but is usually uniform in the cells of any one section. Under phase contrast the jacket material closely resembles mitochondrial substance and is distinctly different in appearance to both grana and stroma of the chloroplast.

Chloroplasts are oriented such that the concavity in their cup-shaped structure faces toward the cell wall. Looking down on a cell, the chloroplasts can be resolved with great clarity on the top surface of the cell, and with less clarity on the bottom surface. Many cells in a section can be found in which every chloroplast on the top surface can be brought into clear focus, and when this is achieved, the chloroplasts are observed to vary in size, and in the number that are encased in a single jacket. It is a striking circumstance that in all the plants examined, chloroplasts are observed to be fixed in position within the cell while the jackets, mitochondria, and spherosomes are free to move about. This observation suggests that the chloroplasts are in some way attached to a rigid structure within the protoplasm. At least a thin layer of cytoplasm separates the chloroplast from the cell wall because spherosomes can be seen to stream between the chloroplast and the cell wall.

In living cells, every chloroplast that can be brought into clear focus is found to contain grana (Plate 1, Figs. 1 and 2). The grana are dispersed in depth, because changing focus will bring a group of grana into sharp focus while throwing other groups out of focus. There appears to be no overlapping of individual grana as the chloroplast is explored in depth. The grana are invariably seen as dark objects and are never green in appearance. There may be some suggestion of a faint tinge of green emanating from the intergrana regions. When the chloroplast is examined by fluorescence microscopy, grana emit radiation characteristic of that expected for chlorophyll, and as far as critical observation will permit, the fluorescence is almost entirely confined to the grana. Again, there may be a suggestion of a very slight fluorescence coming from the stroma, but we consider it probable that scattering of light from the grana into the stroma is responsible for the effect both with phase contrast and fluorescence microscopy.

(*b*) *Appearance of Isolated Chloroplasts*

(i) *Isolated Chloroplasts in* 0·4M *Sucrose.*—Two distinct classes of chloroplast are seen when chloroplasts are isolated in a buffered solution containing 0·4M sucrose, 0·05M Tris, pH 7·8, and 0·01M NaCl. One class (class I) is characterized by a bright, highly reflecting appearance, and the fact that neither phase-contrast nor fluorescence microscopy succeeds in a clear resolution of the grana (Plate 2, Fig. 3). These chloroplasts may appear highly folded, and as a result possess an irregular outline and a

reduced surface area. Class II chloroplasts (Plate 2, Fig. 4) correspond closely to the appearance of chloroplasts observed in living cells (Plate 1, Figs. 1 and 2) except that they possess no obvious jacket. They are slightly concave, circular, or ellipsoidal disks with regular outlines, the grana being distinct in all planes of focus through the chloroplast. The distribution of the two classes in the standard chloroplast preparation is about in the ratio of 40 class I to 60 class II chloroplasts.

Small protuberances resembling those seen in living cells are often seen on the class I chloroplasts (Plate 2, Fig. 5). This fact, together with their behaviour on swelling described below, indicates that class I chloroplasts are those which have retained in the isolated state the jackets of mitochondria-like material which surrounds them in the cell. Class II chloroplasts represent those plastids which either were not jacketed in the cell or which have lost their jacket material during isolation.

(ii) *Isolated Chloroplasts under Mild Swelling.*—When the sucrose concentration in which isolated chloroplasts are suspended is reduced from 0·4 to 0·2M the appearance of the chloroplasts is transformed in several ways. Firstly, the proportion of class I (jacketed) chloroplasts in the suspension is greatly reduced, and in the majority of chloroplasts the grana are now distinct. The few remaining class I chloroplasts have now assumed a very regular spherical shape and the chloroplast itself is seen to be enclosed in a slightly swollen jacket (Plate 2, Fig. 6). Plate 2, Figure 7, shows such a chloroplast, in which the grana are now apparent. The majority of chloroplasts are transformed into a third class. These chloroplasts have the same general shape as class II, but the overall size is slightly increased, their outlines are less distinct, and their stroma is less dense. The most striking transformation is in the appearance of the grana. These now appear both smaller (approximately one-third the area) and more widely spaced in their distribution throughout the chloroplast (Plate 2, Fig. 9). Although smaller they are, nonetheless, still sharply defined objects.

If 0·1 or 0·2M sucrose is introduced under the cover-slip on a microscope slide on which are mounted chloroplasts in 0·4M sucrose, the gradual transformation of class I chloroplasts can be observed. A swollen mass appears, with blue-grey appearance characteristic of the jacket material of chloroplasts in the living cell, and within this swollen jacket the green folded chloroplast can now be seen to unfold as in Plate 2, Figure 6. At the same time the grana which could not be seen at the class I stage now become apparent (Plate 2, Fig. 7). If dilution of the medium is gradual the chloroplast is often seen to swell within the swollen jacket (Plate 2, Fig. 8). The simple explanation of this transformation from class I to II seems to reside in some change in the properties of the chloroplast jacket. It is envisaged that in class I chloroplasts the jacket material has contracted greatly compared to its state in the cell. This contraction forces the saucer-shaped chloroplast into a somewhat folded form with a corresponding reduction in the apparent surface area of the plastid. In this state the optical properties of the jacket material are such as to obscure, by reflection, any details of grana structure within the chloroplast. Lowering the sucrose concentration of the solution causes the jacket material to swell or disperse in such a way that the chloroplast unfolds and assumes the saucer shape seen in the living cell, and grana now become quite distinct. At the next stage of swelling of such

a chloroplast the surrounding jacket may rupture and can sometimes be seen as a collapsed remnant loosely attached to the chloroplast. The chloroplast itself is rapidly transformed into class III with small grana (Plate 2, Fig. 9).

(iii) *Isolated Chloroplasts in less than* 0·2M *Sucrose.*—In 0·2M sucrose the predominant form is the class III chloroplast. When the sucrose concentration is reduced to below 0·2M the formation of blebs is observed. Initially a single bleb is seen swelling out from the margin of the chloroplast. As the sucrose concentration is further decreased the number and size of blebs around a single chloroplast increases. The result is a variety of most bizarre configurations, some of which are illustrated in Plate 3, Figure 10. It should be emphasized that the blebs are seen sharply *at different planes of focus* through the chloroplast. They appear to arise most commonly from the margin of the chloroplast, but are also seen to arise from the upper and lower surfaces. The size of an individual swelling may eventually exceed that of the original chloroplast.

The photomicrographs in Plate 4, Figures 14–22, show the sequence of events in the swelling of a particular chloroplast. The chloroplast, initially suspended in 0·1M sucrose and corresponding to class III in type, was caused to swell gradually by the addition of small volumes of water under the cover-slip. The distortion of the spherical shape of the large blebs is due to the flow of water across the slide. This sequence shows the great extensibility of the membranes.

A suspension of chloroplasts in 0·02M or lower sucrose concentrations under phase contrast is characterized by the presence of large blebs now swollen into balloons often twice the diameter of the original chloroplasts. Such balloons can be either free or attached to a chloroplast remnant of varying size (Plate 3, Figs. 11 and 12).

The extent of bleb formation appears to depend in part on the rate of swelling, more gradual swelling favouring extensive bleb formation. Rapid swelling, as when a chloroplast pellet is suspended in water, results in many chloroplasts with an open, spongy appearance, and less extensive formation of large blebs.

(iv) *Appearance of the Grana during Swelling.*—Our observations support the view that grana in spinach chloroplasts are relatively resistant to osmotic swelling. It has already been mentioned that in the transformation from class II to class III the grana appear to be reduced in size (but not in number) at a time when the overall size of the chloroplast is increasing. With further swelling the grana become obscure, and at a later stage, when the original shape of the chloroplast is no longer recognizable, they become clear again. Restoration of the clarity of the grana at the balloon stage (Plate 3, Figs. 11 and 12) is attended by an apparent loss in numbers of grana, but those that remain attached to the swollen blebs are often grouped together. Parallel observations by phase contrast and fluorescence microscopy of drastically swollen chloroplasts revealed that the chloroplast remnants attached to large balloons contain numerous, apparently intact, grana. Other grana can be seen around the circumference of isolated blebs. Under ultraviolet illumination no evidence could be found of chlorophyll distributed within or around the surface of the balloons other than in these recognizable grana. When such a suspension of chloroplasts is allowed to dry out under the cover-slip the grana are seen even more distinctly

in a faint matrix of collapsed membranes (Plate 3, Fig. 13). A similar effect is observed if a suspension of swollen chloroplasts is treated with 2M sucrose.

Throughout the swelling process grana appear fixed with respect to stroma or to swollen blebs. This is in marked contrast to starch grains which are stationary in the living cell and in class II isolated chloroplasts, but which are seen in active Brownian motion in class III chloroplasts and in all later stages of swelling. No Brownian motion has been seen under fluorescence microscopy. This emphasizes the intimate structural relationship between the grana and stroma lamellae.

(v) *Properties of the Inner and Outer Surface of the Stroma Blebs.*—Two observations combine to reveal the differing physical properties of the inner and outer surfaces of the stroma blebs. Firstly, observation of a suspension of swollen chloroplasts shows that individual swollen blebs can become extremely distorted by pressure from neighbouring blebs, and that deformation of shape can be quite extreme without causing rupture of the bleb. Further we have never observed such blebs to coalesce with each other when their outer surfaces are brought together under these conditions. Secondly, if a swollen bleb becomes distorted in such a way that the inner surfaces touch, there is immediate fusion and the eventual formation of two separate swollen balloons. During swelling, blebs frequently become detached from the chloroplast margin and float away as isolated, still inflated, balloons. The non-coalescence of neighbouring blebs on the one hand and the self-sealing feature of an individual bleb on the other indicate that the inner and outer surfaces of the stroma blebs possess markedly different physical properties.

(vi) *Reversibility of Swelling.*—It should be emphasized that swelling of chloroplasts is not a reversible, osmotic phenomenon in the classical sense applied to the behaviour of living protoplasts. On the contrary, a swollen chloroplast in our hands has never returned to its original compacted condition as the result of restoring the original osmotic environment. A number of experiments were carried out in which chloroplasts were exposed to water for periods ranging from 10 sec to 15 min and rapidly returned to the original sucrose concentration. Even the briefest treatment resulted in an irreversible change in the appearance of the chloroplast. It is true that some degree of shrinkage or collapse of the swollen blebs did occur, but internal organization of the chloroplasts was greatly modified. Thus the membrane of the bleb, although highly extensible, is not elastic. The term "lysis" is frequently used to describe chloroplasts treated with water. If this is meant to imply a process analogous to the lysis of red blood cells, in which an outer membrane ruptures and the contents are released into solution, it is clearly an erroneous concept.

(c) *A Proposed Model of the Structure of a Grana-containing Chloroplast*

To facilitate an interpretation of the above observations, a model has been constructed to depict the probable structure of a grana-containing chloroplast typical of higher plants. The information necessary to arrive at average values for the number of stroma lamellae and of grana lamellae has been derived from an examination of published and unpublished electron-micrographs of thin sections of higher plant chloroplasts. While it could be desired that more pictures of spinach chloroplasts were available to us to permit a closer correspondence with our light-

microscope observations, there appears to be a reasonable degree of uniformity in chloroplast architecture from one higher plant to another.

Whether or not stroma lamellae are continuous with grana lamellae is still a debatable issue. As recently reviewed by Thomas (1960), a number of possibilities have been proposed on the basis of evidence from electron microscopy. It seems likely that the relationship between grana and stroma lamellae may vary in detail from one species to another. We have constructed the model so that grana lamellae appear both inside and outside of the double lamellae. Other arrangements could be accommodated by the model without altering its basic features. The model does require structural continuity of some kind between the lamellae within each granum, and between the grana and the surrounding stroma lamellae. The number of grana is an average value obtained by direct counting of grana in spinach chloroplasts viewed under phase contrast.

(i) *Description of the Model.*—A photograph of the model is shown in Plate 5, Figure 23. The model has been constructed on a scale such that 1 μ is equivalent to 2·54 cm, with the longest semi-axis equivalent to 10 μ, the shortest equivalent to 5 μ, and a maximum thickness of about 1·25 μ. These are the approximate dimensions of an average chloroplast found in living cells of spinach leaf.

Viewed from an angle, the chloroplast presents a laminated structure because the model is formed from 25 individual flat bags layered on top of each other. The whole chloroplast is thus made up of 50 lamellae with each pair of lamellae fused at the outer margin to make an individual flat sealed bag. These bags will be referred to as stroma bags. Both grana and stroma lamellae have a thickness equivalent to 150 Å. Although in the model each bag extends right through the horizontal plane of the chloroplast it is possible that any particular horizontal plane is occupied by several adjoining stroma bags.

The grana are distributed in a nearly random manner within the confines of the chloroplast structure. Distribution is random both in the horizontal plane (the plane of the lamellae) and in the vertical plane. There is a total of 50 grana of diameter equivalent to 0·75 μ. The grana are composed of a variable number of lamellae, ranging from the equivalent of 3 to 20 stroma bags in depth.

Chlorophyll is entirely confined to the grana and the pigment concentration is sufficient to provide for total absorption of light as it passes perpendicularly through a granum.

The individual stroma bags are held in position by the grana which traverse a number of stroma bags and to which these bags are cemented. In the intergrana regions the individual bags are unrestrained, and may even separate from each other to some extent. The grana thus bestow structural continuity to what would otherwise be a stack of unconnected stroma bags.

There is no all-encompassing membrane of chloroplast origin enclosing the layered structure. Not shown in the model, but illustrated in Plate 5, Figure 24, is an outer jacket of variable thickness which encases the chloroplast and which insulates to some extent the loose structure of the stroma bags from the effects of swelling agents. The jacket is composed of material which resembles mitochondrial substance both optically and in the changeability of its form.

The model can be sectioned in almost any plane and still reveal grana as laminated structures connected with stroma lamellae. Only in the case of a cut exactly parallel to the flat plane of the lamellae would some grana appear as composed of homogeneous material. The model is not entirely faithful to the appearance of chloroplasts in living cells, since it could not be bent to conform with their characteristic saucer shape. It also fails to depict the plasticity of a chloroplast and thus to reveal the scope for folding the whole structure and for a less rigid orientation of the stroma bags in the horizontal plane.

Plate 5, Figure 24, which represents an oblique cut through the model, also illustrates the variety of profiles which the cylindrical stacks of grana lamellae can present in such a section, as compared to a vertical profile depicted in Plate 5, Figure 25.

IV. Discussion

The prominence of the chloroplast jacket, as seen in many leaf sections viewed by phase contrast microscopy, suggest that a corresponding structure should be seen in sections of chloroplasts *in situ* viewed by electron microscopy. After a period of controversy it is now generally agreed from electron-micrograph studies (Sager 1959; Mercer 1960) that chloroplasts are bounded by a membrane, although this membrane is seen more readily in some sections than in others. We suggest that the apparent absence of the chloroplast jacket in electron-micrographs can be explained if one equates the jacket seen by phase contrast with those structures seen by electron microscopy which are external to the lamellar system of the chloroplast. In many electron-micrographs the outer membrane is in close contact with the lamellar system, in which case this membrane would be the only structural equivalent of the jacket. However, a number of published electron-micrographs can be cited in which the outer membrane is some distance removed from the lamellar system of the chloroplast. In these cases there is a more direct correspondence between the dimensions of the chloroplast jacket in living cells and the outer, extralamellar zone of the chloroplast seen by electron microscopy. Von Wettstein (1957, fig. 2), shows an electron-micrograph of a section through a barley chloroplast, which appears to have been cut through a protuberance of the jacket material such as is shown in Plate 1, Figures 1 and 2. The contents of the protuberance have a different appearance to the stroma of the chloroplast. Also, Finean, Sjostrand, and Steinmann (1953, fig. 3*a*) show a section through an *Aspidistra* chloroplast with an outer, extralamellar zone which is limited by the double membrane and is of different appearance to the interlamellar stroma. Kahn and von Wettstein (1961, fig. 4) show an electron-micrograph section of an isolated spinach chloroplast which corresponds closely to what might be expected in a section through the highly folded, jacketed chloroplast in our Plate 2, Figure 5. It is possible that fixing and embedding procedures cause shrinkage of jacket material and for this reason the outer double membrane is commonly the only apparent structural equivalent of the jacket which can be seen in electron-micrograph sections.

The concept of the chloroplast jacket is not new. Thomas (1960, p. 536) discusses the view, first put forward by Senn (1908) and supported by Strugger (1956), that the chloroplast is surrounded by a mobile peripheral layer which is distinct from the inner stroma. Senn named this layer the peristromium, and Strugger suggests

that it is a sol phase, in contrast to the inner gel phase of the chloroplast. Although the outer layer is described as stroma it seems likely that both observations refer to the surrounding jacket of mitochondria-like material which we have observed in living cells.

It is possible to reconstruct in terms of the proposed model the sequence of events which are observed when isolated chloroplasts are exposed to increasingly hypotonic conditions. Firstly, the jacket of mitochondria-like material, which during isolation in hypertonic solution has contracted and forced the chloroplast into a folded, irregular shape, swells or disperses. In its swollen state the jacket material is distinguished by a bluish hue, while the chloroplast appears green; a colour difference which corresponds to that seen in the living state. With dilution of the sucrose concentration to 0·2M the swollen jacket ruptures. Chloroplasts isolated in 0·4M sucrose without any obvious jacket (class II) show no evidence of a totally enclosing membrane during their subsequent swelling.

Once the chloroplast jacket has swollen and ruptured there remains the chloroplast proper as depicted in Plate 5, Figure 23. The 25 layered stroma bags represent a collection of individual osmotic units held together by their attachment to common grana. The small blebs which are seen to form at all levels around the margin of the chloroplast upon further dilution most logically represent swellings at the edge of individual stroma bags. The later stages of swelling where large balloons are formed are merely an extension of this process as seen by the sequence in Plate 4, Figures 14–22. The particular pattern of swelling of the stroma bags would be determined in part by the degree of stapling provided by the grana which traverse vertically the layers of bags in any given area. Being more resistant to swelling the grana columns provide structural continuity between the individual stroma bags, and hence restraint to their swelling. Plate 5, Figure 25, depicts diagrammatically the early stages of swelling and bleb formation in terms of the proposed model.

It is appropriate to ask whether it is the individual stroma bags which are being seen under the light microscope. Recalling that the dimensions of the stroma lamellae are approximately 150 Å in thickness, it is clear that such a dimension would be far beyond the theoretical limit of resolution of the light microscope. However, as the swellings develop and become physically separated from each other, it would appear that resolution is no longer the critical physical parameter. Even if the bags were bounded by a monomolecular layer, they could still be seen because of their ability to reflect light. A useful analogy can be made to an oil droplet spread into a monolayer on the surface of water. The limits of the oil layer can be seen by virtue of light reflected at its boundary.

The proposed model of chloroplast structure is essentially in agreement with current notions based on electron-microscope studies, and at the same time is compatible with chloroplast behaviour as seen by phase contrast under a variety of conditions. The concept that the stroma double lamellae form a flat sealed bag has been proposed from electron-microscopy studies (Sager 1959; Menke 1961). Menke (1961) attaches great significance to the stroma bag as a structural unit and proposes for it the name thylakoid. The greater resistance of grana to swelling, which is an important feature of the model, has also been observed (Neish 1939; Frey-Wyssling and Steinmann 1953).

Our observations on the swelling of higher plant chloroplasts are consistent with many earlier studies. Most of these have described the appearance of chloroplasts in water, where the large balloon form predominates (e.g. Zirkle 1926). Granick and Porter (1947) found well-preserved grana in a dried suspension of spinach chloroplasts which had been isolated in 0·05M phosphate and washed with water. Electron-micrographs of such preparations revealed the presence of large blebs associated with the grana. These are presumed to be the same structures that we have seen under phase contrast. The stability of the grana under this treatment is further evidence of their considerable structural strength and resistance to osmotic damage.

The most detailed earlier study of the appearance of grana-containing chloroplasts under various conditions is that of Mudrack (1956) on chloroplasts from *Viola*. Using a range of glucose concentrations he has observed by phase contrast many of the stages of swelling that we have described for spinach chloroplasts. The presence of potassium thiocyanate and physostigmin in the external medium favoured the formation of large petal-like blebs. Following Strugger (1956) he envisages that, upon isolation, the chloroplast is transformed into a sol and a gel phase, in which the chloroplast is subject to osmotic influences. The sol phase appears equivalent to the jacket material described by us and the gel phase to the chloroplast proper. He interprets the blebs formed on the gel phase (chloroplast) as precipitation membranes rather than as swollen stroma bags.

The range of plants examined suggests that our observations may be typical of most grana-bearing chloroplasts. The swelling pattern of chloroplasts which do not contain grana is somewhat different to that described above. Mercer *et al.* (1955) have made an extensive study, by both light- and electron-microscopy, of isolated *Nitella* chloroplasts in a range of hypotonic solutions. The most notable difference is the apparent strength of the outer chloroplast membrane of *Nitella*. This membrane remains intact when chloroplasts are suspended in distilled water even though the plastid has at this stage swollen to many times its original size. This is in marked contrast to the spinach chloroplast in which the enclosing membrane (jacket) ruptures in 0·2–0·1M sucrose. This suggests that the outer membranes are of a different nature in these two types of chloroplast. On the other hand, the apparent resistance of the grana of spinach chloroplasts to osmotic damage suggests that the granum is the structural equivalent of the *Nitella* chloroplast, and supports the view (Mercer *et al.* 1955; Gibbs 1960) that the grana-free *Nitella* chloroplast might be regarded as a single large granum.

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Explanation of Plates 1–5

All material illustrated is from leaves of spinach (*Spinacia oleracea*), and all photomicrographs have been taken under phase contrast. For any one plate, all figures are at the same level of magnification. *J*, jacket, *G*, granum, *S*, starch, *M*, mitochondria, *SP*, spherosome, *P*, protuberance, *C*, chloroplast, *B*, bleb

Plate 1

Fig. 1.—A portion of a living leaf cell showing chloroplasts containing grana and starch grains and surrounded by jackets of dense mobile material.

Fig. 2.—A portion of a living leaf cell showing chloroplasts with jackets, some of which are extended into long mobile protuberances.

Plate 2

Fig. 3.—Isolated chloroplasts of class I in 0·4M sucrose. Grana are not seen clearly in any plane of view. Chloroplasts appear to be more folded than in the living cell. These chloroplasts have retained the jacket material seen in Plate 1, Figures 1 and 2.

Fig. 4.—Isolated chloroplasts of class II in 0·4M sucrose. Grana are distinct. These chloroplasts closely resemble non-jacketed chloroplasts seen in the living cell.

Fig. 5.—An isolated spinach chloroplast in 0·4M sucrose which has retained the jacket with protuberance, similar to those chloroplasts seen in Plate 1, Figure 2.

Figs. 6, 7, and 8.—Isolated, jacketed (class I) chloroplasts swelling under mildly hypotonic conditions (0·1–0·2M sucrose). Figure 6 shows an early stage in swelling of the jacket. Figure 7 shows the swollen jacket, within which is the chloroplast proper; the grana are now apparent. Figure 8 shows a swollen jacket containing a chloroplast which is itself undergoing swelling.

Fig. 9.—Isolated chloroplasts in 0·1M sucrose (class III). Compared to class II (Plate 2, Fig. 4) these chloroplasts are in general slightly larger. The grana are still present but appear smaller than in class II.

Plate 3

Fig. 10.—Isolated chloroplasts in 0·05–0·1M sucrose. Note the extensive formation of blebs at all planes of focus.

Figs. 11 and 12.—Isolated chloroplasts in 0·02M and lower sucrose concentrations. Note the extensibility of the membrane forming the blebs and the grana on the surface of the large blebs. These were identified as grana by their fluorescence under ultraviolet illumination.

Fig. 13.—A suspension of swollen chloroplasts such as is shown in Figures 11 and 12 which has been allowed to dry out on the slide. Grana are now seen clearly in a matrix of collapsed bleb membranes.

Plate 4

Figs. 14–22.—A sequence of photographs of a particular chloroplast of class III, initially suspended in 0·1M sucrose and caused to swell by the addition of small volumes of water to the suspending medium under the cover-slip. The distortion of the spherical shape of the blebs is due to water flow across the slide. The initial small blebs can be seen to swell into large balloons as big as the original chloroplast.

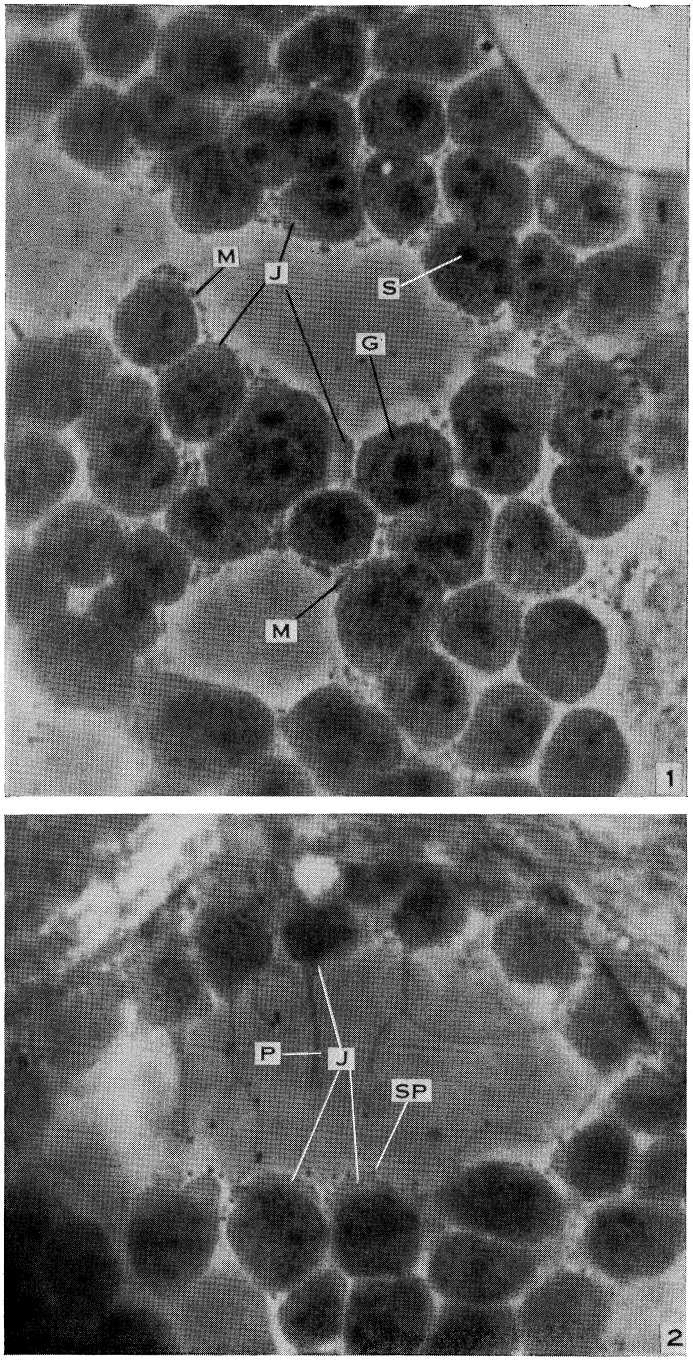
Plate 5

Fig. 23.—A scale model of a chloroplast. The chloroplast consists of a stack of 25 individual flat stroma bags stapled together by the grana columns to which they are fused. *SL*, a pair of stroma lamellae sealed at edge to form stroma bag.

Fig. 24.—A schematic drawing of an oblique section through a jacketed chloroplast. Note the variety of profiles presented by the cylindrical grana.

Fig. 25.—A schematic diagram of a chloroplast in early stages of swelling and bleb formation. Bleb formation is envisaged as the swelling of individual stroma bags.

STRUCTURE OF GRANA-CONTAINING CHLOROPLASTS



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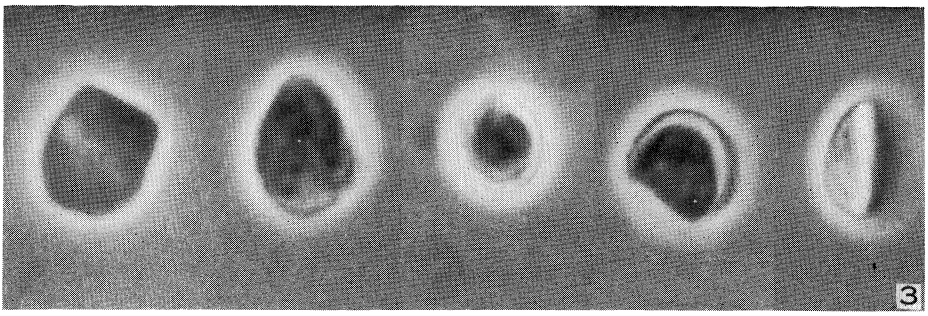

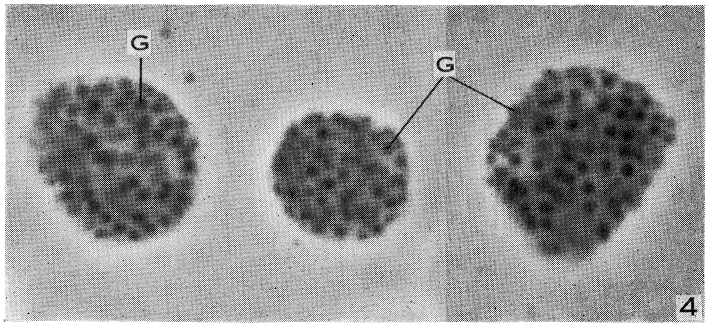

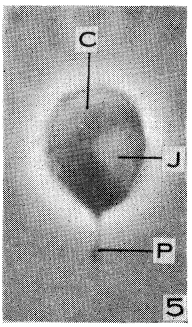

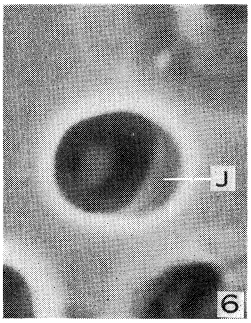

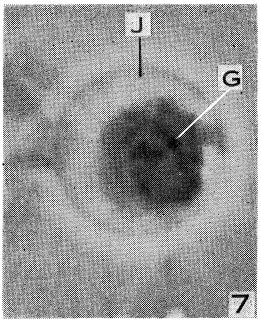

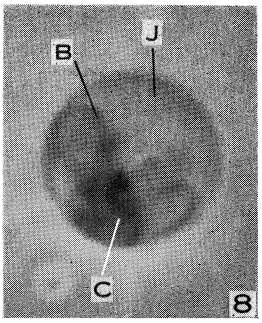

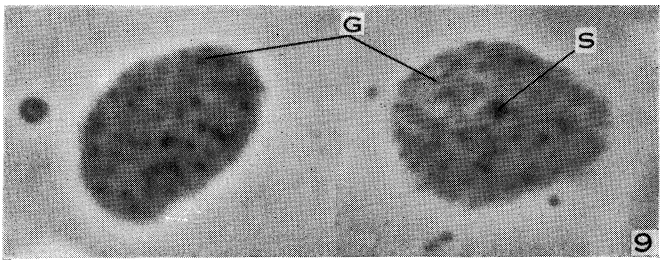

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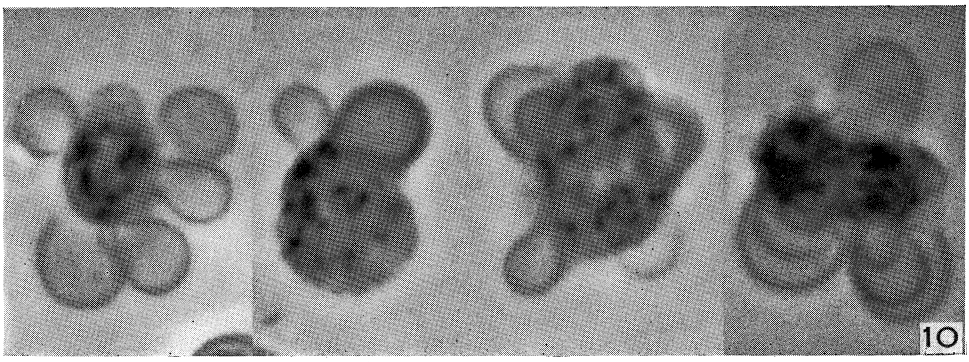

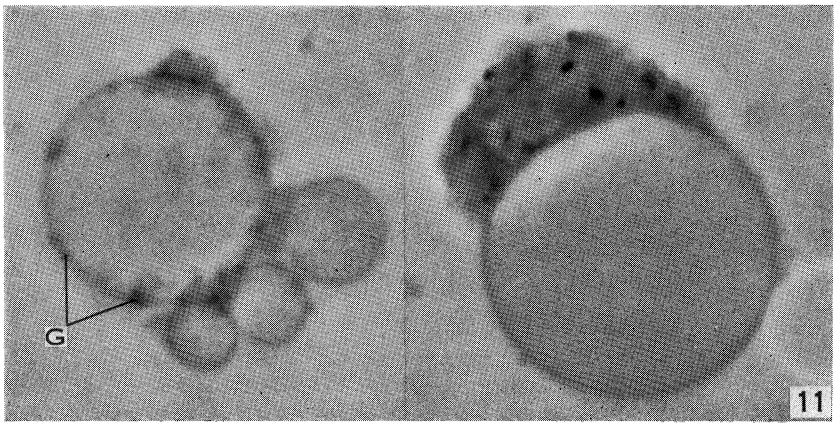

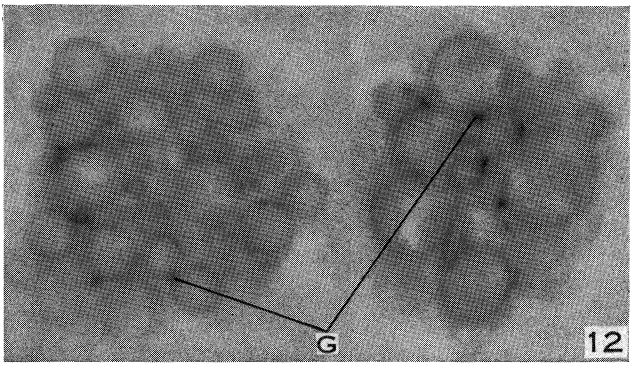

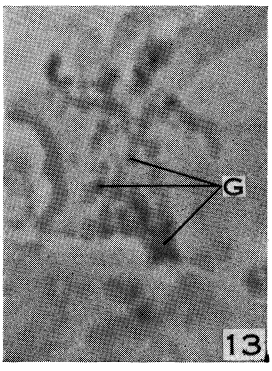

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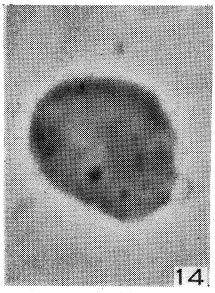

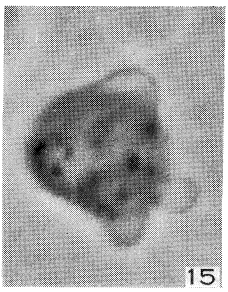

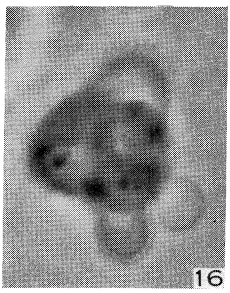

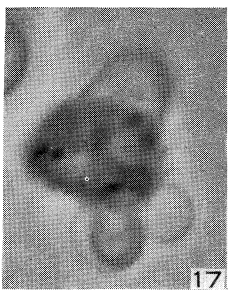

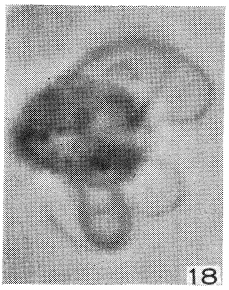

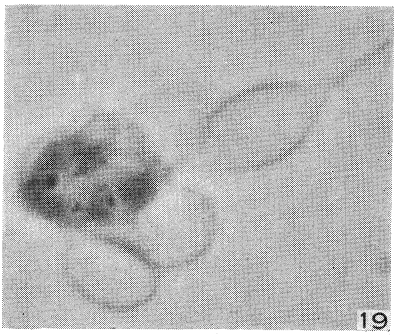

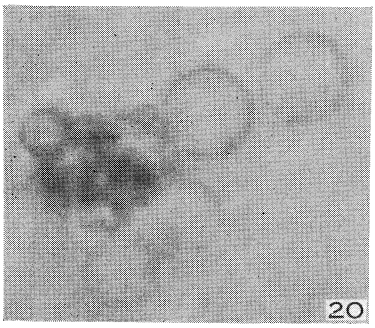

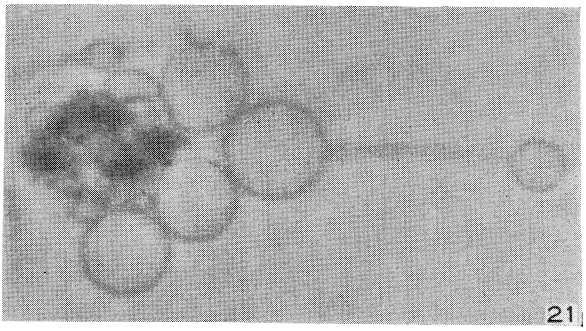

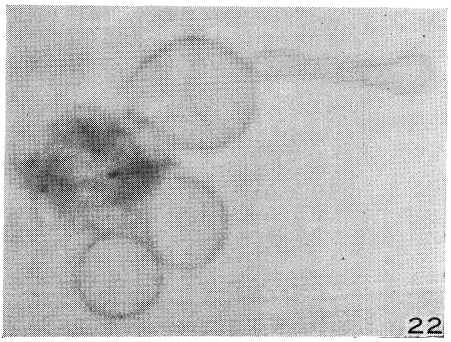

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