# FINE STRUCTURE OF PROTEIN-STORING PLASTIDS IN BEAN ROOT TIPS

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ABSTRACT

The fine structure of leucoplasts in root tip cells of *Phaseolus vulgaris* L. has been studied in material fixed in glutaraldehyde followed by osmium tetroxide and poststained in uranyl acetate and lead citrate. Plastid development has been followed from the young stages in and near the meristematic region, through an ameboid stage, to the larger forms with more abundant storage products in the outermost cells. The plastids contain a dense stroma penetrated by tubules and cisternae arising from the inner membrane of the plastid envelope. Also located in the stroma are lamellae, ribosome-like particles, phytoferritin granules, and fine fibrils in less dense regions. In some elongate plastids microfilaments run lengthwise in the stroma near the surface. The same plastids store both starch and protein, but in a strikingly different manner. The starch is deposited in the stroma, while the protein always is accumulated within membrane-bounded sacs. These sacs arise as outgrowths from a complex of interconnected tubules which in turn appears to originate by coalescence and proliferation of tubules and cisternae arising from the inner plastid membrane. This "tubular complex" bears a strong resemblance to the prolamellar body of etiolated chloroplasts, but is smaller and ordinarily less regularly organized, and is apparently light-insensitive. Crystallization of the protein commonly occurs in the sacs and occasionally takes place within the tubules of the complex as well. The fine structure of the leucoplasts is discussed in relation to that of etiolated chloroplasts. Suggestions are made concerning the function of the tubular complex, role of the ameboid plastid forms, and manner of accumulation of the storage protein in the plastids.

## INTRODUCTION

With the notable exception of work on the lamellar organization of chloroplasts, there has been little investigation of the fine structure of plastids despite their ubiquity in plant cells and the interesting genetic and physiological problems they pose. A number of recent reviews of the changing concepts of the origin, relationships, and interconvertibility of different plastid types are available (6, 7, 21, 34, 35). Recent advances in fine structure techniques, including the introduction of glutaraldehyde as a fixative (29), provide the opportunity for further clarification and extension of these concepts.

The plastids of the bean root tip include young forms in the meristematic cells as well as somewhat older plastids which have undergone a certain amount of differentiation. In the course of the present investigation it was discovered that many of these plastids store protein as well as starch, and do so in a quite distinctive manner. Whereas the starch bodies form in the stroma, the protein always is deposited within membrane-bounded sacs. These sacs generally appear to arise as swellings from the margins of a body of interconnecting tubules which forms in the plastid. This body, or "tubular complex" as it is termed here, may be

organized into a regular lattice, but more frequently appears to be relatively disorganized.

The root tip plastids qualify as the "proplastids," "leucoplasts," "amyloplasts," and "proteinoplasts" of the classical literature, yet seem to be members of a single developmental sequence and are clearly quite similar to one another in their fine structure. Indeed, in some cases a single plastid occurring in a meristematic cell and containing both starch and protein can serve as an example of all four types. Investigation of the fine structure of this plastid series thus assumes particular interest in view of our lack of information about the genesis and interrelationships of plastid types.

These plastids, furthermore, are related structurally in a unique and suggestive way to another plastid type, the young chloroplast of etiolated leaves, since the "tubular complex," with which the protein-storing sacs are associated, bears a strong resemblance to the prolamellar body of the chloroplast. Future work on the organization and behavior of these two plastid types under a variety of experimental conditions should prove rewarding in determining the extent to which they are related structurally and functionally.

## MATERIALS AND METHODS

Seeds of bean (*Phaseolus vulgaris* L. var. Dwarf Horticulture) were obtained from Olds Seed Co., Madison, Wis. Following germination the plants were grown under greenhouse conditions for 3 wk in a modified Hoagland solution. Iron as ferric tartrate was added as needed to prevent chlorosis. After removal of the plants to the laboratory, tips of both main and lateral roots were obtained by immersing the ends of intact roots in 3% glutaraldehyde in a depression slide, and by quickly cutting off the terminal 2–3 mm. Fixation, rinsing, and postfixation in osmium tetroxide were carried out at room temperature in 0.025 M phosphate buffer at pH 6.8. Fixation for a period of 1.5 hr was followed by washing in four changes of buffer over a period of 1 hr. The root tips then were postfixed in 2% osmium tetroxide for 2 hr, dehydrated in an acetone series, and embedded in Araldite-Epon (22). Silver-gray sections were cut on a Servall MT-1 Ultramicrotome with a diamond knife and mounted on 300 × 75 Athene-type grids. The sections then were stained with aqueous 2% uranyl acetate for 10 min followed by lead citrate (28) for 5 min, and viewed in a Hitachi HU-11A microscope at 50 or 75 kv with a 20 or 30 $\mu$ objective aperture.

## RESULTS

### *General Observations*

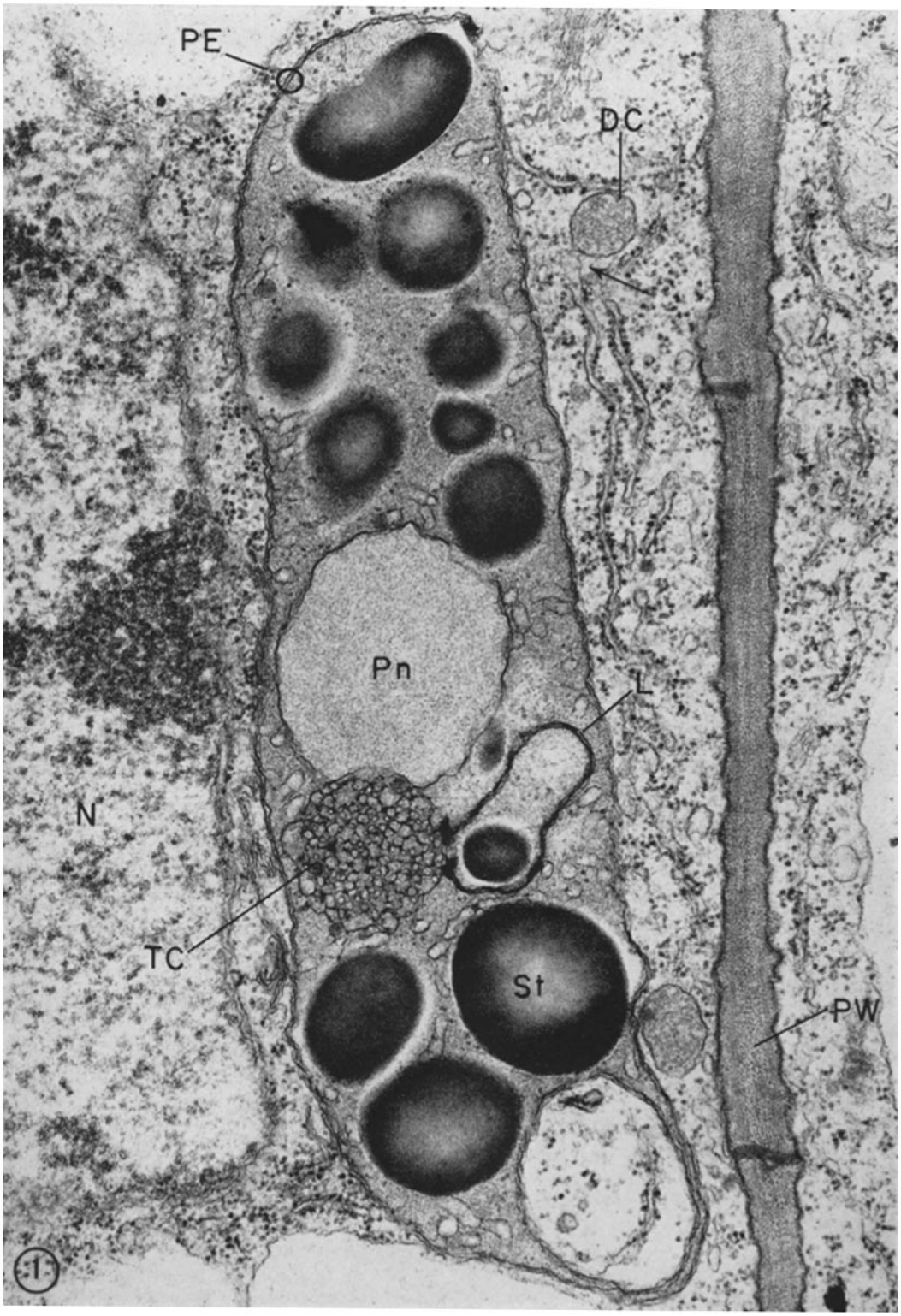
The present report is based largely on an examination of root tips taken from bean plants cultured in complete nutrient solution for about 3 wk. Plants were grown and sampled on four separate occasions. Several root tips from 2-day old seedlings also were examined.

Proceeding from the meristematic region of the root tip laterally into the cortical layers of cells and apically through the quiescent zone into young

*Abbreviations*

*C*, cisterna
*DC*, dilated cisterna
*F*, fibrils
*L*, lamella
*M*, mitochondrion
*Mf*, microfilaments
*Mt*, microtubule
*N*, nucleus
*OD*, osmiophilic droplet
*PB*, prolamellar body
*PE*, plastid envelope
*Pf*, phytoferritin
*Pn*, protein
*PR*, plastid ribosome
*PW*, primary wall
*S*, stroma
*St*, starch body
*T*, tubule
*TC*, tubular complex
*V*, vacuole

FIGURE 1 A large plastid in a root cap cell of bean. A large accumulation of protein (*Pn*) in the plastid is enclosed by a membrane arising from the tubular complex (*TC*). One of the numerous starch bodies (*St*) in the plastid stroma lies within a loop formed by a lamella (*L*) arising from the complex. A fragment of ER lies in a pocket at one end of the plastid. To the upper right is a body (DC) believed to consist of protein within a cisterna of the ER. Its possible attachment to the ER is seen at the arrow. A similar body is present at lower right. × 47,000.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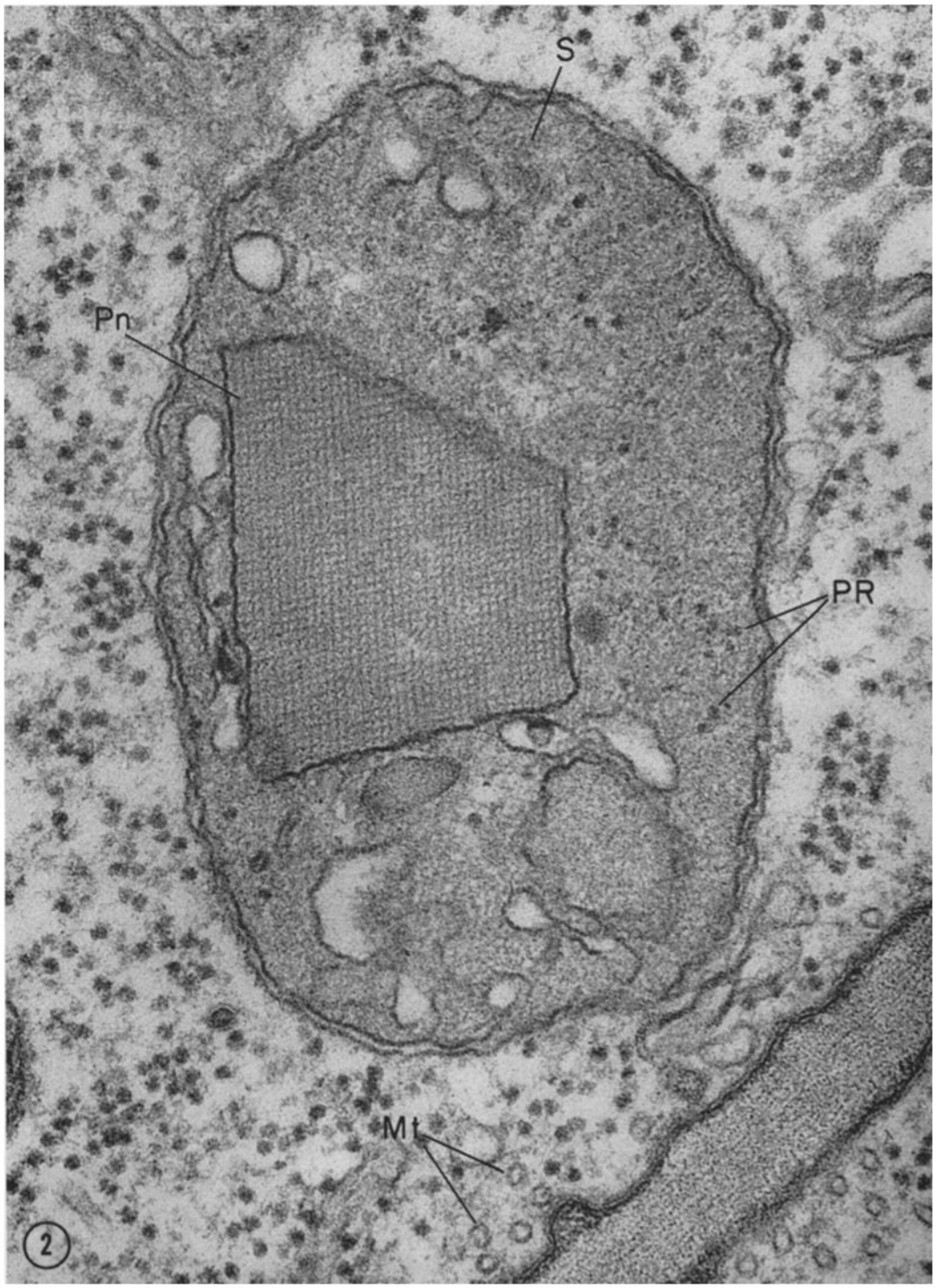



FIGURE 2 Young plastid in a meristematic cell of a bean root tip. The plastid contains a large membrane-bounded protein crystal (*Pn*) and plastid ribosomes (*PR*) lying in the stroma (*S*). Fibrils are present in less dense regions within the stroma. × 130,000.

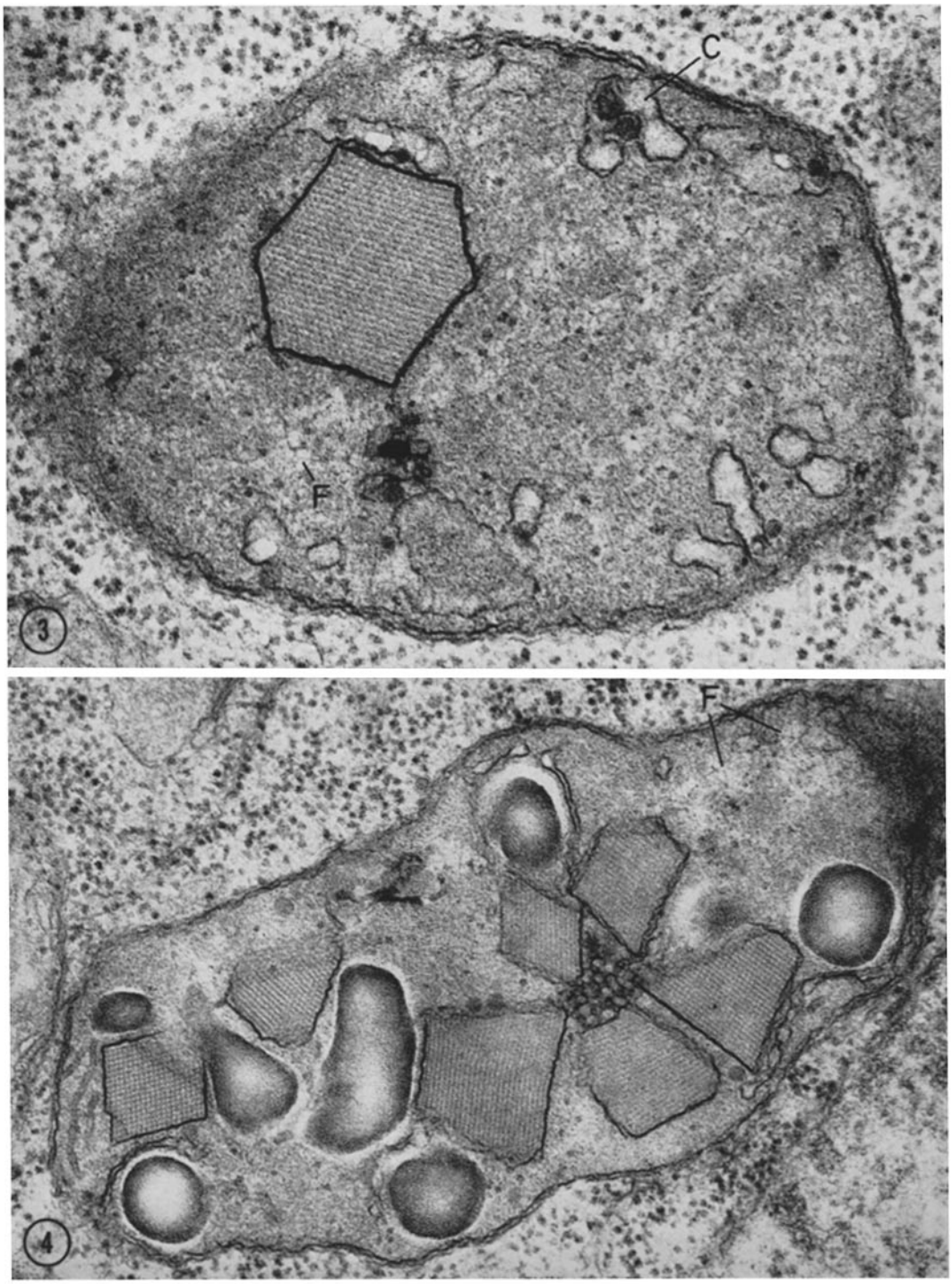



FIGURE 3 Young plastid containing a prominent protein crystal surrounded by a dense membrane. A cisterna (*C*) can be seen arising as an invagination of the inner plastid membrane. Fibrils (*F*) occur in less dense regions of the stroma. × 90,000.

FIGURE 4 A plastid containing several crystalline protein deposits as well as starch bodies. Several of the protein bodies are oriented around a tubular complex. Fibrils (*F*) are present in less dense regions within the stroma. × 77,000.

and then older cells of the root cap, a progressive but loosely defined developmental gradient can be observed in the plastids with respect to size, morphology, and amount of stored products. The young, compact forms (Figs. 2 and 3), which predominate in the meristematic cells in the center, peripherally and distally grade into somewhat larger plastids exhibiting a great variety of ameboid shapes (Figs. 8–10). Finally, the largest plastids with the greatest amount of stored reserves are encountered in the cortical layers and older cap cells (Fig. 1). Starch and protein can occur in the plastids in any of these regions; protein commonly predominates in the subcortical and young root cap cells; starch is most abundant in the cortical and older cap cells. Incidental observations suggest that the starch and protein undergo breakdown in old root cap cells. It seems likely that the starch, at least, is utilized in the formation of the great amount of mucilaginous material secreted by the older cells of the root cap (17, 26).

### *Leucoplast Structure and Ontogeny*

The variety and abundance of inclusions in a mature plastid of a root cap cell are illustrated in Fig. 1. Numerous starch bodies as well as small, ribosome-like particles lie in the dense, finely granular stroma. Of special interest is the labyrinthine, tubular complex from which arises a large sac of granular protein enclosed by a membrane. Lamellae originate from the same complex and extend in a loop enclosing a portion of the stroma and a small deposit of starch. Located at one end of the plastid is a pocket of cytoplasm containing a fragment of rough endoplasmic reticulum.

Lying near the plastid in Fig. 1 are two membrane-limited, moderately electron-opaque bodies, one of which appears to be connected to the endoplasmic reticulum (ER). These inclusions are numerous in the bean root cells and may correspond to the accumulations of protein within cisternae of the ER found in radish root cells (2). They differ from the latter, however, in that they lack polyribosomes on the outer surface of the membrane. Conceivably some of these bodies may be degraded by the plastids, as is discussed below and illustrated in Fig. 10.

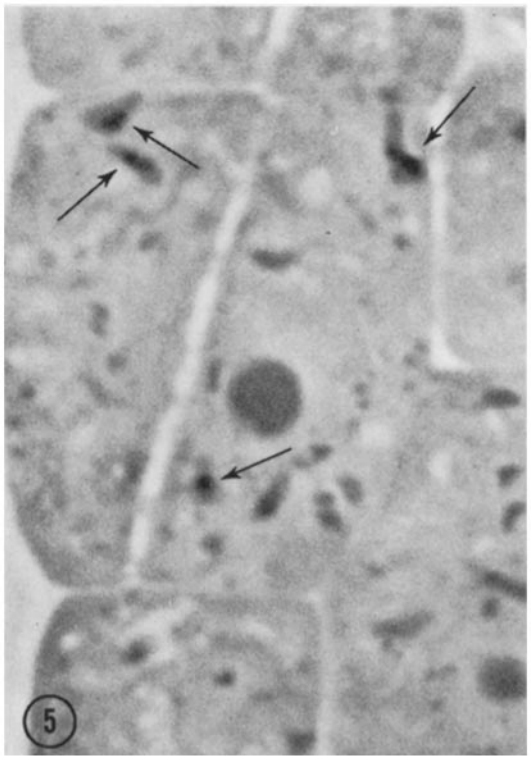
The typically spherical to ovoid plastids of the meristematic region correspond to what are generally termed proplastids (Figs. 2, 3, and 6). These young forms are quite similar in structure to the older, larger plastids into which they soon develop. As illustrated in Fig. 6, they are readily distinguishable from mitochondria. The membranes of the plastid envelope, for example, are thicker and more prominently stained. No evidence has appeared, in the present study, for the existence of still smaller bodies of different structure from which plastids might develop. Occasionally, elongate plastids constricted near the middle are observed. These may be sections of twisted forms, or may represent stages in plastid division.

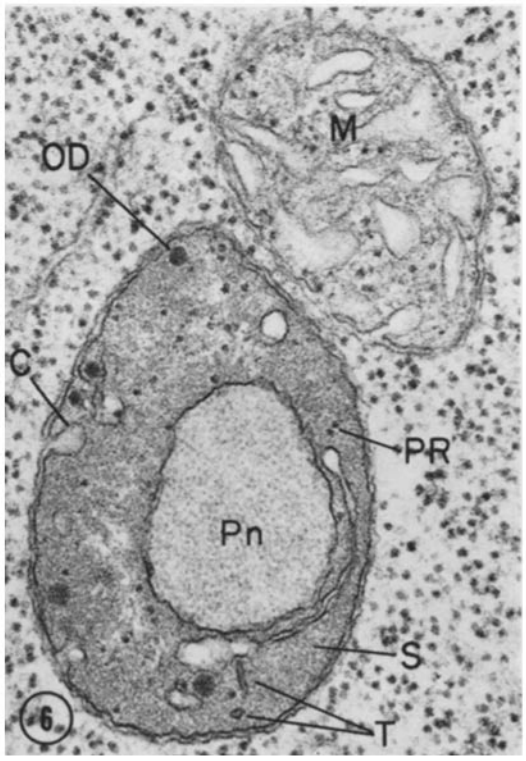
The inner membrane of the plastid envelope gives rise to two distinct types of structures penetrating into the stroma, one consisting of pouchlike invaginations or cisternae whose faintly stained interiors are of highly variable dimensions and are continuous with the space between the membranes of the envelope (Figs. 2, 3, and 7). Many of these cisternae ramify, branch, or coalesce with others in apparently haphazard fashion. Others are pouchlike where they arise from the inner plastid membrane but grade into lamellae consisting of pairs of closely spaced membranes as they penetrate more deeply into the stroma (Figs. 16–18). There appears to be no sharp distinction between the cisternae and lamellae, and intermediate forms are common (Figs. 4 and 6).

FIGURE 5 Photomicrograph of cells in epidermal and subepidermal layers of root tip. Plastic section was cut at 1 $\mu$, then stained with mercuric bromophenol blue. The plastids are stained and contain very strongly stained regions (at arrows) indicative of protein. × 2,700.

FIGURE 6 Young plastid adjacent to a mitochondrion (*M*) in a meristematic cell of a bean root tip. The two organelles are differentiated easily. An osmiophilic droplet (*OD*), cisterna (*C*), tubules (*T*), and plastid ribosomes (*PR*) are present in the stroma (*S*) of the plastid. × 67,000.

FIGURE 7 Portion of a plastid containing a granular protein body (*Pn*) as well as two crystalline protein bodies exhibiting different lattice patterns. × 91,000.

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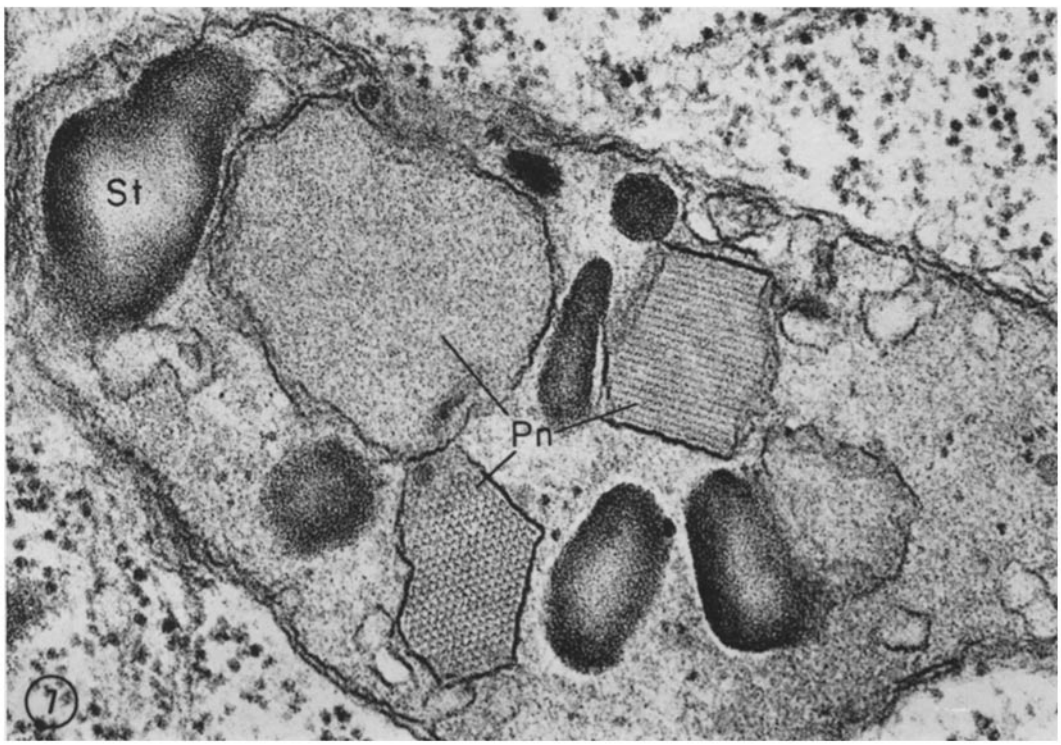
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A second type of structure originating at the inner plastid membrane consists of tubules which run into the stroma singly or in groups in which they vary in number up to at least 12 or 14 (Figs. 6, 11–13, and 17–19). These tubules have an over-all diameter of 190–220 A, with an electron-opaque cortex 50–70 A thick and a less dense core 80–120 A across. The cortex of the tubules does not appear to be an infolding of the inner plastid membrane, and does not closely resemble the latter in appearance.

Among the small particulate inclusions in the stroma are three distinctively different kinds of bodies which are probably phytoferritin, plastid ribosomes, and osmiophilic droplets. The granules (Fig. 14) presumed to be phytoferritin are similar in size and appearance to those previously isolated from bean and shown by Hyde et al. to be phytoferritin (14). They commonly occur scattered through the stroma in clusters of various sizes. No particular localization of these has been noticed although a large cluster frequently is observed at one end of an elongate plastid. The individual granules are similar to phytoferritin particles in size, are highly electron-opaque, and at high magnification exhibit the angular profiles and less dense center previously reported for phytoferritin (14). Similar phytoferritin granules have been observed by Jacobson et al. in the proplastids of etiolated leaves of *Zea mays* (15).

The ribosome-like particles in the plastids commonly appear to be distributed at random in the stroma (Figs. 2, 3, and 6), although occasionally they occur in clusters possibly representing polysomes (Figs. 8 and 9). The diameter of the plastid ribosomes is only about two-thirds that of the ribosomes in the general cytoplasm. The smaller size of plastid ribosomes has been noted by others (see references 8 and 15), and may be a widespread phenomenon. The osmiophilic droplets lie in the stroma (Fig. 6), and frequently are located near or within the tubular complexes (Figs. 17, 19, and 21). They are characterized by an approximately spherical shape and a structureless, uniformly electron-opaque appearance. They are variable in diameter and are not membrane-bounded.

Fine fibrils frequently can be detected in relatively clear regions within the stroma (Figs. 2–4). These are interpreted as DNA fibrils since they are similar in appearance to the fibrils which are believed to contain DNA and which have been seen in chloroplasts and mitochondria by previous workers. Also, the staining procedure used in the present work is known to be effective in revealing structures containing DNA (13).

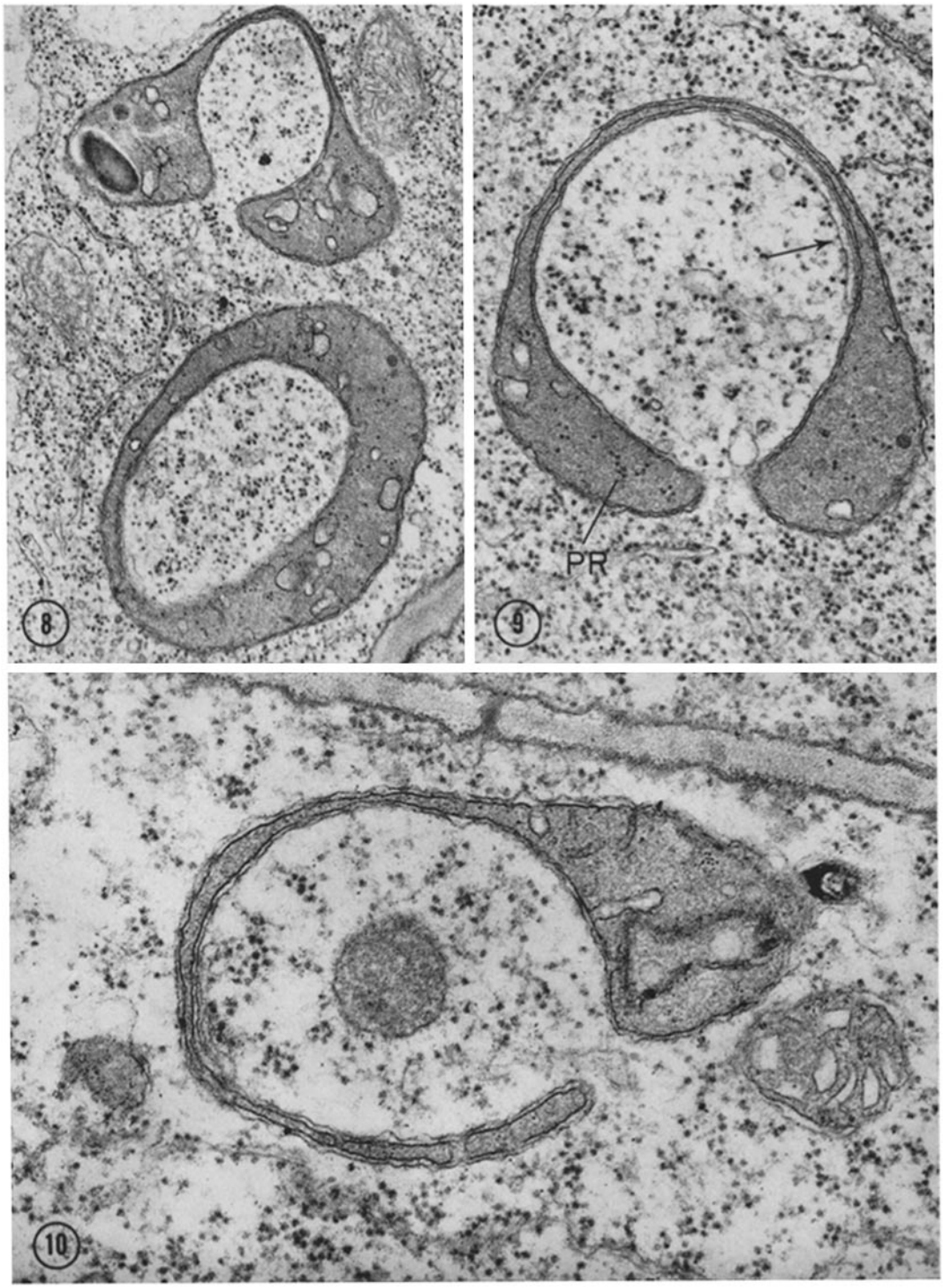
Occasionally in large, elongate plastids, microfilaments 50–60 A in diameter are observed running lengthwise in the stroma near the plastid surface (Fig. 17). Individual fibrils curving smoothly in conformity to the plastid outline can be traced for a distance of 1 $\mu$ or more. These fibrils resemble in size and appearance the microfilaments implicated by Nagai and Rebhun (25) in cytoplasmic streaming in *Nitella* and by Wohlfarth-Bottermann (37) in motility in *Physarum*. In the plastids they may be associated with changes in shape or with flexures of the organelle resulting in movement.

### *Ameboid Forms of the Plastids*

Ameboid forms of the plastids are found in many of the cells of the root tip, but are especially prominent in the cells located between the meristematic region and the outermost layers. In section they appear highly variable in shape, and commonly include ring-, horseshoe-, and dumbbell-shaped forms (Figs. 8–10). The cytoplasm encircled by these plastids contains ribosomes and sometimes portions of endoplasmic reticulum and even mitochondria. Observation of a number of the plastids, including some in serial section, suggests that the cytoplasmic material is not surrounded entirely, but lies in pockets or embayments. Many of the profiles probably represent sections through cup-shaped or umbonate plastids similar in shape to the umbo-mitochondria discovered in the egg cells of *Pteridium* by Bell and Mühlethaler (1). The engulfed cytoplasm usually appears less dense than that outside, owing to a lower concentration of ribosomes and apparently to less background material.

In Figs. 9 and 10 a fragment of endoplasmic reticulum is surrounded by a plastid. In both cases the plastid is appressed closely to the *ER*, and the *ER* membrane adjacent to the plastid envelope is devoid of ribosomes. In Fig. 10 a pincer-shaped plastid encircles an electron-opaque, membrane-limited body believed to be a derivative of the ER like the bodies in Fig. 1. There is some indication of the presence of material running radially from the body toward the enfolding plastid.

The ameboid forms possess the typical internal structure shown by the more compact plastids as described above, and may contain small starch




FIGURES 8 and 9 Ameboid forms of plastids in bean root tip cells. The encircled cytoplasm appears less dense than the outside cytoplasm. In Fig. 9, the arrow points to a remnant of ER to which the plastid is appressed. In both figures the plastid ribosomes (*PR*) tend to occur in clusters. Fig. 8, × 32,000; Fig. 9, × 45,000.

FIGURE 10 Ameboid plastid encircling a body believed to be an accumulation of protein in a cisterna of the ER. On the left side, a fragment of ER is appressed to the plastid. × 70,000.

granules (Fig. 8). Tubular complexes and proteinaceous deposits are not encountered frequently, however. The larger plastids of the outermost cells of the root also may be quite elongate and may include pockets of cytoplasm (Figs. 1 and 13).

### *The Tubular Complex and Associated Protein Bodies*

In addition to, or instead of starch, many of the plastids store a granular or crystalline material. This appears to be protein, based on use of phase contrast to examine sections cut at a thickness of 1 $\mu$ and then stained for protein with mercuric bromophenol blue (20). In sections so treated, only the nucleoli and plastids are stained prominently. Additionally, some of the plastids contain more darkly stained regions or spots (Fig. 5). The localization of the latter plastids in the section coincides with that observed for plastids with the presumptive protein deposits, as determined on adjacent thin sections with the electron microscope. In the root tip used for Fig. 5, the plastids containing more deeply stained regions were confined largely to the two outermost layers of cells. When sections serial to the thick section used for the figure were examined electron microscopically, the plastids containing the membrane-limited granular and crystalline deposits also were observed largely in the two outermost cell layers. Fig. 7 was obtained from one of the sections and shows both granular and crystalline bodies in a plastid in a subepidermal cell. Thus it seems quite probable that these accumulations are proteinaceous.

As the figures illustrate, the protein deposits always are membrane-bounded. Where more than one occur in a plastid, they may be separate or confluent. In thin sections a large proportion of the profiles of these bodies or sacs are observed to be associated closely with a compact, more or less organized, tubular complex (e.g., Figs 1, 4, 13, 17, and 18). The bounding membrane of the protein body is continuous with the membranes of one or more subunits of the complex lying near or adjacent to it (Figs. 17–19). Occasionally a body is connected to and apparently arises from two or more complexes (Fig. 17). Conversely, two or more of the storage bodies occasionally arise from a single complex (Figs. 4 and 24).

Frequently a large protein deposit is observed without an associated tubular complex (Figs. 2 and 3). It is assumed that in many such cases a complex is connected to the sac at a different level of the plastid, and in some instances this has been substantiated by comparison of successive sections. It appears likely that in other instances some of the protein bodies arise directly from tubular and cisternal invaginations of the inner plastid membrane. The younger, smaller plastids in or near the meristematic region of the root tip frequently contain small complexes in association with granular or crystalline protein deposits, while the large plastids in the outermost cells more frequently contain large complexes to which are attached quite large sacs of granular protein (Figs. 1 and 13).

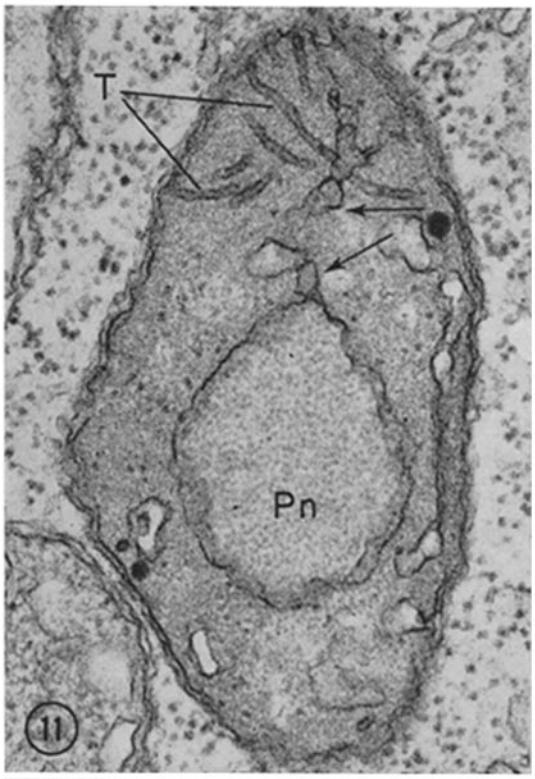
Starch deposits lying near the complex are sometimes partially or completely encircled by lamellae which arise from its edges (Figs. 1, 17, and 19). The starch lies in the stroma, however, and not within the tubular system. Starch bodies also are encircled sometimes partially or completely by lamellae arising directly from the inner membrane of the plastid envelope, as in the case of the uppermost starch grain in Fig. 17.

In that the membranes surrounding the protein sacs of the root tip plastids clearly arise from the

FIGURE 11 Young plastid with several tubules (*T*) running into the stroma from the inner membrane of the envelope. A passageway that may lead into the sac of protein (*Pn*) is indicated by arrows. This and the associated tubules may represent an early stage in the formation of the tubular complex. × 77,000.

FIGURE 12 Portion of a plastid with what may be a tubular complex (*TC*) in process of formation. Two tubules (*T*) run from the envelope to the complex. A lamella (*L*) arising from the complex forms a loop encircling some starch bodies. × 56,000.

FIGURE 13 An elongate plastid containing a tubular complex with which a membrane-bounded mass of protein is associated. A tubule (*T*) runs from envelope to complex. × 45,000.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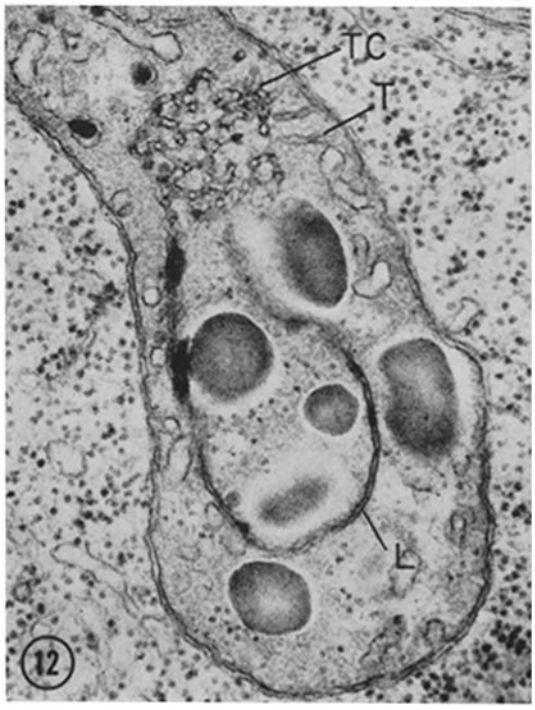
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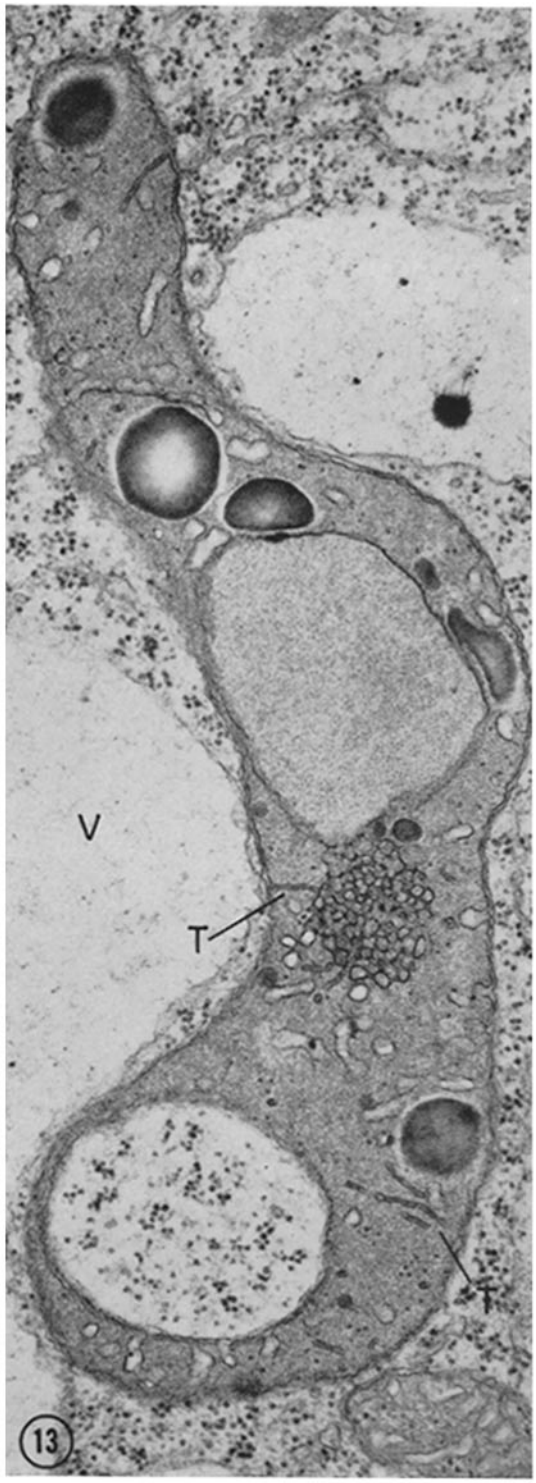
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tubular complex, the latter structure appears to be analogous to the highly organized prolamellar body of the young etiolated chloroplasts from which lamellae arise upon illumination. For comparison with the tubular complex, a typical prolamellar body in a leaf chloroplast from an etiolated bean seedling is shown in Fig. 20. The two types of plastids involved are also similar in that both contain starch bodies surrounded by loops of lamellae arising, respectively, from the tubular complex (Fig. 21) or the prolamellar body (Fig. 22). However, the incipient formation of grana which usually can be detected along the interrupted loops in the chloroplasts (Fig. 22, arrow) never is observed in the root tip plastids.

### *Origin and Structure of the Tubular Complex*

The tubular complex appears to be an elaboration of tubules and cisternae (or lamellae) which arise from the inner membrane of the plastid envelope. In many of the younger plastids, tubules running into the stroma from the envelope are conspicuous features of the fine structure (Fig. 11). They may occur singly, but are commonly in groups of two or three to as many as 12 or 14. Frequently the members of a group appear to fuse with one another and with cisternal or lamellar membranes in the stroma (Fig. 12). In somewhat older plastids where a well-developed tubular complex is present, both tubules and cisternae frequently are observed in its vicinity (Figs. 13, 19, and 21), and occasionally can be traced to a connection with it (Figs. 13, 15, and 17). In cross-sectional appearance and dimensions the tubules in the complex closely resemble those in the stroma.

Typically the tubular complex appears disorganized (Figs. 1, 13, and 17), or organized somewhat irregularly (Fig. 19). Occurrence in a more regular latticework, such as is illustrated in Figs. 18 and 24, thus far has been encountered infrequently. The structure in Fig. 18 appears to be a somewhat distorted lattice in which the smaller circular profiles represent cross-sections of tubules and the larger represent the interiors of the system at corners where tubules converge. The interconnected tubules lie in a matrix continuous with the stroma in the remainder of the plastid. A more definitive description of the complex must await the discovery of conditions under which it occurs more frequently as a crystalline lattice. Of interest are the recent studies of Gunning (9) on the structure of prolamellar bodies in etiolated oat leaves and the studies of Wehrmeyer (36) on these structures in etiolated bean leaves.

Study of the less regularly organized complexes strongly suggests that they also consist of a meshwork of interconnecting tubules penetrated by stroma (Figs. 17, 19, and 23). Since these observations were made on root tips receiving diffuse light during growth, it seemed reasonable to suppose that the irregularity of structure of the complex might have resulted from exposure to light and that the complex might prove to be more highly organized in root tips kept entirely in the dark. These suppositions are based upon the effect of light on the crystalline structure of the prolamellar body of young chloroplasts in etiolated leaves (18). However, when bean seeds were germinated for 3 days in complete darkness and the root tips then were excised and fixed in weak, green light, it was found that the tubular complexes in the plastids also were organized irregularly and were similar in appearance to the great majority of those in root tips of older plants as described above.

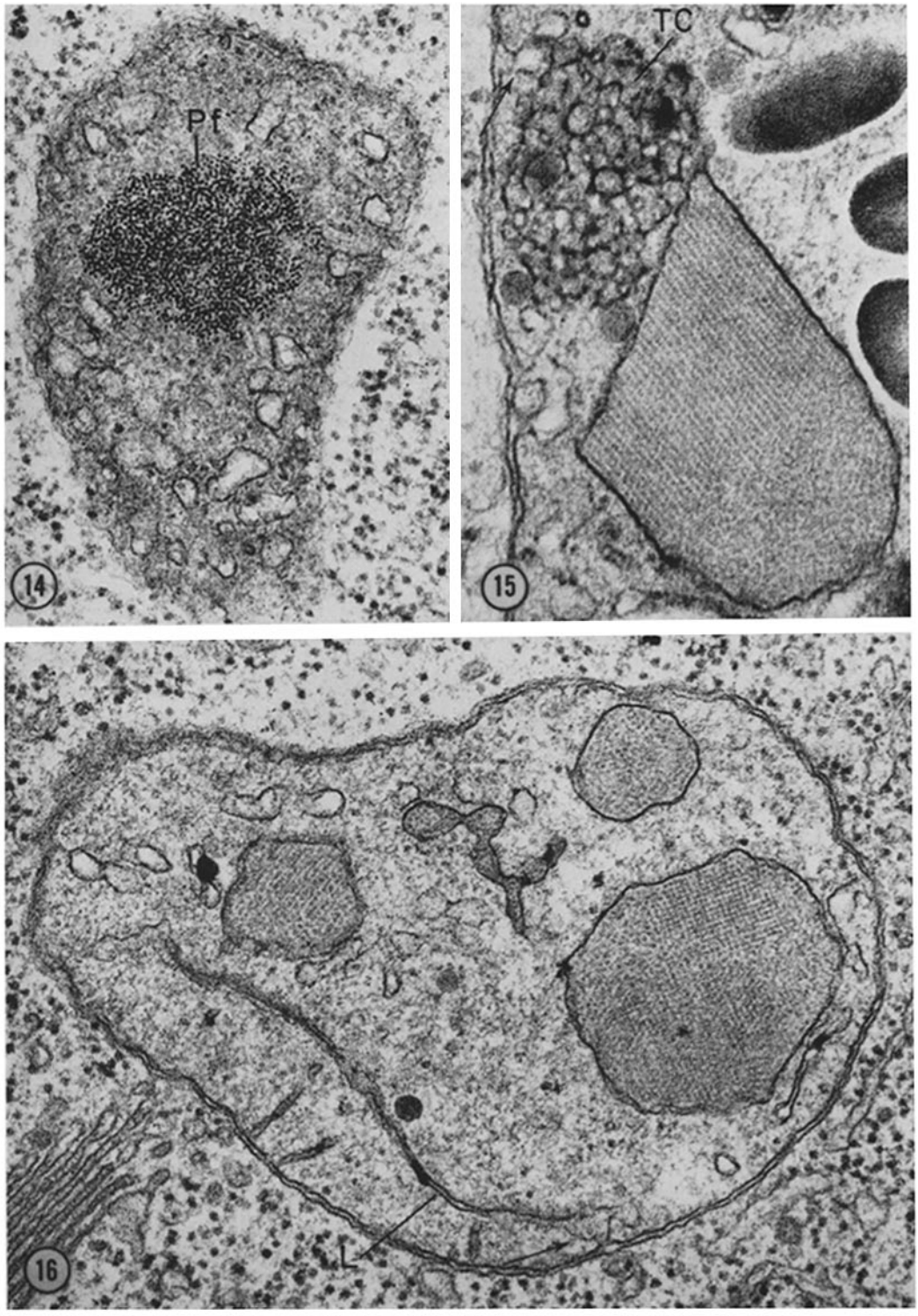
### *The Storage Protein*

The storage protein ordinarily is deposited first in the form of granules or spherules about 85 A in diameter within sacs arising from the tubular com-

FIGURE 14 A cluster of phytoferritin granules in a young plastid of a bean root tip. × 70,000.

FIGURE 15 Portion of a plastid showing a tubular complex (*TC*) and an associated deposit of protein. A cisterna running from the inner plastid membrane to the complex is indicated by an arrow. In that portion of the deposit which has crystallized, the bounding membrane conforms to the angles of the crystal. × 104,000.

FIGURE 16 Plastid containing several sacs in which the protein is in process of crystallization. An invagination of the inner plastid membrane begins as a cisterna (see arrow) but becomes a lamella (*L*) deeper in the stroma. × 73,000.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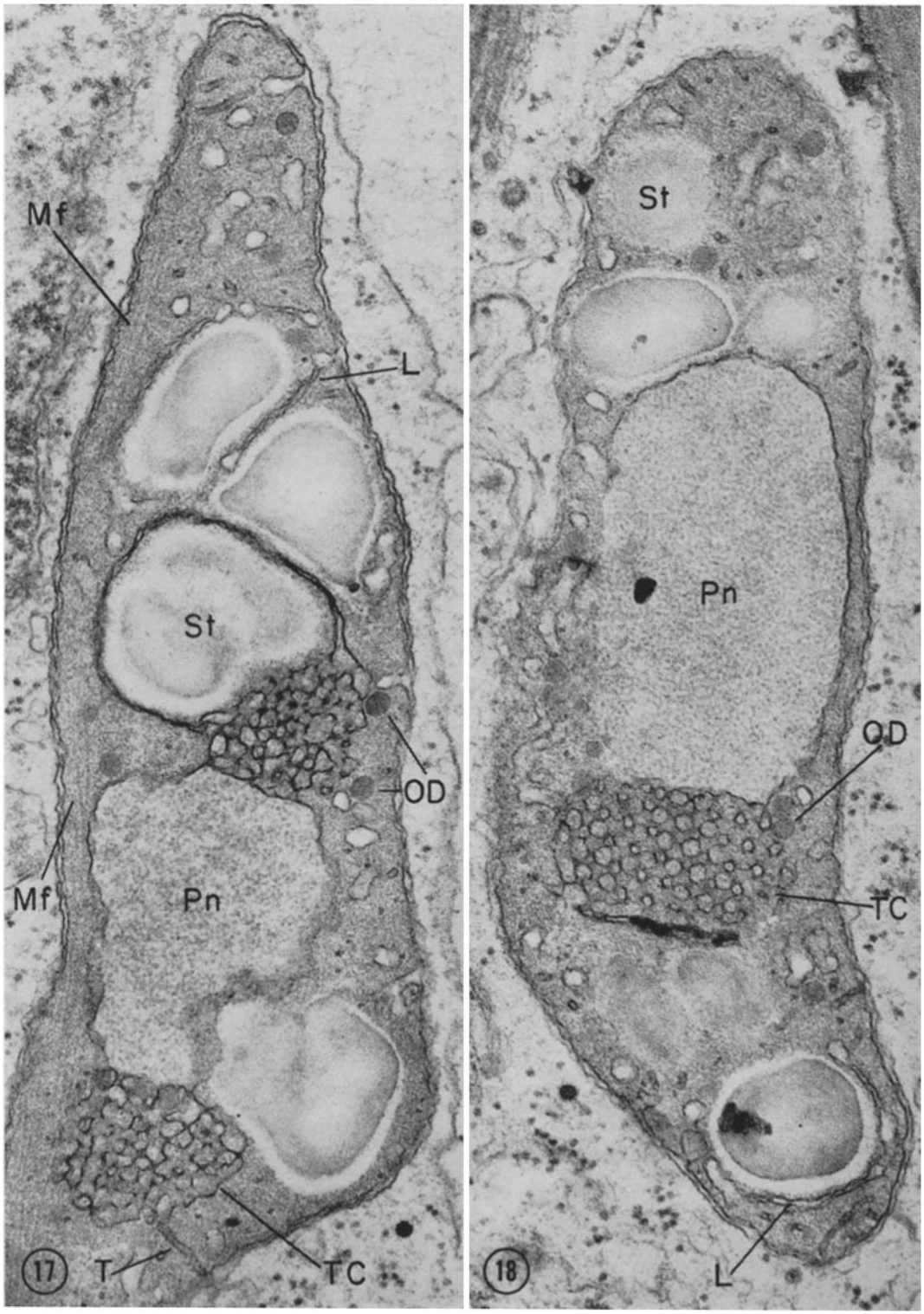



FIGURE 17 Plastid showing a protein sac associated with two different tubular complexes. The origin of the sac membrane from the complexes can be seen clearly. Microfilaments (*Mf*) run lengthwise in the stroma beneath the plastid envelope. Two osmiophilic droplets (*OD*) lie near one of the complexes. × 65,000.

FIGURE 18 Plastid in which the tubular complex is organized more regularly than is typically the case. × 70,000.

plex. Each granule appears to consist of a small ring with electron-transparent center, or possibly of a group of several smaller particles (Figs. 6 and 17–19). Various stages can be observed in the assembly of these granules into a crystalline lattice (Figs. 15 and 16). Crystallization occurs early in some plastids and later or not at all in others, so that those in or near the meristematic cells may bear sacs in which the protein is crystallized completely (Figs. 2 and 3). More peripheral plastids contain uncrystallized material (Figs. 1, 13, and 17). The outermost cap cells in particular commonly contain large sacs of uncrystallized, granular protein. The plastid in Fig. 7 is somewhat unusual because it possesses two completely crystallized protein bodies adjacent to one showing no evidence of crystallization.

It is assumed from these observations that the uncrystallized and crystallized deposits represent the same storage protein. The fact that many of the older plastids contain large sacs of material remaining in the granular state may indicate, however, the presence of a mixture of proteins or extraneous material inhibiting crystallization.

Crystallization of the protein deforms the bounding membranes of the sacs into angular patterns conforming to the crystal outlines; this results in striking profiles (Figs. 2–4 and 24). Where the contained protein is crystallized only partially, the membrane may be rounded in one region and angular in another (Fig. 15).

Of great interest are occasional plastids in which the protein accumulation and crystallization appear to have taken place even within the tubules of the complex, causing the enlargement and deformation of the tubules into angular profiles (Figs. 23 and 24). In Fig. 23 granular material, similar in appearance to that in the sacs, can be traced within the tubular complex to a region near the surface of the plastid, where an invagination (marked by an arrow) appears to lead into the complex from the inner plastid membrane.

Owing to different orientations of the lattice with respect to the plane of section, a number of lattice patterns, among which two are common, have been observed in the crystalline bodies. One of these consists of a set of parallel electron-opaque lines of granular appearance (Figs. 4, 7, and 24). The spacings between these vary somewhat from crystal to crystal, presumably owing to the tilt of the crystal with respect to the plane of section. The spacings between lines in the largest body of Fig. 24 average 114 A. The second frequently encountered pattern consists of lines disposed along two axes which most commonly intersect at an angle of approximately 78°, as observed in Figs. 2 and 4, and in the lower left body of Fig. 24. The spacings between lines are almost the same along both axes; in Fig. 2, for example, they average 113 A vertically and 105 A laterally. A third and less commonly encountered lattice orientation is seen in the lower crystalline body of Fig. 7. This may be similar to the pattern observed in the crystal exhibiting the striking hexagonal profile in Fig. 3.

## DISCUSSION

The unsatisfactory state of plastid nomenclature has been recognized for some time (21). In particular the term "proplastid" appears of doubtful utility, at least when used so broadly and indiscriminately as it is at times to designate plastids, observed in the electron microscope, in diverse stages of development and activity in a wide variety of plant tissues. During the 1950s, when the resolution of structure in thin sections of plant tissue was frequently unsatisfactory and permanganate was used widely as a fixative, it was believed commonly that recognizable young plastids, or proplastids, themselves arose from still smaller initials which were indistinguishable from mitochondrial precursors. The effect of permanganate in simplifying the structure of plastids by destroying some of their most interesting and important features also tended to perpetuate and extend the use of the term.

Although the plastids in the meristematic cells of bean root tips are smaller and less variable in shape and contain fewer cisternae and fewer stored food reserves than plastids of older cells, they nevertheless possess typical plastid ultrastructure and are readily distinguishable from mitochondria. Since no clear fine structural distinction can be made between possible initials or proplastids on the one hand, and older forms on the other, it seems more satisfactory in the present instance to refer to "young," "juvenile," or "undeveloped" plastids, or simply to "plastids."

Perhaps of greatest interest in this study are the questions raised about plastid types, relationships, and interconvertibilities, and the possibilities suggested for further exploration. Are the young plastids of bean root tips essentially similar to those of etiolated leaves; are they capable of differentiating into chloroplasts, and the juvenile chloroplasts into leucoplasts under suitable conditions; or are the two kinds of plastids no longer interconvertible

due to irreversible changes occurring in them during differentiation of root and shoot in embryo and seedling development? Obviously, much work will be required to clarify these relationships. Experimentation to modify the formation and subsequent development of the specialized tubular associations found in these plastids in a study of their potentialities, similarities, and differences might be an especially rewarding approach.

A wide variety of terms has been applied to the organized aggregate of tubules found in etiolated chloroplasts. The commonly used term "prolamellar body" may be a suitable designation for this structure in the chloroplast. It is much less suitable for the analogous tubular meshwork in plastids of the root tip, the structure of which occasionally gives rise to lamellae that do not arise with any detectable consistency or develop into grana or other organized structures. On the other hand, this structure in plastids of the root commonly is associated closely with protein bodies whose bounding membranes clearly arise from it. Sitte (31) used the term "plastid center" to describe a structure observed in plastids in root tips of pea. However, the object seen by Sitte resembles an aggregate of phytoferritin granules rather than the structure described here, and is not associated with protein bodies. Sitte's term seems inappropriate, for the additional reason that in a plastid two or more complexes frequently are associated with one to several protein bodies and are not central either in location or in function. It may be satisfactory to designate the aggregate as a "tubular complex," however, since this rather noncommittal term is sufficiently descriptive to permit distinguishing the structure in the root tip plastids from the prolamellar body of chloroplasts and since it serves to postpone adoption of a more definitive term until the functions of tubular aggregates in plastids and the relationship of these aggregates to one another are better understood.

It is not clear at present whether tubular complexes will prove to be more than infrequent components of leucoplast architecture. Possibly they will be found under special conditions, e.g., where protein storage occurs, or in particular species, e.g., in those whose root plastids turn green in the light. Buvat[1] observed a "centroplast" consisting of a lattice of tubules in protein-storing leucoplasts in roots of *Phajus wallichii*. Buvat suggested that the storage protein of the plastids, which occurred in the form of fibrous bundles, arose from the stroma and was spatially unrelated to the centroplast. There was no evidence of a membrane bounding the protein. Murakami (24) observed a prolamellar body without associated storage protein in plastids of root cells of barley seedlings grown in the light.

In addition to their tubular aggregates, the root tip plastids resemble young plastids of etiolated bean leaves in a number of fine structural features. The envelope, stroma, and envelope-derived cisternae and tubules are similar in appearance; ribosome-like particles, phytoferritin, fine fibrils, osmiophilic droplets, and starch deposits occur in the stroma of both.

An obvious dissimilarity is in the concentration of ribosomes, which in the stroma of the etiolated chloroplasts is many times that observed in the root leucoplasts (Figs. 20 and 22). This difference may be in part a reflection of the much greater amount of membrane protein required in the syn-

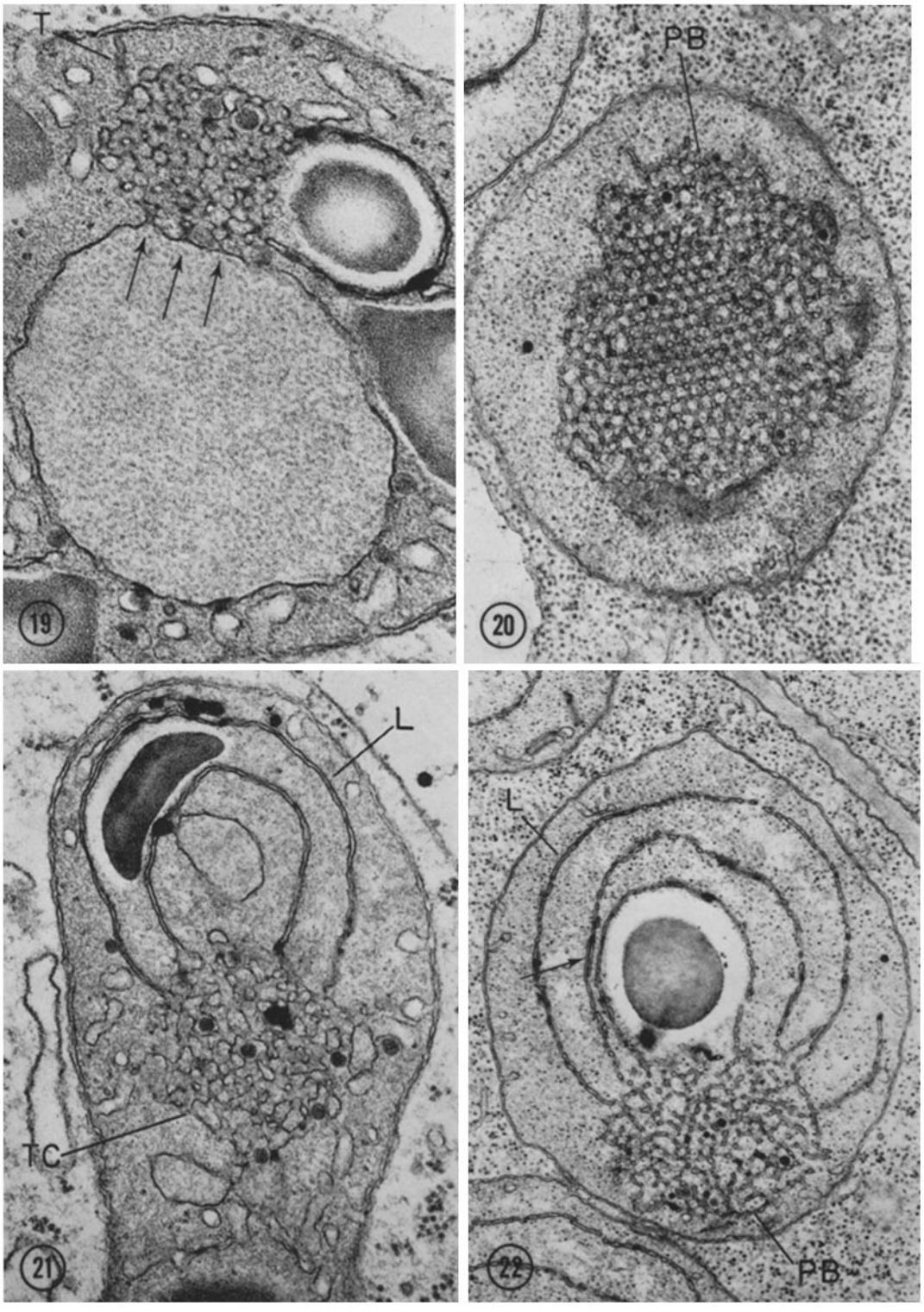
[1] Buvat, R. 1959. *Compt. Rend.* **249:** 289.

FIGURE 19 Portion of plastid illustrating the accumulation of protein within a membrane arising at multiple points from the tubular complex. Arrows indicate the origin of membrane from the complex in the plane of the section. × 85,000.

FIGURE 20 Young chloroplast in an etiolated primary leaf of a bean seedling. The plastid contains a large, regularly organized, prolamellar body (*PB*). × 40,000.

FIGURE 21 Tubular complex (*TC*) and associated loops of lamellae (*L*) in a root tip plastid. Compare with chloroplast in Fig. 22. × 55,000.

FIGURE 22 Young chloroplast from the same etiolated leaf as that of Fig. 20. The prolamellar body (*PB*) is not organized so regularly as in Fig. 20, and has given rise to perforated loops of lamellae (*L*). It thus superficially resembles the root tip plastid of Fig. 21. An early stage in the formation of a granum is indicated at the arrow. × 30,000.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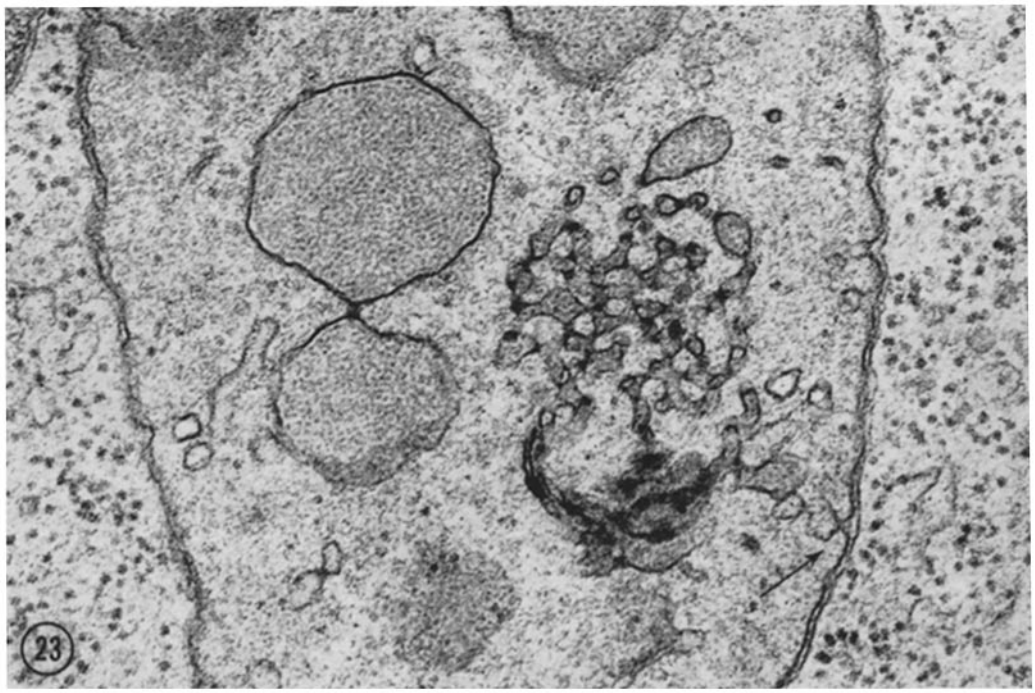


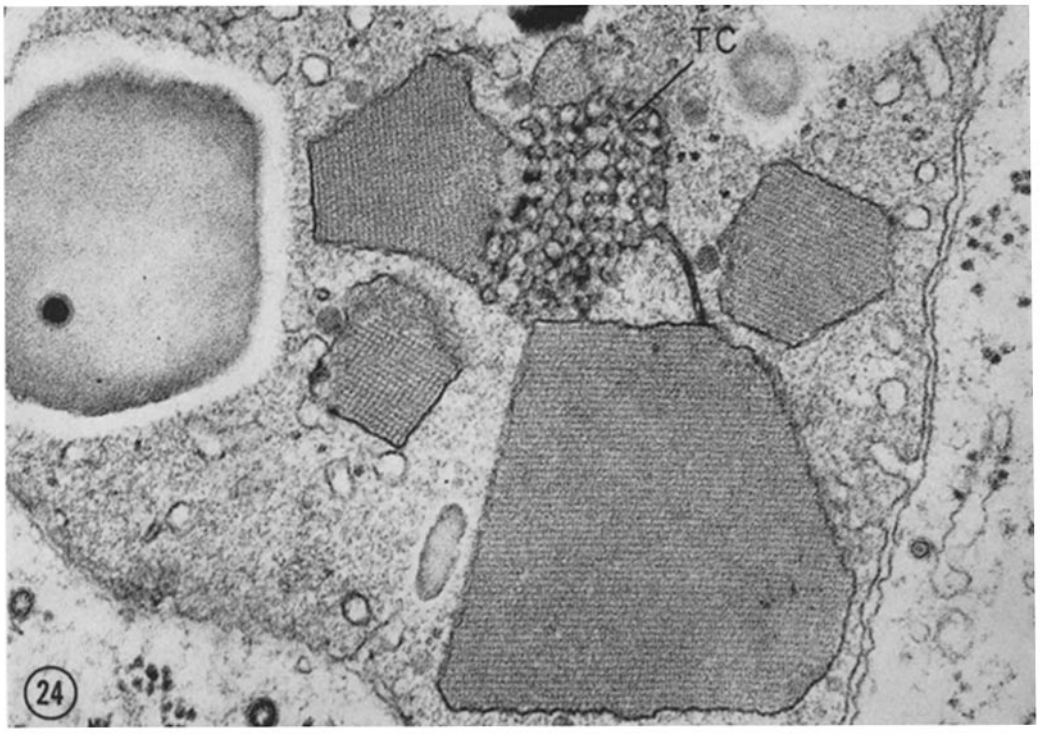


FIGURE 23 Portion of a plastid showing the presence within the tubular complex of granular material similar in appearance to that in the nearby sacs. The arrow indicates an infolding of the inner plastid membrane which appears to lead into the tubular complex. × 85,000.

FIGURE 24 Portion of a plastid showing several crystalline deposits of protein associated with the tubular complex (*TC*). The angularity of the tubules and the appearance of their contents strongly suggest that crystallization of protein also has occurred within the complex. × 80,000.

thesis of the prolamellar bodies and subsequently developed grana than in the synthesis of the relatively small tubular complexes and associated storage sacs.

It may be useful to call attention to some of the more obvious differences between the tubular aggregates of the leucoplast and etiolated chloroplast. The tubular complex is smaller and less highly organized, and appears to lack the ribosome-like particle which has been demonstrated by Gunning (9) in the stroma of each unit cell in chloroplasts of etiolated oat seedlings. Also, preliminary work indicates that the organization of the complex and the outgrowth of protein sacs from it in bean root plastids are not affected by light as is the prolamellar body of chloroplasts.

The light-induced disorganization of the chloroplast prolamellar body and the initiation of lamellar outgrowths from it are accompanied by a conversion of the associated protochlorophyllide to chlorophyllide *a* (18). Thus light sensitivity appears to be related to the association of protochlorophyll with the tubule membranes. Whether the tubular complex in the leucoplasts lacks protochlorophyll is not known. The complex does appear to be light-insensitive, however, and in addition seems to be specialized to collect protein and concentrate it for storage in associated sacs. It appears likely, therefore, that there are basic differences in chemical composition, organization, and response between the leucoplast complex on the one hand, and the chloroplast prolamellar body on the other.

Further differences between the two types of tubular aggregation become apparent during subsequent plastid development. Existence of the prolamellar body is confined largely to the early stages of chloroplast development in the dark; during plastid maturation in the light it soon is utilized as lamellae grow out from it and develop into the system of interconnected grana. In plastids of the root tip, however, development of the tubular complex is a continuing accompaniment of plastid development, and the complex not only persists in older cells in association with the sacs which arise from it, but also commonly appears to undergo further elaboration.

Tubules arising from the inner membrane of the plastid envelope probably play a role in the aggregation of the tubular complex. Sections in which one or more tubules can be traced from the plastid envelope to the complex have been observed repeatedly. Also, young plastids with groups of tubules which arise from the envelope and appear to converge on one another deeper in the stroma are encountered frequently; this raises the possibility that the complex is formed through the merger of a number of tubules with one another and with cisternae and lamellae, followed by local membrane proliferation.

Tubules similar both in appearance and in their origin from the inner plastid membrane were observed by Schnepf (30) in plastids in the floral gland cells of *Passiflora*, and recently have been described by Jensen (16) in amyloplasts in micropylar cells of the cotton nucellus. There is no evidence that in these plastids tubules are interconnected to form complexes, however. It also should be pointed out that there are a number of published observations of plastids not containing tubules, so that, on the basis of the evidence available at present, it is not clear that tubules will prove to be important components of plastid structure generally.

Although Mühlethaler and Frey-Wyssling (23) described the origin of the prolamellar body of juvenile chloroplasts from invaginations of the inner membrane of the envelope, the details by which this is accomplished do not appear to have been explored. In the present study, young plastids of the primary leaves of etiolated bean seedlings were examined, and the convergence of groups of tubules in a particular region of the stroma was noted, suggesting the possibility that tubules are involved in formation of the prolamellar body.

Interesting questions are raised when the site of synthesis of the storage protein and its pathway of movement into the plastid sacs are considered. It seems likely that this synthesis takes place outside the plastids. Ribosomes are not numerous in the plastid stroma, and appear to be no more abundant in plastids with large protein bodies than in plastids with no storage protein. Considering the requirement of ribosomes for general plastid maintenance, a considerably greater abundance than is observed might be expected if ribosomes are responsible also for synthesis of the large amount of storage protein.

Conceivably, some of the protein could be obtained by the plastids during the ameboid stage. Isolation of cytoplasm within pockets would provide a mechanism for keeping secreted enzymes localized and concentrated, and for preventing breakdown products from rapidly diffusing away before absorption. However, what proportion of the leucoplasts, in fact, do undergo an ameboid

stage is not known, and at any rate some of the young plastids contain protein deposits before they reach this stage. Also, hydrolysis of protein isolated in the pocket of cytoplasm would require a plastid-localized mechanism of resynthesis. Aside from the question of the storage protein, however, the possibility remains that the ameboid plastids may represent a "feeding" stage in which cytoplasmic material is digested and assimilated, particularly since the engulfed cytoplasm frequently appears degraded partially.

If the storage protein is synthesized outside the plastids, it then presumably passes through the outer membrane of the plastid envelope, moves via a tubule into the tubular complex, and thence into a sac opening off the complex. This assumes that the protein is confined to the space bounded by the inner membrane and its tubular and saccate extensions and that it remains isolated completely from the stroma. As protein accumulates in the complex, it causes swellings at the margins which may occur partly at the expense of pre-existing membrane in the complex, and partly by new membrane synthesis.

The tubular complex might, alternatively, function to concentrate and purify the protein by removing water and extraneous substances. Possibly the crystallization, which occasionally appears to have taken place within the tubules of the complex itself, is indicative of such processes.

The outer root cap cells of bean, like those of other plants (17), lie embedded in an abundant secretion of mucilage. Dictyosomes and associated vesicles are numerous in these cells, and probably are involved in the synthesis of mucilage at the expense of the starch bodies in the plastids (26). The fate of the protein stored in the plastids is not known, although in some micrographs of the older root cap cells the protein bodies appear digested partially.

Since fine structural evidence for it has not appeared previously, the storage of protein in crystalline form in root tip plastids probably is not a common phenomenon, although it possibly may prove to be so in some groups of plants or under particular environmental conditions. There are, however, a number of recent reports of the accumulation of protein or of crystalline material suspected to be protein in plant cells, and it is of interest to compare these reports with the mode of storage described here. Heslop-Harrison (11) observed protein crystals lying in an electron-transparent space in plastids in tapetal cells of hemp. Recently, Price et al. (27) have reported the presence of crystalline bodies within membrane-bounded sacs in chloroplasts of the coconut palm. Tubular complexes in association with the sacs were not seen. Heinrich (10) has examined the fine structure of "proteinoplasts" in the lower epidermis of *Helleborus* leaves fixed in permanganate or osmium-dichromate solutions. It seems likely that the large vacuoles that he has observed in these plastids are protein sacs, like those reported in the present study, contents of which have been destroyed by the fixatives.

In several studies crystalloids or proteinaceous deposits have been observed in plastids in sieve elements. Hohl (12) observed crystalloids in sieve element plastids of *Datura*, and Srivastava and O'Brien (32) have recently identified protein crystalloids in sieve element plastids of *Pinus*. Falk (5) and Esau (4) have demonstrated proteinaceous material in the form of thick, fibrous rings in plastids of sieve elements of *Tetragonia* and *Beta*, respectively. In all these observations involving sieve element plastids, the material has been located in the stroma and has not been enclosed within a membrane.

Protein accumulation within dilations of the endoplasmic reticulum also has been reported recently. Bonnett and Newcomb (2) have described the formation of large masses of protein in ER cisternae in radish root cells, and Jensen (16) has demonstrated similar accumulations in collar cells of the cotton nucellus.

Finally, several papers have established that organelles consisting of a crystalline body surrounded by a single membrane are present in the cytoplasm of a variety of plant cells (3, 19, 33). Cronshaw (3) has reported the presence of these bodies in several cell types and has proposed that they represent storage granules of hydrolytic enzymes. Marinos (19), who has found them in dormant potato tubers, has suggested that they consist of storage protein and may arise from the ER. It remains for future investigation to determine to what extent these bodies, the above mentioned ER cisternae, and the plastids described in this paper are related by common features in the accumulation, storage, and utilization of protein in plant cells.

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