AN ELECTRON MICROSCOPIC EVALUATION OF CROWN GALL TUMORS IN TOMATO PLANTS

by

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INTRODUCTION

STATEMENT OF THE PROBLEM

Crown gall tumors of plants have been studied extensively from the physiological and biochemical point of view. Detailed basic structural studies concern gross morphology and histology, but the numbers of electron microscopic studies is limited. The purpose of this thesis is to compare tumor and normal cells at the cellular and subcellular levels by means of electron microscopy. In this way it may be possible to draw some conclusions as to the possible identity of the unknown tumor-inducing principle.

REVIEW OF THE LITERATURE

Cancerous growths have been the object of speculation since long before the birth of Christ. The Papyrus Ebers (1500 B.C.) mentions this problem, as does some of the oldest literature of India and Persia (27). Through the centuries various causes of cancer have been proposed. During the time of Hippocrates, imbalance of the humors of the body--blood, mucus, and bile--were thought responsible for cancerous growths. Wolf's theory of cellular architecture of tissues and Rapail's discovery that growth of tissues resulted from the multiplication of cells launched the search for the "cancer cell" in the early 1800's (27).

One of the most popular theories of the causative agent of cancer is the parasitic theory. Epidemiological studies in Europe had proposed an infectious agent in the early 1800's. Protozoa, many species of bacteria, spirochetes, slime molds, yeasts, and bread molds were all

described as specific agents in cancer, but each in turn failed to possess the necessary ability to stimulate somatic cells to repeated multiplication (27, 60).

In 1907 Erwin F. Smith and C. O. Townsend wrote an article titled "A Plant-Tumor of Bacterial Origin". Smith and Townsend successfully isolated a bacteria from galls of Paris daisy. The bacteria, which they named *Bacterium tumefaciens*, was cultured, reinoculated into the daisy, and reisolated from the resulting tumorous growth, thus fulfilling Koch's postulates and establishing it as the etiological agent. The importance of this discovery was realized by Smith as he wrote, "A tumor- the origin of which is known- may be of some interest to animal pathologists in determining the origin of cancerous growth" (92).

Smith felt there was a definite parallel between crown gall and certain malignant diseases of man: the primary tumor gave rise to metastasis or secondary tumors, there appeared to be a variable resistance to crown gall in different species of plants, and many tumors contained embryonic characteristics of teratomas (91). Therefore, Smith proposed that animal cancers must be due to a similar cause, an intracellular microorganism yet undiscovered (90).

Not all researchers shared Smith's enthusiasm for his analogy between plant and animal cancers. Levin felt that crown gall formation was a protective reaction similar to an inflammatory response, and should be considered a normal defence mechanism rather than neoplastic cellular growth (58). C. O. Jensen, a Danish biologist, proved that bacteria were not necessary for the perpetuation of the crown gall tumor. He grafted bacteria-free tissue into healthy plants and observed vigorous

tumor formation in the host tissues in the absence of any bacteria (22).

Agrobacterium tumefaciens.

Because the transformation from normal plant cells to autonomously growing crown gall tumor cells is dependent upon the presence of a viable strain of *Agrobacterium tumefaciens*, considerable effort has been made to determine why the agent is tumorigenic.

Agrobacterium tumefaciens (Smith and Townsend) Conn. is a unicellular, non-sporulating, gram-negative, rod-shaped organism, occurring singly or in pairs, encapsulated and motile with 1 to 6 flagella. The bacteria frequently have fimbria-like structures measuring 60 to 80 Å in diameter. The fimbriae are longer, thinner, and more fragile than the flagella, which measure 100 to 120 Å in diameter (18, 21, 86).

Smith observed the bacteria within the tumor cells (89); however, it has since been shown that the bacteria are definitely located in the extracellular spaces. Hill found bacteria to move rapidly as a zoogloea through intercellular spaces (36), both above and below the injection site (37). This movement is passive, as no difference in movement has been observed between alive and killed bacteria (42, 94). Riker injected bacteria into healthy tomato plants and observed tumor formation to follow sterile woundings distant from the site of injection, and concluded that the bacteria travelled from the site of injection through the tracheal system. This concept was supported by isolation of the bacteria from serial sections of inoculated plant stems (75). Bacteria have been isolated from secondary tumors in tobacco, which suggested that extracellular movement of bacteria was the probable cause of these distant tumors (80).

Stonier demonstrated the intercellular location of bacteria in sunflower and *Kalanchoë* by using P^{32} labelled bacteria. Bacteria move primarily up and down the stem with little lateral migration. The tumors arise only in the site of wounding and this migration of bacteria alone will not give rise to additional tumors spontaneously (94). Bacteria can be injected intracellularly and are found to cause no tumor, although the bacteria proliferate in media composed of cell sap (34). The relationship of tumor formation and wounding will be discussed later in this paper.

Five of the seven known strains of *A. tumefaciens* cause crown gall in plants (86). Avirulent strains of *A. tumefaciens* grown on media composed of filtered tumor tissue become virulent. Subcultures of these newly tumorigenic bacteria remain virulent. Avirulent *A. tumefaciens* grown in the presence of DNA isolated from pathogenic strains of bacteria become virulent; DNAse causes the nucleic acid to lose this ability to transform avirulent strains of bacteria to virulent strains. Isolated virulent DNA injected into a plant gives no response. The tumorigenic qualities can even be transmitted to *Rhizobium leguminosarum*, a closely related nitrogen-fixing intracellular bacteria (47), implicating a genetic factor in virulence determination.

Ribonuclease A inhibits the tumorigenic properties of virulent *A. tumefaciens* (17). Schilperoort cited an increased formation of complexes between DNA isolated from tobacco crown gall tumors and RNA complementary to *A. tumefaciens* DNA (84), as evidence for the transmission of some genetic material from the bacteria to the plant cell.

External factors influence the tumorigenic properties of *A. tumefaciens*. The bacteria are unable to produce tumors in plants if maintained

at temperatures in excess of 30° C (77), although the bacteria grow well at this temperature. Treating *A. tumefaciens* cultures for short periods of from 39° C to 48° C destroys the tumor-inducing principle (TIP). This inactivation is time-related and reversible. Theories as to the nature of inactivation include: protein denaturation and heat inactivation of virus nucleoproteins (67), induced depolymerization of DNA (47), or decreased metabolism of the bacteria (95).

Twenty to thirty transfers of *A. tumefaciens* colonies on media containing increasing amounts of glycine result in apparent loss of TIP for 4 years (97). Anti-tumor drugs 5-fluorouracil, cytosine arabinoside hydrochloride, and others (1, 56), along with antibacterial agents streptomycin (25) and aureomycin (26), all inhibited tumor formation. A positive correlation was found in all studies between bacterial growth inhibition and the effects of the drugs on the tumor-inducing ability of the bacteria.

Wounding:

The necessity of tissue wounding as well as the presence of virulent *A. tumefaciens* for the formation of tumors was first established by Riker in 1923 (75). Viable bacteria spread on the surface of tobacco plants do not cause tumors, although bacteria can be subsequently demonstrated within the leaf (71). Plants grown in heavily infected soil remain tumor free if not wounded. A suspension of bacteria injected into the hollow pith of a bean plant causes tumor only at the site of injection, although the bacteria flow for a considerable distance. Bacteria injected into a plant adjacent to an area of wounding will induce tumor formation in the wounded area, but not in nearby healthy tissue

(75). The size of a puncture wound has a direct relationship to the size of the resulting tumor, regardless of the size of the bacterial inoculum (34, 76).

Using the heat inactivation technique of Riker (77), Braun studied the critical time necessary for tumor induction. Bacteria need a minimum of 10 to 14 hours in contact with wounded plant cells, but the cells must be "conditioned" a minimum of 32 to 36 hours following wounding (15, 63). Plants wounded and injected with virulent bacteria, and kept at 32° C for 5 days, and then returned to an optimal 25° C don't develop tumors (8, 15, 77). This inhibition is also true of plant tissue *in vitro* (26). Bacteria are present and viable in these plants following 5 days of heat treatment, but a fresh wound is no longer present.

Hildebrand, using micropipettes, was able to inject one cell with 100 bacteria, thus providing no surrounding wound response, and there were no resulting tumors. One bacterium injected into a large wound, however, caused a large tumor to form (34).

Braun showed that plant cells had variable susceptibility to the tumor-inducing properties of the *A. tumefaciens* at different intervals following wounding. In these experiments, virulent bacteria were injected into susceptible plants. The tumor inducing principle (TIP) of the bacteria was stopped at any desired time by transferring the plant to a chamber at a temperature of 32° C, thus inactivating the TIP. Plant and bacteria were allowed to remain at the optimal 25° C for variable times prior to temperature inactivation of the TIP. Braun showed plant cells "transformed" at 32 hours (i.e., TIP heat inactivation after 32 hours) after wounding developed slow growing tumors, while plant cells

"transformed" at 60 to 70 hours after wounding formed rapidly growing tumors. These rapidly growing tumors would continue to grow *in vitro* on a basal medium capable of supporting neither normal tissue growth, nor the tumor induced at 32 hours (7, 8, 14).

Sections of beet tissue washed for 10 hours following wounding are not transformed to tumor cells by virulent *A. tumefaciens*, nor is there callus formation which is usually observed as a response to wounding. Application of wound sap to these sections restores their tumor-producing potential, as well as the ability to form callus (48). X-ray suppression of the wound healing response inhibits tumor formation (57). Wounding apparently not only gives bacteria access to plant cells, but more importantly, releases substances from the cells which stimulate surrounding healthy tissue to divide. The maximum host sensitivity is closely related to the first wound-induced cell division (63). After 60 to 70 hours after wounding, sensitivity falls off so that by 120 hours plant cells are no longer transformable (7, 15).

The latent period of 10 to 12 hours required for the bacteria to transform wound-responding cells into tumor cells is apparently not related to time required to change into an infective spheroplast. Spheroplasts caused by glycine media (2) or ultraviolet light (83) have no shorter transformation period than normal *A. tumefaciens*.

Normal wound response--The normal wound response has been described by Bloch (3, 4), and Rash, Klein and Swift (73). On the first day of wounding the ruptured cells form a layer of sap and cell debris following the line of the puncture. After two or three days this sap effectively seals off the surrounding cells from the wound and cell death

occurs in those cells disrupted by wounding. Approximately 60 to 70 hours after wounding the first wave of mitotic figures is seen (63), and by 4 to 5 days the perimedullary cells have begun active repair by cell division parallel to the axis of the wound surface. By the fifth day one-third of the parenchymal cells surrounding the wound have divided. Parenchymal cells of the central cylinder initiate a secondary area of growth (the wound periderm) which produces small, flattened, parenchymal derivatives from the wound meristem. These dividing cells continue to proliferate until wound repair is complete in 30 days (73). Riker felt the stimulus of wounding in tomatoes lasted only five to ten cell divisions (75). Twisting vascular elements, especially tracheae, are common in callus formation, as are nuclear irregularities (4).

Tumor Response:

The first 24-hour cellular response to a puncture wound containing viable *A. tumefaciens* is the same as the response to a sterile wound (73). The first observed response is an enlargement of those cells surrounding the wound. This is especially marked in the cortex (52). All living cells except epidermis and mature xylem and phloem become affected by 9 days after wounding (53, 73). These cells either continue enlarging, becoming progressively more polyploid, or divide, giving rise to nests of small, atypical dividing cells (51, 52). The division planes of the proliferating cells cease to parallel the path of the wounding needle, as in the normal wound response, and by the eighth day, orderly arrangement disappears (52). The growth of the "stimulated" cells fills in the space left by wounding, and proliferating cells reach the outer wound surface, growing into an irregular globular mass of tissue (59).

As the mass enlarges, the epidermis and outer cortex react. Most of these cells divide, forming small cells which fuse with the tumor mass as it emerges from the stem. The larger cortical cells are usually shoved aside, but may be dragged into the growing mass (52). The innermost proliferative islands of small cells cease to divide and differentiate into trachea-like structures. These lignify and form the core for a widening mass of cells with lignified secondary walls (52, 73). The marginal islands of small cells continue to divide and give rise continuously to new growth centers. These marginal centers of growth are responsible for the tumor's nodular surface (51, 59).

The tumor tissue is disorganized with great cellular variation, giant cells with varying degrees of polyploidy, and irregular twisting cells which are rarely of uniform size (53).

During the first 3 days Rasch, Klein and Swift (73) observed the bacteria within the wound; no direct indication of bacteria within the resulting tumor tissue was noted. The difficulty of locating bacteria with the light microscope within tumor tissue has been noted by Smith and Riker (75, 89). Riker (75) thought the difficulty resulted from the increased "stainability" of the cell wall adjacent to inciting bacteria and the resulting masking of the bacteria. The location of the bacteria determined which cells begin to divide: the cells adjacent to bacteria were transformed to the small, rapidly dividing cells (76, 94).

Crown Gall Teratomas:

Not all crown gall tumors present as the classical warty mass of disorganized tissue. A "teratomatous" form of tissue has been studied by Armin Braun of the Rockefeller Institute of Medical Research, and it

is his studies which are here presented (6, 9, 10).

A tobacco plant's stem is cut so as to remove a transversely oriented notch of tissue. Both the basal (lower) and apical (upper) surfaces of the notch are painted with a suspension of *Agrobacterium tumefaciens* of moderate virulence. The tumors forming on the two cut surfaces are distinctly different. The tumor arising from the apical surface of the notch is the "typical" irregular unorganized tumor mass. The tumor arising from the basal surface of the notch is a complex tumor called a "teratoma".

The "teratoma" is composed in part of highly abnormal leaves and buds in varying stages of development. The tissue is morphologically and histologically more organized than the "typical" crown gall tumor. This teratomatous tissue, when transferred to artificial media, proliferates on basal medium which will not sustain growth of normal tissue, and morphologically different areas of the tumor give rise to morphologically identical cultures *in vitro*.

When this teratomatous tissue is transplanted to the apex of a tobacco plant after removal of the apical bud, the resulting tumor continues to grow as a teratoma. However, when the teratomatous tissue is transplanted to a position on the stem of a plant, the resulting tumor tissue loses the organized features and assumes the irregular, warty form. The presence of a functional apical bud apparently suppresses the organizational activity of the grafted tissue. If this suppressed teratomatous tissue is once again transferred to artificial media, the teratomatous morphology reappears.

None of the tumors formed by a highly virulent strain of bacteria have this teratomatous morphology. When the experiments were duplicated on sunflower, which has less regenerative capacity than tobacco, the tumors were invariably unorganized and without teratomatous formation. Braun concluded that the morphology of crown gall is determined by three factors: 1) the virulence of the initiating strain of bacteria, 2) the position the tumor occupies on the host plant, and 3) the inherent potentialities of the affected cell for regeneration.

Nuclear Changes of Tumor Cells.

Aside from the structural abnormalities noted in light microscopic studies of crown gall tumors, nuclear changes have been constantly mentioned. Although there is some difference of opinion, most authors agree that there seems to be an increased incidence of chromosomal polyploidy in tumor tissue when compared to normal (52, 53, 59).

Biochemical analysis of this nuclear change led Klein *et al.* (50) to report a 30 percent increase in DNA in "transformed" tumor cells which preceded all other events in the initiation of tumor formation. Subsequent researchers have been unable to demonstrate this immediate rise in DNA; however, microphotometry of Fuelgen-stained tissue has documented a marked increase in DNA as well as RNA in actively growing tumors (52, 73). The increased values were felt to reflect the increased metabolic activity of the cells and the progressive accumulation of nuclei with higher degrees of polyploidy.

Comparisons of crown gall and normal tissue in tissue culture indicate that tumor cells contain more DNA than does normal tissue. Some workers suggest the increase is not due to chromosomal DNA, but to the

presence of a non-chromosomal DNA (41, 81).

Cytoplasmic Changes of Crown Gall Cells.

The presence of coarse cytoplasmic granules in the active regions of tobacco tumors has been described. Levine (59) observed them as "dense globular bodies which may be distributed equally through the cytoplasm or piled up about the nucleus." Riker (78) felt they were metabolic products which stained with osmic acid. Crystals were seen in the cytoplasm of tumor cells of tomato and felt to be more frequent than in controls (78).

Electron microscopic studies of crown gall cells have been few. The most comprehensive electron microscopic study of crown gall is the work of Hohl (39). Studying tomato, datura and sunflower, this author concluded that the only abnormal organelles in tumor tissue were the plastids. He observed hypertrophic proplastids, plastid invaginations, plastids containing inclusion bodies, and degenerated plastids. Gee's work (30) with sunflower supported Hohl's original findings in regard to plastids.

Inclusions of chloroplasts in both crown gall tumor and auxin-induced tumors have been reported. Such inclusions may not be diagnostic of crown gall tumor. The endoplasmic reticulum of crown gall cells is felt to be more prominent than that of non-tumorous cells (64, 65).

Biosynthetic Systems of Tumor Cells.

Crown gall tumors resulting from "transformation" by a virulent strain of *A. tumefaciens* early in the wound healing response (36 hrs) differ from tumors resulting from "transformation" later in the healing process (60 hrs) (14). The later tumors grow more rapidly and are able

to sustain rapid growth on a basal medium which will support only retarded growth of the earlier tumors. Thus the transformation from normal plant cells to tumor cells is considered to be a progressive, rate-limited process leading gradually to "fully transformed" autonomous tumor cells (5, 8, 15).

Fully transformed crown gall tumor cells can maintain rapid growth on the mineral salts and sucrose contained in White's basic medium (99). Partially transformed cells are able to grow at a lesser rate on the basic medium. The addition of an auxin (such as naphthalene acetic acid) and inorganic salts to the medium enables the growth rate of the partially transformed crown gall cells to approach that of the fully transformed cells. Normal cells which are unable to grow on White's basic medium attain the rapid tumor-like growth of crown gall cells if the medium is supplemented with an auxin, a kinin, inositol and additional inorganic salts. This information led Henry Wood to propose that the transformation to tumor cells involves either changes in membrane permeability or in ion-transport systems, and as a result, "essential ions penetrate to the proper loci in tumor cells but are unable to do so in a normal cell unless the concentration of such ions is raised significantly in the medium" (102-104).

It has long been recognized that crown gall tumors seemed to produce growth stimulators. The plant-supporting tumors demonstrated: 1) epinasty of leaf petioles, 2) initiation of adventitious roots, 3) stimulation of combined growth, 4) inhibition of lateral buds, 5) petiole abscission, all conditions felt to be indicative of the presence of greater than normal amounts of growth stimulators (68). Serial

sections of plant stem removed from the tumor region of a stem showed a gradation of root formation on artificial medium, demonstrating the diffusion of a "growth substance", both up and down the plant stem (23).

Plant callus cells cultured *in vitro* with tumor grew profusely, while alone on similar culture medium there was no growth (23, 24, 93). Extracts of bacteria and the application of exogenous growth hormones have been successful in increasing the growth of normal cells to a level approximating crown gall proliferation; however, the normal cells revert back to normal growth as soon as the supply of hormone is discontinued (12, 49, 61, 66, 88). The growth hormones produced by crown gall have been identified as two factors necessary for active growth of any plant cell: auxin, the factor necessary for cell enlargement, and kinin, the factor required for cell division (11, 13, 16). Anti-auxin, isolated from plant tissue and applied to plants prior to injection with *A. tumefaciens*, suppressed the growth of the tumors, as well as all the previously mentioned systemic effects of high growth hormone production (79, 96).

Although much has been written and said about the crown gall tumor, little is known about the origin of the tumor inducing principle proposed by Braun. The field of research which seems to offer the most promise currently involves the biochemistry of cytokinins. Cytokinins are powerful growth stimulators recognized as N^6 substituted adenines. They show profound effects on general control of plant form as well as the rate of cell division. Cytokinins govern the production of DNA, RNA and protein; they regulate organ formation, phloem transport, and metabolite mobilization (32). Cytokinin activity due to 6-[γ,γ-dimethylallylamino]purine has been localized in transfer RNA preparations

from *E. coli* (87). The same substance has been isolated from *Corynebacterium fasciens*, another bacterial pathogen known to cause abnormal growths in plants (33, 46). This is the first demonstration known to this author of a substance, found in bacteria, which can apparently be incorporated into another cell and stimulate its growth.

Theories as to the Cause of Crown Gall Tumors.

Erwin Smith felt that *Agrobacterium tumefaciens* was directly responsible for crown gall tumors (90). Though this original theory has not been disproved, it has been modified. The bacterium is not necessary for continuation of the tumor, only its initiation. The nature of the principle which the virulent strains of *A. tumefaciens* contain, or produce, and which is capable of initiating a plant cell to uncontrolled division and "malignant growth", is unclear.

deRopp (23) proposed a viral etiological agent after finding secondary tumors arising on healthy sunflower stalks, above which were grafted bacteria-free crown gall tumor tissue. White and Braun were unable to cause tumor formation following usual virus transfer techniques (101). The variability of the tumor forms (rapid and slow growing), and the heat inactivation of *A. tumefaciens* at 32° C with crown gall tumor cells which still retained their growth patterns at 47° C, both indicate the improbability of the viral causal theory (10). Riker (75, 76) and Stonier (94) both determined that the location of the bacteria in the wound caused the surrounding cells to become tumorous. This observation and the necessity for the bacteria to be metabolically active (1) raised the possibility that the etiological agent was a diffusable substance

produced by the bacteria. Repeated tests with media in which bacteria have been cultured failed to cause tumorous growths (66).

The possibility that chromosomal changes resulted in tumorigenesis was a popular theory. The concept arose that all plant cells were potential tumor cells, but were held in check by genetic repressors. Braun (6, 10) placed tumor cells on apical portions of plants, and thereby forced the tumor cells to divide even faster than usual, and converted tumor cells back to normal cells by continued stimulated division. This finding indicated the tumor inciting material or change must lie in the cytoplasm or at least not be tightly attached to genetic material as previously thought. Braun felt the cells had divided faster than some cytoplasmic element, thereby diluting this tumor-inducing material out of the cell line. Braun explained the different degrees of "malignancy" by assuming that "wounding generates a cytoplasmic component which generally increases during early stages of wound healing and reaches a maximum in 2 to 3 days." He proposed that this component was no longer produced by the cells and as they divide during the healing process they progressively diminished the amount of this component found in each cell.

The tumor-inducing principle of the bacteria must influence this cytoplasmic element to become self-perpetuating and thus give rise to constantly dividing cells (10). Hohl proposed the plastid inclusion may be this cytoplasmic component (40).

There is a large amount of data suggesting transfer of genetic material from the bacteria to the plant cell. Schilperoort (84) demonstrated the presence of genetic material from the inducing bacteria

within the crown gall cell. Braun (17) inhibited the production of tumors with ribonuclease-A and proposed that TIP may be bacterial RNA. Klein and Klein (47) showed that the tumorigenic ability could be passed from a virulent strain of bacteria to an avirulent strain. They also transmitted the tumorigenic property to a *Rhizobium leguminosarum*, which is an intracellular symbiont. Skoog demonstrated the cytokinin activity of bacterial transfer RNA (87) and this activity has been demonstrated in bacteria capable of inducing tumors on plants (33).

MATERIALS AND METHODS

PLANTS

The plant material studied was obtained from commercial seed sources and consisted of tomato (*Lycopersicon esculentum*), Marglobe and Bonny Best varieties. *Bryophyllum* plants were obtained from the Botany and Plant Pathology Department at Oregon State University through the generosity of Dr. Tom Allen. *Bryophyllum* were propagated by leaf sproutings. Both tomato and *Bryophyllum* plants were rooted in sand, transplanted into clay pots, and maintained in a greenhouse in a temperature range of 22-29° C. Some plants were kept inside the laboratory under a bank of fluorescent lights containing two Sylvania Gro-Lux^{R} lamps.

BACTERIA

A virulent strain of *Agrobacterium tumefaciens* (Smith and Townsend) Conn. was obtained from Dr. Ira Deep of the Department of Botany and Plant Pathology at Oregon State University. The culture was maintained on potato dextrose agar (PDA). New transfers were made about every three months and cultures were maintained at 10° C.

Potato Dextrose Agar (PDA).

The following recipe was used.

Dissolve in 500 ml of water in which 200 gm of potato have been boiled:

Bacto agar.....15 gm,

Dextrose.......20 gm.

Dilute to 1000 ml with water.

Autoclave for 15 min at 15 psi.

Pour into sterile culture bottles.

INOCULATION OF PLANTS

The cultures of *A. tumefaciens* were reinoculated into new bottles of PDA and allowed to remain at room temperature for 48 to 60 hours. At this time the streak of bacteria was easily visible and there was ample material for inoculation.

An anatomical dissecting needle was used to wound the plant and transfer the bacteria into the wound. The needle was flamed over an alcohol lamp and a portion of the viscous bacterial material collected on the needle. The bacteria were deposited upon the surface of the plant stem and the needle introduced into the plant tissue through the bacteria. The plants were inoculated at the nodal area on the stem. The needle was introduced approximately three-quarters of the way through the stem, withdrawn and reinserted into the wound in an attempt to carry a maximum amount of bacterial inoculum into the depths of the wound. The plants were usually 20 to 30 cm in height at the time of inoculation. The injected nodes were always removed from the growing tip by at least two other nodes. Lower nodes were never utilized.

TEMPERATURE CHAMBER

Attempts were made to establish a gradation of tumors from slow growing to rapidly growing by use of variable incubation periods with the virulent bacteria and termination of this period with heat (15). A standard growth chamber was used (NAPCO model 330). The temperature was

32° C and humidity was kept high with an open pan of water in the chamber. Potted plants were placed in the chamber and kept there for the remainder of their transformable period following wounding--or until 120 hr post-wounding.

The tomato plants were uniformly unable to withstand the heat of the chamber and consistently wilted and died. *Bryophyllum* plants were more successful in withstanding the heat treatments, especially after the addition of a fluorescent light source outside the glass-enclosed chamber. The addition of the light source was not tried with tomato plants.

TISSUE CULTURE

Tissue culture methods were used to obtain a source of actively dividing non-tumor cells to compare with the rapidly dividing active tumor cells.

The plant stem was transected with a razor blade and all leaves and stems were cut off flush with the surface of the stem. The pieces of stem were cut into 1 to 2 cm sections for ease in handling and placed into a 5 percent solution of Phisohex[R] and sterile distilled water. The sections of stems were agitated for 5 min to remove dirt and help reduce surface tension. The stem fragments were rinsed in sterile distilled water, then agitated in a 10% Chlorox[R] solution for 10 min and rinsed 4 to 5 times in sterile distilled water. The plant material was then placed in a bacteria- and virus-free hood and cut aseptically into 1 cm sections. The sections were then split longitudinally in half so each piece of stem was 1 cm long and consisted of one-half of a stem.

The pieces of stem were then transplanted three-quarters of the way into culture bottles containing tissue culture media. The necks of the bottles were plugged with cotton and covered loosely with aluminum foil. Within 2 to 3 weeks the apical portions of the transplanted stems were covered with a warty, yellow friable gall formation which could be easily removed and transplanted into similar culture bottles. The bottles of plant cells were kept at room temperature (22-25° C) and under the Sylvania Grow-Lux lights. The cultures were transplanted every 3 to 4 months.

Attempts to establish a crown gall tumor cell line on artificial media failed. Tissue fragments were removed aseptically by breaking open a surface-sterilized tumor nodule and curetting out a small segment of tissue with a sterile, sharpened ear-curette. The cultures were consistently overgrown with bacteria contaminants from the tumor tissue felt to be *Agrobacterium tumefaciens*. A few attempts were made to heat the plant to 46 to 47° C for 5 days to kill the bacteria (100), but the plants were unable to withstand these temperatures. Tumor tissue was transferred to tissue culture media and heated to 46-47° C for 5 days in an attempt to eradicate the bacteria, but the tissue failed to grow. Penicillin, 200 units per ml, and streptomycin, 200 μg per ml, were added to the tissue culture media and the bacteria were suppressed, but the plant tissue transplants did not grow.

Tissue Culture Media.

The artificial medium used was modified from four sources (35, 43, 44, 99).

The medium is made up in a series of stock solutions which may be frozen and mixed when medium is needed. The first stock solution, the mineral salts, is made in 5 separate solutions, I-V, as follows.

I.	Double distilled water.....	250.0	ml
	$Ca(NO_3)_2$..................	3.0	gm
	KNO_3......................	0.8	gm
	KCl........................	0.65	gm
II.	Double distilled water.....	250.0	ml
	$MgSO_4 \cdot 7\ H_2O$................	7.5	gm
	Na_2SO_4.....................	2.0	gm
III.	Double distilled water.....	250.0	ml
	$Na_2H_2PO_4 \cdot H_2O$...............	0.19	
IV.	Double distilled water.....	250.0	ml
	$MnSO_4$.....................	0.05	gm
	$ZnSO_4$.....................	0.03	gm
	Boric acid................	0.015	gm
	KI........................	0.0075	gm
V.	Double distilled water.....	100.0	ml
	$CuSO_4 \cdot 5\ H_2O$................	0.04	gm
	MoO_3......................	0.004	gm

Mix solutions I, II, III, and IV in a 2 liter flask. Add 0.25 ml of solution V and autoclave at 15 psi for 15 minutes. Pipette 100 ml of the mixture into sterile bottles. The bottles are stored in the frozen state and used as a source of stock mineral salt solution.

Vitamin solution:

1) Add to 100 ml distilled water:

300 mg glycine,

50 mg nicotinic acid,

10 mg thiamin,

10 mg pyridoxin.

2) Filter through a Millipore filter and pipette into sterile test tubes a sufficient amount to be able to use the solution in 1 ml aliquots. Store frozen.

Indicator Solution:

1) Dissolve 100 mg chloramphenol red in 25 ml N/100 NaOH.

2) Dilute to 250 ml with double-distilled water.

3) Adjust pH to 6.0.

4) Filter with Millipore^R in vapor hood.

5) Store frozen in 10 cc samples.

Final Medium:

1. Mix in a 2 liter flask:

    a. 100 ml of mineral salt stock solution.

    b. 1.0 ml of vitamin stock solution.

    c. 10 cc of indicator solution.

    d. 20 gm sucrose.

    e. 150 ml coconut milk (can be stored frozen in large amounts).

    f. 2.5 $MgFe(SO_4)_3$.

    g. 6.0 gm Bacto agar.

    h. Dilute to 1000 cc with double-distilled water.

2. Autoclave at 15 psi for 20 minutes, remove, and allow to cool partially.

3. Add aseptically while media is still warm:

   a. 10 cc of 50% ETOH containing:

      1) 2.5 mg calcium pantothenate.

      2) 0.1 mg indole acetic acid.

      3) 6.0 mg 2-4-D ([2,4-dichlorophenoxy]acetic acid).

This method allows the growth stimulators to be added aseptically, but prevents their inactivation by autoclaving.

FIXATION OF TISSUE FOR ELECTRON MICROSCOPY

The tissue to be examined was removed from the plant with an alcohol washed razor blade and placed as small pieces into drops of fixative on a wax surface and sliced to approximately 1 to 2 mm cubes. The blocks of tissue were then moved to vials of fixative and kept there for 2 to 5 hours.

A variety of fixing methods were used. Figures 15, 16, 17, 21, 22, 25, 26, and 28 represent tissue fixed in 6.5% glutaraldehyde in 0.07 M sodium phosphate buffer, pH 7.0, for 3 hours at the temperature of melting ice. The tissue was washed overnight at 4° C in 0.07 M sodium phosphate buffer and post-fixed for 1 hour with 2% aqueous osmium tetroxide at room temperature. Dehydration was accomplished by a graded acetone series. The tissue was embedded in Araldite^{R}.

Figures 6, 7, 8, 9, 10, 18, 19, 20, 23, and 24 are tissue fixed in Karnovsky's fixative (45) for 3 1/2 hours at room temperature, washed overnight at 4° C in 0.2 M sucrose and 0.1 M cacodylate buffer, pH 7.4, and post-fixed in 1% aqueous osmium tetroxide in a veronal acetate buffer, pH 7. Dehydration was carried out through a graded ethyl alcohol

series and the tissue was embedded in Araldite. Figure 24 was similarly fixed except the overnight wash was done with 0.1 M sodium cacodylate only.

The tissue demonstrated by Figures 11, 12, 13, and 14 was fixed in Karnovsky's fixative (45) for 3 1/2 hours at room temperature under a vacuum of 15 mm mercury. The tissue was washed overnight in a veronal acetate buffer, pH 7, at a temperature of 4° C, post-fixed for 1 hour in 1% aqueous osmium tetroxide in veronal acetate buffer (pH 7.35), dehydrated in graded ethyl alcohol washes, and embedded in Araldite.

The Araldite was polymerized for 6 days at 60° C and sections for electron microscopy were cut on an LKB ultrotome, type 4802A, using glass knives. Sections were mounted on carbon-coated copper specimen grids and stained for 3 1/2 hours on a drop of saturated aqueous solution of uranyl acetate, rinsed with distilled water, and stained 10 minutes on a drop of lead citrate (74). The sections were viewed and photographed in an electron microscope, RCA model EMU-3G.

BACTERIAL PHOTOS

Electron micrographs of the bacteria *Agrobacterium tumefaciens* were prepared as follows. The bacteria were inoculated into 1% peptone Bacto agar and placed at 23 to 28° C with slight agitation for 24 hours. A drop of medium was placed in a dental wax slab and a parlodion-covered carbon-shadowed grid was inverted onto the drop of the medium. The grid was removed from the drop and the adherent medium absorbed onto filter paper. The dried grid was placed into a vacuum evaporator and shadowed with platinum-plutonium at a 15° angle. The grids were then viewed in the electron microscope.

RESULTS

The data will be presented as the ultrastructural comparison of three tomato (*Lycopersilum escalentum* Mill.) tissues. The tissue types will be referred to as: "normal", being samples from the nodal area of non-tumor, healthy tomato plants; "stimulated normal", referring to non-tumor tomato cells maintained on artificial medium containing growth stimulators; and "tumor" cells, being cells located within the crown gall formation resulting from the injection of virulent *Agrobacterium tumefaciens* (Smith and Town) Conn.

NORMAL DIFFERENTIATED TOMATO TISSUE

Morphology.

The normal tomato stem tissue is composed of two cell types: differentiated cells, and undifferentiated or meristematic cells (Figs. 6, 7, and 8).

The majority of cells from the tomato stem are the differentiated cells, cylindrical in shape, and measuring 20 μ by 6 μ. The differentiated cell is bounded externally by a prominent cell wall 0.4 μ to 0.7 μ in thickness. Applied to the inner surface of the cell wall is a unit membrane, approximately 100 Å thick, the plasmalemma. The plasmalemma is irregular in contour and contains frequent infoldings surrounding small vesicles measuring 0.15 μ to 0.2 μ in diameter (Fig. 8). Adjacent to the internal surface of the plasmalemma is the thin rim of cytoplasm containing cytoplasmic structures and a peripherally located nucleus. The rim of cytoplasm is bounded internally by the tonoplast,

or limiting membrane of the large (18 μ by 4 μ) central vacuole, and externally by the plasmalemma and cell wall. The central vacuole is devoid of any identifiable secretory products or structures and is considered characteristic of the differentiated cell.

Cytoplasmic Elements.

Endoplasmic Reticulum:

The cytoplasm of the differentiated tomato cell contains both smooth and granular endoplasmic reticulum (ER). The smooth ER consists of closed membranous sacs which are not surfaced by ribosomes. The granular ER is conspicuously present; ribosomes are attached to the outer surfaces. The sacs measure up to 2.1 μ in greatest dimension (Fig. 7). There is no consistent topographical relationship observed between ER and mitochondria, nuclei or plastids.

Ribosomes:

Ribosomes appear as osmiophilic granules measuring 100 Å to 150 Å in diameter, and are observed attached to membranes or lying free in the cytoplasmic matrix. The techniques and magnification of this work do not allow meaningful comparison of ribosomal concentrations.

Mitochondria:

Mitochondria are elliptically shaped structures ranging in size from 0.7 μ to 1.2 μ with an infrequent elongated form measuring up to 1.7 μ in length (Fig. 8). Mitochondria are bounded by a double membrane with the inner membrane folded into tubules which extend into the mitochondrial matrix. Osmiophilic granules (measuring approximately 300 Å in diameter) are observed within the matrix of the mitochondria (Figs. 6 and 9).

No consistent topographical relationship is observed between mitochondria and other cellular components.

Plastids:

The normal differentiated tomato cell contains normal chloroplasts measuring 4 μ to 5 μ in length. A double membrane surrounds an inner matrix containing a system of closely approximated lamellae consisting of double membranes. These lamellae enclose occasional osmiophilic droplets (0.1 μ to 0.3 μ) called plastoglobuli, and starch grains (1.0 μ by 0.4 μ). Within the uniform parallel pattern of the thin stroma lamellae are denser areas composed of thickened lamellae called grana lamellae (Figs. 9 and 10). There are peripheral clear vesicles (700 Å) (Fig. 10), part of the peripheral reticulum system which gives rise to the lamellar system (82). The chloroplasts of normal tobacco (Fig. 25) and *Bryophyllum* (Fig. 27) exhibit the same basic pattern.

Nucleus:

The nucleus of the differentiated normal cell is eccentric in location and usually not seen on cross section because of the displacement by the large central vacuole (Fig. 8). The nucleus measures 8 μ by 4 μ and is surrounded by a double membrane, the nuclear envelope. The chromatic material of the nucleus stains densely and is concentrated into an interrupted ring directly inside the nuclear envelope (Fig. 8). Contained within the nucleus are one or two circular, coarse, granular nucleoli measuring 0.7 μ and 2.4 μ in diameter.

NORMAL MERISTEMATIC TOMATO TISSUE

Morphology.

The meristematic tomato cell is smaller (5 μ to 7 μ in diameter) and more rectangular or oval in cross section than the differentiated cell (Fig. 6). The cell wall and plasmalemma have the same close relationship as described for the differentiated cell. The cytoplasmic components appear similar except for the loss of the prominent central vacuole in the meristematic cell.

Cytoplasmic Elements.

Endoplasmic Reticulum:

The granular ER of the normal meristematic tomato cell (Fig. 6) is quantitatively and qualitatively similar to the granular ER of the differentiated cells.

Mitochondria:

The mitochondria of the meristematic cells are oval and measure, as in the instance of the mitochondria of the differentiated tomato cells, 0.7 μ in length. They are enclosed by a double membrane. The inner membrane forms tubules which extend into the mitochondrial matrix. Osmiophilic granules, measuring approximately 300 Å in diameter, are seen in the mitochondrial matrix.

Plastids:

Meristematic normal tomato cells do not contain the mature form of chloroplast seen in the differentiated cells (Fig. 6). The plastid form observed is round to oval in shape, measuring 1.7 μ in length. Within a homogeneous matrix are small (0.4 μ) inclusions interpreted as

starch granules. There is also a granular appearing material suggesting osmiophilic plastoglobuli, but no suggestion of the lamellar structure characteristic of differentiated chloroplasts. The structure of these bodies corresponds closely to previous descriptions of proplastids (20).

Nucleus:

Because of the small size of the normal meristematic cell, the nucleus is frequently observed. Nuclei measure approximately 3.5 μ in diameter and frequently occupy a central location in the cell. The nuclear envelope is clearly a double membrane (Fig. 6). The chromatin is clumped as an interrupted band located at the periphery of the nucleus. Nucleoli are common, measuring 1.0 μ in diameter, and are composed of osmiophilic granular material without any limiting membrane.

STIMULATED NORMAL TOMATO TISSUE

Morphology.

The normal tomato tissue grown on artificial medium (stimulated) is composed of a more homogeneous population of cells. The stimulated normal cell measures 12 μ to 16 μ in diameter; the cytoplasm is usually without vacuoles or contains multiple small ones (Figs. 11, 12, and 14). Few cells contain the central vacuole of the differentiated cell. The cell walls of stimulated tissue are not as prominent as in normal cells, and there are many thin (0.14 μ) newly formed cell walls (Fig. 11).

Cytoplasmic Elements.

Endoplasmic Reticulum:

The ribosomal associated endoplasmic reticulum, although present

in stimulated tissue (Fig. 14) is not as frequently demonstrated as in normal, non-stimulated tissues. The structure of the ER is the same as in non-stimulated tissue.

Mitochondria:

Mitochondria of stimulated normal tissue are generally similar to mitochondria in non-stimulated tissue. The mitochondria measure about 0.7 μ in length, but occasional long, filamentous forms are observed. These measure up to 1.7 μ in length (Fig. 11). The previously mentioned osmiophilic granules (300 Å) are seen within the mitochondria of stimulated normal cells (Figs. 13 and 14).

Plastids:

Stimulated normal tomato cells contain a variety of plastids not observed in normal cells.

Some plastids, measuring 2.8 μ in length, contain starch grains and an occasional osmiophilic granule (Fig. 11), but are without a lamellar system. These plastids appear to contain inclusions which are similar to the ground plasm and may be artifacts (Fig. 11).

Other stimulated normal cells possess large plastids measuring 4.4 μ by 2.5 μ. Such plastids contain up to eight starch grains (1.4 μ by 0.7 μ). In these starch-containing plastids there is no lamellar system though single strands of osmiophilic material are seen between the starch grains and through the plastid matrix (Figs. 12, 13, and 14). In sections of plastids demonstrating little starch, poorly formed osmiophilic plastoglobuli are found, but there are no lamellae. These starch containing plastids represent stages of development between proplastids and starch-storing amyloplasts. The peripheral reticulum is prominent

in these intermediate plastid forms (Figs. 12 and 13).

Nucleus:

The nuclei of normal stimulated cells measure about 9.8 μ by 6.3 μ (Figs. 12 and 14). The peripheral clumping of chromatic material is not as marked as described in the unstimulated normal tomato tissue. Distribution of nuclear osmiophilic granular material appears much more homogeneous than in the normal tissues. Nuclei frequently contain a nucleolus measuring 3.2 μ in diameter.

CROWN GALL TUMOR TISSUE OF TOMATO TISSUE

Morphology.

Tomato crown gall tumor tissue is composed of nests of undifferentiated vacuole-free cells measuring approximately 7 μ in diameter (Fig. 15) surrounded by large differentiated cells measuring 20 μ by 7 μ containing characteristically prominent central vacuoles (Fig. 24).

There is more variability in cell size as well as cell shape in the tumor tissue (Fig. 19) when compared to normal tissue. Tumor cells are the only cells seen dividing (Figs. 19 and 20) with the formation of a new cell wall formed by the apparent coalescence of cytoplasmic vacuoles.

Cytoplasmic Elements.

Endoplasmic Reticulum:

The granular endoplasmic reticulum is strikingly abundant in the tumor cells, and exceeds in amount the granular ER of normal cells of comparable size (Fig. 15). The structure of the granular ER of tumor cells is in no way different from that of normal cells. There are no

consistent topographical relationships between granular ER, nucleus, mitochondria or plastids. There is only one observed instance of an association between granular ER and osmiophilic cytoplasmic granules (to be discussed later).

Dictosomes:

The classical concentric series of flattened, cup-shaped, double membrane-enclosed sacs, called dictosomes, or Golgi zones, are seldom observed in either normal tomato tissue or in stimulated normal tissue. The tumor cells contain numerous (5-10 per section) dictosomes, averaging 0.5 μ in length (Fig. 15). Small (circa 0.1 μ) empty vesicles are observed near the terminal ends of the flattened sacs.

Mitochondria:

The mitochondria of tumor cells average 0.9 μ in length and appear similar to previously described mitochondria of normal tissues (Figs. 16 and 17).

Figure 18 demonstrates a close topographical relationship between the mitochondria and the nuclear envelope. This close relationship was seldom observed in tumor tissue.

Plastids:

The plastids of tomato crown gall tissue differ from those of normal tissue. The plastids of crown gall range from 0.8 μ to 1.7 μ in length. Some long filamentous plastid forms are observed measuring up to 3.8 μ in length (Figs. 15, 16, 17, 18, and 22).

Crown gall tissue plastids contain little starch in contrast to the amounts seen in plastids from normal and stimulated tissue. Some tumor plastids contain a few strands of an apparent lamellar structure (Fig.

18). Other plastids possess bizarre collections of osmiophilic material with no evidence of lamellar structure (Figs. 15, 16, and 22). Finely granular inclusions (0.5 μ to 1.0 μ) are seen within the matrix of some plastids (Figs. 23 and 24). Peripheral vesicles related to the peripheral reticulum are prominent (Figs. 15, 16, 17, and 22).

Crystals:

Regularly arranged crystal lattices are found in crown gall tumor tissue. These structures are also seen in normal tissues but not in stimulated normal tissues. The crystals are most frequently observed in tumor tissue, are rectangular in shape, and measure up to 0.7 μ by 0.5 μ. The crystals are often enclosed by a membrane (Figs. 16 and 22), but are commonly seen free in the cytoplasm (Fig. 21). There appears to be no difference in the structure of the crystals of tumor and normal tissues.

Cytoplasmic Granules:

Unique to the cytoplasm of the tumor cell are densely osmiophilic granules measuring about 0.15 μ in diameter. These granules are found in close approximation to membrane-enclosed vacuoles. The vacuoles range in size from 0.3 μ to a size approximating that of central vacuoles. The granules appear to be unlimited by any membrane, but are seen either flattened against the vacuolar membrane or free floating in aggregates within the confines of the vacuole. The granules are not observed in normal or stimulated normal cells, and are therefore the only cytoplasmic entity unique to the tumor cell (Figs. 15, 16, 22, 23, and 24).

Nuclei:

The nuclei of tomato tumor cells are relatively large (measuring

up to 9.8 μ) and contain clumped chromatin distributed along the inner aspect of the nuclear envelope. The nucleoli frequently number 2 to 3, and measure from 0.5 μ to 2.3 μ in diameter.

TOBACCO TISSUE

A preliminary examination of the plastids of normal and crown gall tissue in tobacco plants demonstrates the following.

The normal tobacco chloroplasts of the differentiated tobacco cell (Fig. 25) measure about 7.5 μ by 2.3 μ and contain laminated stroma and grana lamellae surrounding starch grains (3.5 μ by 1.5 μ) and plastoglobuli (0.4 μ).

The chloroplast observed in the crown gall tumor of the tobacco plant (Fig. 26) is circular and measures approximately 2.3 μ in diameter. The stroma and grana lamellae are compressed to the periphery of the chloroplast by a large (circa 1.9 μ) coarsely granular central inclusion. The stacks of grana lamellae appear to be composed of the same material as is seen in the inclusion.

BRYOPHYLLUM TISSUE

A chloroplast in the leaf of a *Bryophyllum* plant (Fig. 27) is spindle shaped and measures 6.4 μ by 1.3 μ. The stroma and grana lamellae are seen to surround densely osmiophilic plastoglobuli, 0.4 μ in diameter, and starch grains, 1.6 μ in length.

A chloroplast belonging to a *Bryophyllum* crown gall tumor cell has a different structure. The chloroplast measures 2.4 μ by 1.0 μ and is surrounded by a double membrane which can be seen to invaginate, giving

rise to vesicles of the peripheral reticulum. The lamellar system possesses both stroma and grana lamellae. The terminal portion of the chloroplast in Figure 28 is swollen, containing three empty (electron lucent) vacuoles measuring up to 0.25 μ. These plastids appear to demonstrate the transition from proplastid to chloroplast.

DISCUSSION

In the present study two types of cytoplasmic structures, not present in normal tomato cells, are observed in the crown gall tumor cells. These are 1) abnormal plastid forms and 2) characteristic cytoplasmic granules.

Hypertrophic proplastids as well as plastids with invaginations and inclusion-bodies have been reported in crown gall tissues and considered diagnostic of crown gall by some investigators (30, 39). Plastid inclusions have been reported, however, in sterile, auxin-induced tumors as well as in tumors resulting from *A. tumefaciens* in *Kalanchöe* plants (64). The presence of normal, immature and degenerating forms of plastids in crown gall cells led French researchers to propose the formation of multiple separate lines of plastid development as unique to tumor formation (72). Abnormal plastids do not seem unique to tumor formation, however, as they are observed in normal cells in response to a sterile wound (31, 54) as well as in *A. tumefaciens* inoculated cells kept at 35° C, a temperature which inhibits tumor formations (39).

The use of normal stimulated cells in this study provides a rapidly growing non-tumor tissue to use as a control for comparison with the rapidly growing tumor tissue. This comparison is necessary because of the lack of comparable rapidly growing tissue in the normal tomato stem.

The results of this study indicate abnormal plastid forms are found not only in the tomato crown gall tumor cell but also in normal tissue induced to grow rapidly on artificial media. The chloroplast structure of cultured tobacco cells is reported to correlate with the growth phase

of the cells. Tobacco chloroplasts do not mature during a period of active cell division (55). These data suggest the appearance of abnormal plastid forms is a nonspecific change related to either increased metabolic activity, or to the process of differentiation, or to both.

When plants are kept from sunlight, the developing lamellar structures of the proplastids are arrested, presumably because the metabolic pathways necessary for complete development are not activated in the absence of sunlight. The result is a granular, "quasi-crystalline" structure, the prolamellar body (29, 38). In dividing normal as well as tumor cells, the proplastids fail to mature, possibly as a result of altered metabolic pathways. It may be that during cell division those products necessary for normal plastid maturation are no longer available to the plastid.

The fate of the original plastids is unclear and suggests the necessity for serial time studies at early periods of tumor development. Plastoglobuli, located in plastids, are reported to enlarge as the plastid undergoes degeneration. The process of degeneration is total and the plastid largely disappears, leaving only the plastid membrane, enclosing large plastoglobuli which are apparently composed of lipophilic plastid quinones (29, 62). The plastids observed in this study demonstrate no evidence of total degeneration in the above sense. The presence of a well-defined plastid matrix surrounding starch grains seems more compatible with arrested development than with degeneration.

The osmiophilic cytoplasmic granules noticed in the crown gall cells of tomato in this study have not been reported in previous electron microscopic studies of normal or tumor cells (29, 30, 40, 65,

98). However, cytoplasmic granules have been associated with crown gall tumor cells of tomato in light microscopic studies. Riker noted the presence of small globular inclusions which stained with osmic acid. These inclusions progressed into spheroid bodies and light-colored bodies with black granular centers. Riker attributed these granules to either intracellular bacteria, or to accumulating products of metabolism (78). Dense globular bodies have been described in the cytoplasm of tobacco crown gall cells (58), and "thick walled" structures resembling granule containing vacuoles are reported in normal plant embryos (85).

In this study the particles observed in tomato crown gall tumor cells are always associated with a vacuole of variable size. The osmiophilic granules are found either within the confines of the membrane, or closely approximated to the outer surface of the vacuole. The chemical nature of these granules is unknown. Lawrence Moore (70) has also noted these granules within tomato tumor cells and has succeeded in crudely isolating the granules using gradient centrifugation methods. Moore finds the granules to be quite dense, enabling one to propose that the granules are composed of materials other than the lipophilic plastid quinones which make up the plastoglobuli of the plastids. Plastoglobuli have a density of less than unity and float when centrifuged in aqueous media (62). On the basis of fixed tissue studies using the electron microscope, Moore proposes that the osmiophilic granules move through the vacuolar membrane (70). The vacuoles seem to enlarge and may represent an early central vacuole with the vacuolar membrane developing into the tonoplast. It has been proposed that the tonoplast and central vacuole arise from the granular ER, or from the dictosomes (20, 69), both

of these structures being very prominent in the tumor tissue.

In this study the only non-vacuole associated granules observed were associated with granular ER in one instance.

The subtle changes in cytoplasmic or nuclear genetic material unique to tumor tissue are not recognizable by the methods used in this study. The fimbria structures on the *Agrobacterium* (Fig. 5) are about the same size as type I and sex pili described on *E. coli* by Brinton (19). The identification of sex pili requires phage typing of the pili, and might disclose a possible mechanism for the transfer of a cytoplasmic episome from *A. tumefaciens* to another bacteria (47), or indeed to the potential tumor cells. This concept would support the findings of bacterial nucleic acids in the plant cell (84), the transmitability of tumor-inducing ability (47), and the unexplained time requirement in the production of variable degrees of neoplasia in the plant tumors (8). The transmission of the sex factor of *E. coli* occurs in a time dependent manner.

In none of the sections were bacteria identified, which is consistent with the results of Hohl and Gee (30, 40) who observed bacteria only in the initial 72 hours of tumor induction. However, attempts to get sterile tissue culture samples of tumor tissue in the present study consistently failed because of contamination with gram-negative rods felt to be *A. tumefaciens* apparently contained within the tissue, but not detected morphologically.

Fogelberg (28) observed changes from the granular form of tomato mitochondria to a filamentous form with the transformation from normal to crown gall tissue. This has not been confirmed by other researchers and there was no evidence of filamentous mitochondria unique to tumor

cells in this study. There were no virus-like particles, L-forms of bacteria, or mycoplasma-like structures observed in the present work.

The plastid changes previously attributed to tumor transformation are considered in this work as evidence of a normal meristematic state. The influence of a diffusable growth stimulant is implicated by the observed division of a differentiated cell surrounded by other differentiated cells. Electron radiographic studies of radioactively labeled *A. tumefaciens* and the plant cells during the transformation period should be an exciting avenue of future research. The unique cytoplasmic granules of the tumor cells also require further investigation, and should be isolated and tested for their ability to transform previously avirulent *A. tumefaciens* into tumor-inducing bacteria.

SUMMARY AND CONCLUSIONS

The observance of abnormal plastid forms should not be considered unique or diagnostic of crown gall tumor in tomato plants. Rather, it is probably a condition indicative of a meristematic or undifferentiated cell. Osmiophilic granules associated with variable sized vacuoles were the only cytoplasmic element unique to tumor cells. Further studies regarding the origin of these granules may lead to a clearer understanding of the inciting agent in the production of crown gall.

REFERENCES

1. Beardsley, R. E., and Lipetz, J. Effects of 5-flurouracil and 5-flurodeoxyuridine on growth and tumor-inducing ability of *Agrobacterium tumefaciens*. J. Bact. 92: 346-348, 1966.

2. Beardsley, R. E., Stonier, T., Lipetz, J., and Parsons, C. L. Production and pathogenicity of spheroplasts of *Agrobacterium tumefaciens*. Cancer Res. 26: 1606-1610, 1966.

3. Bloch, R. Wound healing in higher plants II. Botan. Rev. 18: 655-679, 1952.

4. Bloch, R. Abnormal plant growth. Brookhaven Sympos. Biol. 6: 41-54, 1954.

5. Braun, A. C. Studies on tumor inception in the crown-gall disease. Amer. J. Bot. 30: 674-677, 1943.

6. Braun, A. C. Recovery of crown-gall tumor cells. Cancer Res. 11: 839-844, 1951.

7. Braun, A. C. Conditioning of the host cell as a factor in the transformation process in crown gall. Growth 16: 65-74, 1952.

8. Braun, A. C. Plant cancer. Sci. Amer. 186 (no. 6): 66-72, 1952.

9. Braun, A. C. Bacterial and host factors concerned in determining tumor morphology in crown gall. Botan. Gaz. 114: 363-371, 1953.

10. Braun, A. C. Studies on the origin of the crown gall tumor cell. Brookhaven Sympos. Biol. 6: 115-127, 1954.

11. Braun, A. C. The activation of two growth-substance systems accompanying the conversion of normal to tumor cells in crown gall. Cancer Res. 16: 53-56, 1956.

12. Braun, A. C. The biological action of growth substances. Sympos. Soc. Exp. Biol. 11: 132-142, 1957.

13. Braun, A. C. Tissue culture as a tool for studying the development of autonomy in neoplastic plant cells. J. Nat. Cancer Inst. 19: 753-769, 1957.

14. Braun, A. C. Plant tumors as an experimental model. The Harvey Lect. 56: 191-210, 1960-61.

15. Braun, A. C., and Mandle, P. J. Studies on the inactivation of the tumor-inducing principle in crown gall. Growth 12: 255-269, 1948.

16. Braun, A. C., and Naf, U. A non-auxinic growth-promoting factor present in crown gall tumor tissue. Proc. Soc. Exp. Biol. Med. 86: 212-214, 1954.

17. Braun, A. C., and Wood, H. N. On the inhibition of tumor inception in the crown-gall disease with the use of ribonuclease A. Proc. Nat. Acad. Sci. U.S.A. 56: 1417-1422, 1966.

18. Breed, R., Murry, E., and Smith, N. (eds.). Bergey's Manual of Determinative Bacteriology. 7th edition. Baltimore: Williams and Wilkins Co., 1957. pp 288-289.

19. Brinton, C., Gemski, P., and Carnahan, J. Sexpili: A new type of bacterial pilus genetically controlled by the fertility factor of *E. coli* K_{12} and its role in chromosome transfer. Proc. Nat. Acad. Sci. U.S.A. 52: 776-783, 1964.

20. Buvat, R. L'origine et le developpement des vacuoles des cellules vegetales. 5th Intern. Congr. Electron Microscopy, Philadelphia. New York: Academic Press, 1962. 2, W-1.

21. DeLey, J. Approach to an improved taxonomy of the genus *Agrobacterium*. J. Gen. Microbiol. 43: 7-17, 1966.

22. deRopp, R. S. Plant tumors and animal cancers. Nature 160: 780-782, 1947.

23. deRopp, R. S. The growth promoting and tumefacient factors of bacteria-free crown-gall tumor tissue. Amer. J. Bot. 34: 248-261, 1947.

24. deRopp, R. S. The response of normal plant tissues and of crown-gall tumor tissue to synthetic growth hormones. Amer. J. Bot. 34: 53-62, 1947.

25. deRopp, R. S. Action of streptomycin on plant tumors. Nature 162: 459-460, 1948.

26. deRopp, R. S. The comparative growth promoting action of indole-3-acetic acid and *Agrobacterium tumefaciens*. Amer. J. Bot. 37: 352-363, 1950.

27. Ewing, J. Neoplastic Diseases. Philadelphia: W. B. Saunders Co., 1940. pp 1-8 and 113-127.

28. Fogelberg, S. O., Stuckmeyer, B. E., and Roberts, R. H. Mitochondria changes in crown gall. Amer. J. Bot. 44: 454-459, 1957.

29. Frey-Wyssling, A., and Mühlethaler, K. Ultrastructural Plant Cytology. New York: Elsevier Publishing Co., 1965. pp 135-341.

30. Gee, M. M., Sun, C. N., and Dwyer, J. D. Subcellular observations on sunflower crown gall induced by *Agrobacterium tumefaciens*. Amer. J. Bot. 53: 622, 1966 (abstract).

31. Granick, S. Quantitative isolation of chloroplasts from higher plants. Amer. J. Bot. 25: 558-561, 1938.

32. Helgeson, J. P. The cytokinins. Science 161: 974-981, 1968.

33. Helgeson, J. P., and Leonard, N. J. Cytokinins: Identification of compounds isolated from *Corynebacterium fascians*. Proc. Nat. Acad. Sci. U.S.A. 56: 60-61, 1966.

34. Hildebrand, E. M. A micrurgical study of crown gall infection in tomato. J. Ag. Res. 65: 45-59, 1942.

35. Hildebrandt, A. C., Riker, A. J., and Duggar, B. M. Growth in vitro of excised tobacco and sunflower tissue with different temperatures, hydrogen-ion concentrations and amounts of sugar. Amer. J. Bot. 32: 357-361, 1945.

36. Hill, J. B. The migration of *Bacterium tumefaciens* in the tissue of tomato plants. Phytopathology 18: 553-564, 1928.

37. Hill, J. B., Brittingham, W. H., Gibbons, F. P., and Watts, G. W. Further notes on *Bacterium tumefaciens* and its host relationship in tobacco. Phytopathology 20: 179-186, 1930.

38. Hodge, A. J., Mclean, J. C., and Mercer, F. V. A possible mechanism for the morphogenesis of lamellar systems in plant cells. J. Biophys. Biochem. Cytol. 2: 597-608, 1956.

39. Hohl, R. H. Über die Submikroskopische Struktur hyperplastischer Gwebe von *Datura stramonium* L. Phytopathol. Z. 40: 317-356, 1960/61.

40. Hohl-R. H. Plastids and tumorgenesis. Amer. J. Bot. 48: 528, 1961 (abstract).

41. Hope, H. R. Comparison of the nucleic acid, total nitrogen and protein levels of normal and tumor tissue. Plant Physiol. 34: 435-440, 1959.

42. Ivanoff, S. S., and Riker, A. J. Studies on the movement of the crown-gall organism within the stem of tomato plants. Phytopathology 20: 817-829, 1930.

43. Jones, L. E. Personal communication. Botany and Plant Pathology Dept., Oregon State University, Corvallis, Oregon. June, 1966.

44. Jones, L. E., Hildebrandt, A. C., Riker, A. J., and Wu, J. H. Growth of somatic tobacco cells in microculture. Amer. J. Bot. 47: 468-475, 1960.

45. Karnovsky, M. J. A formaldehyde-glutaraldehyde fixation of high osmolarity for use in electron microscopy. J. Cell Biol. 27: 137A-138A, 1965 (abstract).

46. Klämbt, D., Thies, G., and Skoog, F. Isolation of cytokinins from *Corynebacterium fascians*. Proc. Nat. Acad. Sci. U.S.A. 56: 52-59, 1966.

47. Klein, D. T., and Klein, R. M. Transmittance of tumor-inducing ability to avirulent crown-gall and related bacteria. J. Bact. 66: 220-228, 1953.

48. Klein, R. M. The etiology of crown gall. Quart. Rev. Biol. 30: 202-277, 1955.

49. Klein, R. M., and Braun, A. C. On the presumed sterile induction of plant tumors. Science 131: 1612, 1960.

50. Klein, R. M., Rasch, E. M., and Swift, H. Nucleic acids and tumor genesis in broad beans. Cancer Res. 13: 499-502, 1953.

51. Kupila, S. Anatomical and cytological comparison of the development of crown gall in three host species. Ann. Bot. Soc. 'Vanamo' 30: 1-89, 1958.

52. Kupila, S., and Stern, H. DNA content of broad bean (Vinca Faba) internodes in connection with tumor induction by *Agrobacterium tumefaciens*. Plant Physiol. 36: 216-219, 1961.

53. Kupila, S., and Therman, E. Anatomical observations on genetic tumors and crown gall in amphidiploid *Nicotiana glauca* X *langsdorffii*. Ann. Bot. Soc. 'Vanamo' 32: 1-20, 1962.

54. Kupila, S., and Therman, E. Morphogenesis of crown gall. IN Advances in Morphogenesis (M. Abercrombie, J. Brachet, and T. King, eds.), Volume 7. New York: Academic Press, 1968. pp 45-78.

55. Laetsch, W. M., and Stetler, D. A. Chloroplast structure and function in cultured tobacco tissue. Amer. J. Bot. 52: 798-804, 1965.

56. Lang, J., and Baker, R. Influence of compounds with antitumor activity on crown gall. Phytopathology 56: 131-133, 1966.

57. Levin, I., and Levine, M. Sensitivity of radiation on tumor and normal plant tissue. Proc. Soc. Exp. Biol. Med. 15: 24-25, 1917.

58. Levin, I., and Levine, M. The role of neoplasia in parasitic diseases of plants. J. Cancer Res. 7: 171-178, 1922.

59. Levine, M. Studies in the cytology of cancer. Amer. J. Cancer 15: 1410-1494, 1931.

60. Levine, M. Plant tumors and their relation to cancer. Bot. Rev. 2: 439-455, 1936.

61. Levine, M., and Chargaff, E. The response of plants to chemical fractions of *Bacterium tumefaciens*. Amer. J. Bot. 24: 461-472, 1937.

62. Lichtenhaler, H. K. Plastoglobuli and the fine structure of plastids. Endeavour 17: 144-149, 1968.

63. Lipetz, J. Crown gall tumorigenesis: Relations between wound healing and tumorigenic response. Cancer Res. 26: 1597-1605, 1966.

64. Lipetz, J. Fine structure studies of plant tumor cells. J. Cell Biol. 35: 82A, 1967 (abstract).

65. Lipetz, J. Changes in the endoplasmic reticulum of plant tumor cells. J. Cell Biol. 39: 81A, 1968 (abstract).

66. Lippincott, J. A., Heberlein, G. T. The induction of leaf tumors by *Agrobacterium tumefaciens*. Amer. J. Bot. 52: 396-403, 1965.

67. Lippincott, J. A., and Lippincott, B. B. *Agrobacterium tumefaciens*: Thermal inactivation of tumor inducing ability. Science 147: 1578-1579, 1965.

68. Locke, S., Riker, A., and Duggar, B. Growth substances and the development of crown gall. J. Agric. Res. 57: 21-39, 1938.

69. Merinos, N. G. Vacuolation in plants. J. Ultrastruct. Res. 9: 172-185, 1963.

70. Moore, L. Personal communication. Botany and Plant Pathology Dept., Oregon State University, Corvallis, Oregon. March 29, 1968.

71. Patty, F. A. Some experiments with crown gall bacteria. Phytopathology 20: 856, 1930 (abstract).

72. Puiseux-Dao, S., and Hoursiangou-Neubrum, D. Etude de l'evolution des plastes au cours de la tumorisation chez la *Datura stramonium*, solancees, infeste par l'*Agrobacterium tumefaciens*. Electron Microscopy, 1966. Volume II, pp 331-332.

73. Rasch, E., Swift, H., and Klein, R. M. Nucleoprotein changes in plant tumor growth. J. Biophys. Biochem. Cytol. 6: 11-34, 1959.

74. Reynolds. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17: 208-213, 1963.

75. Riker, A. J. Some relations of the crown gall organism to its host tissue. J. Agric. Res. 25: 119-132, 1923.

76. Riker, A. J. Some morphological responses of the host tissue to the crown gall organism. J. Agric. Res. 26: 425-436, 1923.

77. Riker, A. J. Studies on the influence of some environmental factors on the development of crown gall. J. Agric. Res. 32: 83-96, 1926.

78. Riker, A. J. Cytological studies of crowngall tissue. Amer. J. Bot. 14: 25-37, 1927.

79. Roberts, R. H. A naturally occurring antiauxin. Science 117: 456-457, 1953.

80. Robinson, W., and Walker, H. A critical review of crown gall. Ann. Bot. 37: 299-324, 1923.

81. Robson, H. H., Budd, M. A., and Yost, H. T. Comparison of the nucleic acid, total nitrogen and protein nitrogen levels of normal and tumor tissue under similar growth conditions. Plant Physiol. 34: 435-440, 1959.

82. Rosado-Alberio, J., Weier, T. E., and Stocking, C. R. Continuity of the chloroplast membrane in systems in *Zea mays* L. Plant Physiol. 43: 1325-1331, 1968.

83. Rubio-Huertos, M., and Cabezas deHerrera, E. Fixed L-forms of *Agrobacterium tumefaciens* induced by ultra-violet light radiation. Nature 209: 1262-1263, 1966.

84. Schilperoort, R. A., Veldstar, H., Warnhar, S., Mulder, G., and Cohen, J. Formation of complexes between DNA isolated from tobacco crown gall tumors and RNA complementary to *Agrobacterium tumefaciens* DNA. Biochim. Biophys. Acta 145: 523-525, 1967.

85. Setterfield, G., Stern, H., Johnston, F. B. Fine structures in cells of pea and wheat embryos. Canad. J. Bot. 37: 65-72, 1959.

86. Skerman, V. B. D. The Genera of Bacteria, a Guide to the Identification of. Baltimore: Williams and Wilkins Co., 1967. p 142.

87. Skoog, F., Armstrong, D., Cherhyil, J., Hampel, A., and Bock, R. Cytokinin activity: Localization in transfer RNA preparations. Science 154: 1354-1356, 1966.

88. Skoog, F., and Miller, C. Stimulation of normal tissue with artificial means resulting in tumor type colonies. Sympos. Soc. Exp. Biol. 11: 118-131, 1957.

89. Smith, E. F. Crown gall of plants. Phytopathology 1: 7-11, 1911.

90. Smith, E. F. Cancer in plants. FROM The Proceedings of the Seventeenth International Congress of Medicine, 1913. Section III, pp 1-18.

91. Smith, E. F. 20th century advances in cancer research. J. Radiol. 4: 295-317, 1923.

92. Smith, E. F., and Townsend, C. O. A plant-tumor of bacterial origin. Science 25: 671-673, 1907.

93. Steward, F. C., Caplin, S. M., and Shzutz, E. H. Investigations on the growth and metabolism of plant cells: Tumorous growth in relation to growth factors of the type found in coconut. Ann. Bot. 19: 29-47, 1955.

94. Stonier, T. Radioautographic evidence for intercellular location of crown gall bacteria. Amer. J. Bot. 43: 647-655, 1956.

95. Stonier, T., Beardsley, R. E., Parsons, L., and McSharry, J. Effects of thermal shock on bacteria in relation to tumor-inducing ability. J. Bact. 91: 266-269, 1966.

96. Struckmeyer, B. E., and Roberts, R. H. The inhibition of abnormal cell proliferation with antiauxin. Amer. J. Bot. 42: 401-405, 1955.

97. Van Lonen, J. M., Baldwin, I. L., and Riker, J. A. Attenuation of cell stimulating bacteria by specific amino acids. Science 92: 512-513, 1940.

98. Whaley, W. G., Mollenhauer, H. H., and Leach, J. H. The ultrastructure of the meristematic cell. Amer. J. Bot. 47: 401-449, 1960.

99. White, P. R. The Cultivation of Animal and Plant Cells. 2nd edition. New York: Ronald Press Co., 1963. pp 57-63, 113-124.

100. White, P. R. Metastatic (graft) tumors of bacteria-free crown gall on *Vinca rosea*. Amer. J. Bot. 32: 237-241, 1945.

101. White, P. R., and Braun, A. C. Cancerous neoplasm of plants. Cancer Res. 2: 597-617, 1942.

102. Wood, H. N. Regulation of biosynthetic systems in normal and crown gall tumor cells. Trans. N. Y. Acad. Sci. 24: 750-752, 1962.

103. Wood, H. N., and Braun, A. C. Studies on the regulation of certain essential biosynthetic systems in normal and crown-gall tumor cells. Proc. Nat. Acad. Sci. U.S.A. 47: 1907-1913, 1961.

104. Wood, H. N., and Braun, A. C. Studies on the net uptake of solutes by normal and crown-gall tumor cells. Proc. Nat. Acad. Sci. U.S.A. 54: 1532-1538, 1965.

Figure 1:

Bryophyllum plants with typical crown gall tumors pictured five months after inoculation of the healthy plant with *Agrobacterium tumefaciens*. The tumors appear similar on tomato plants.

Figure 2:

A close-up of one of the tumors pictured in Figure 1. There is a "metastatic" tumor growth superior to the large tumor.

Figure 3:

Normal tomato cells grown on artificial media (normal stimulated).

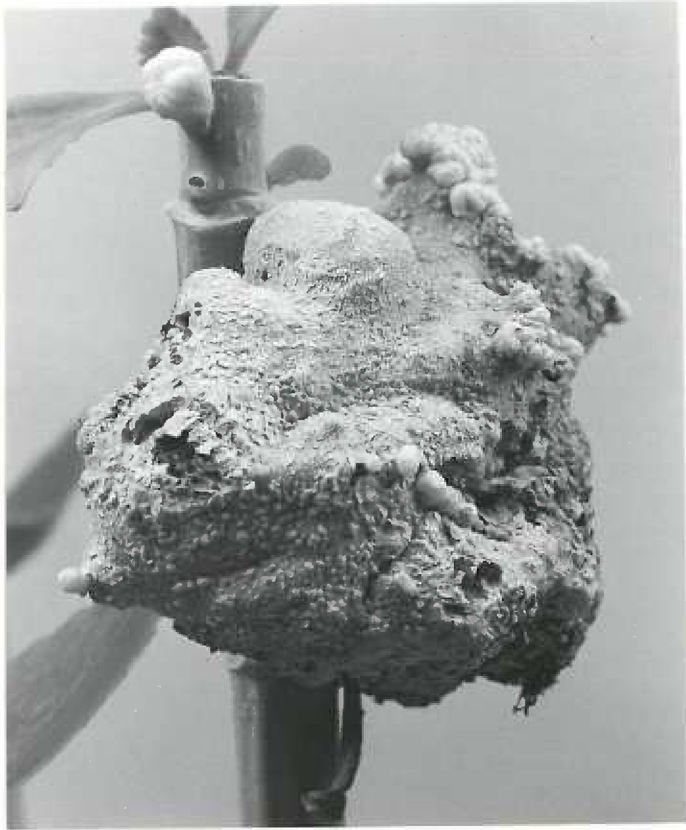


Figure 4:

Agrobacterium tumefaciens, shadowed with platinum-plutonium. Peritrichous flagellae (F). X49,000.

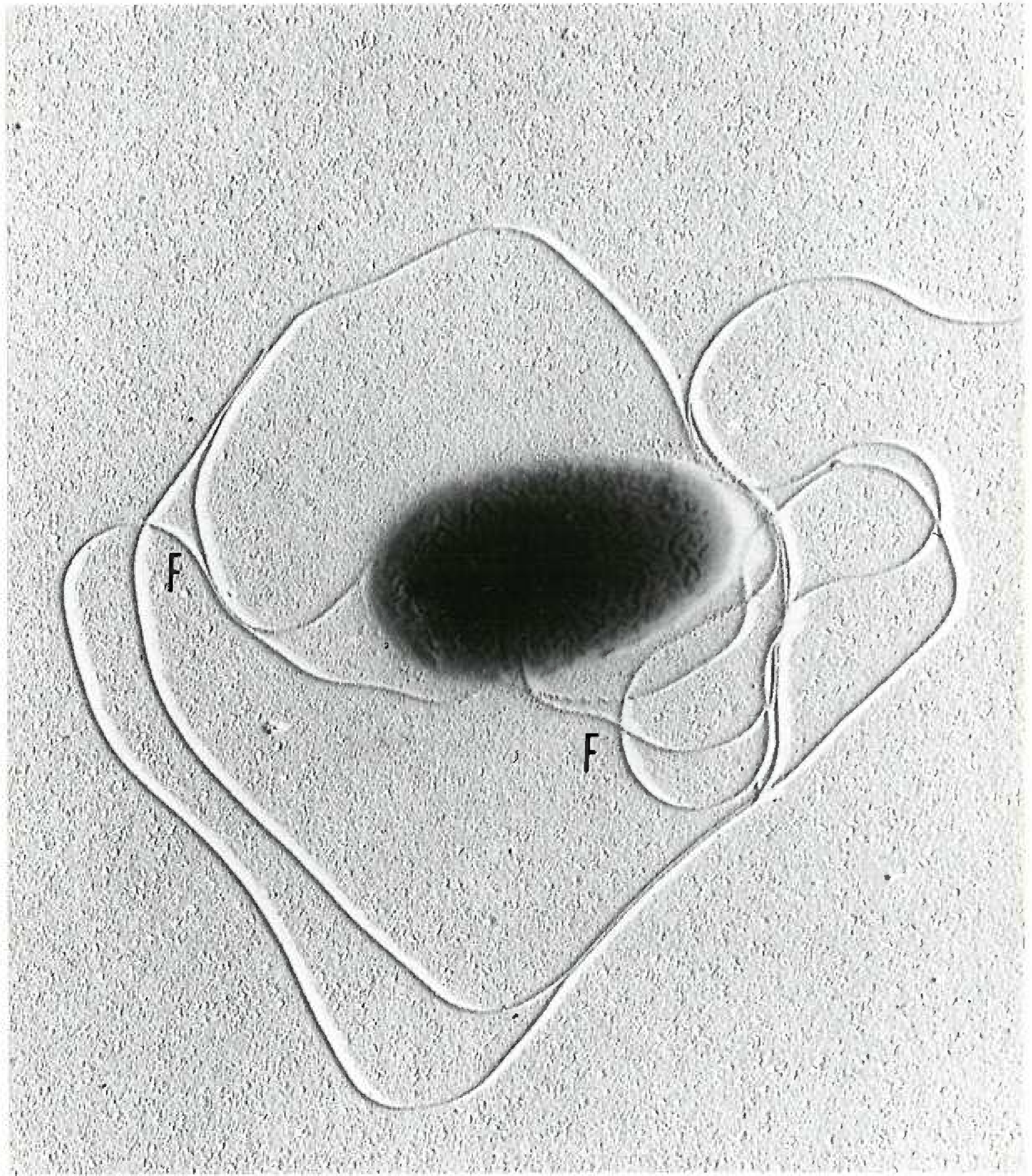

F
F

Figure 5:

Agrobacterium tumefaciens, shadowed with platinum-plutonium. The two bacterial cells are closely approximated. The upper cell has three structures interpreted as fimbria (Fi). X31,600.

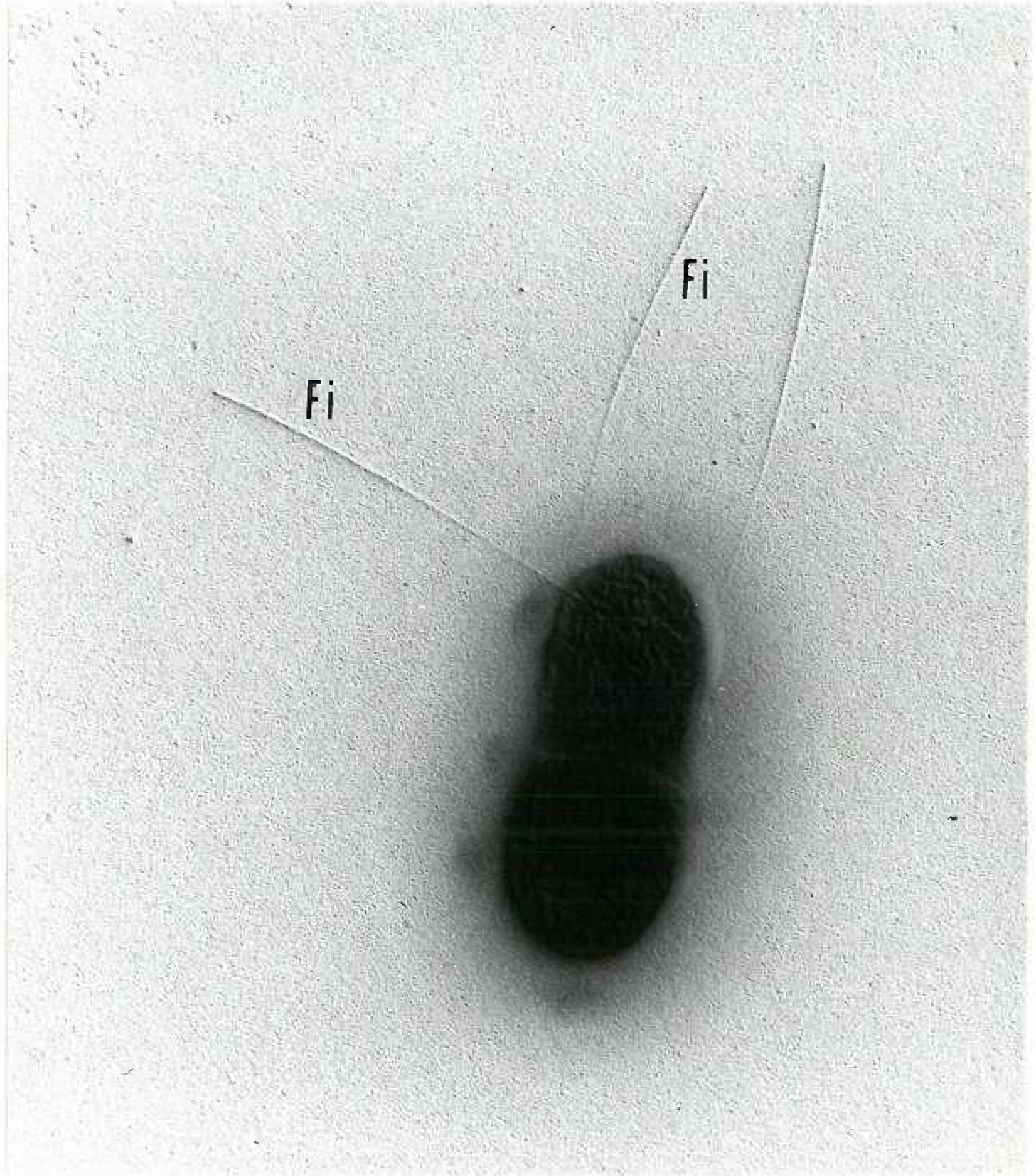
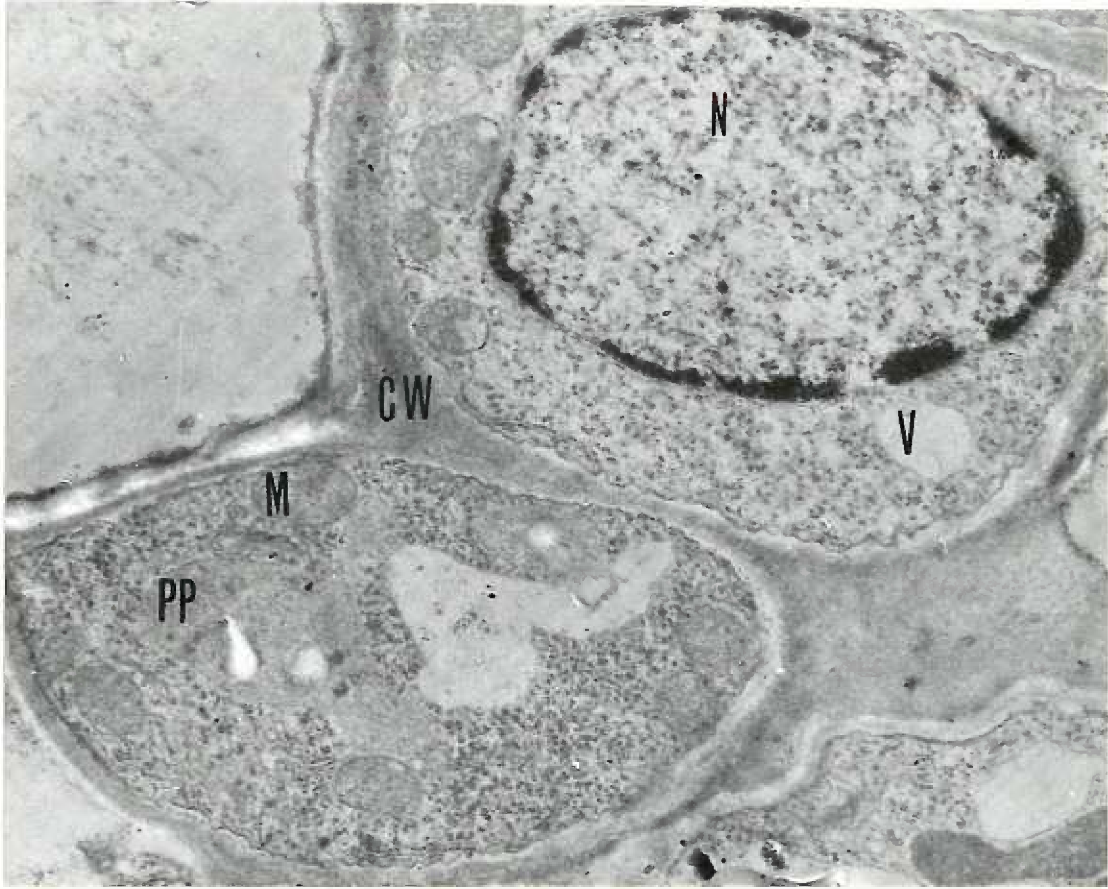

Fi
Fi

Figure 6:

Two undifferentiated (meristematic) normal tomato cells (upper right and lower left). The chromatin of the nucleus (N) is peripherally clumped. The cells contain small vacuoles (V). Cell wall (CW), mitochondria (M), and proplastid (PP). Part of a differentiated cell with a large central vacuol is at the upper left. X20,500.

Figure 7:

Typical differentiated normal cells have large central vacuoles (V), surrounded by thin rims of cytoplasm. The cytoplasm has typical endoplasmic reticulum (ER), mitochondria (M), and ribosomes. X11,600.


N
CW
V
M
PP

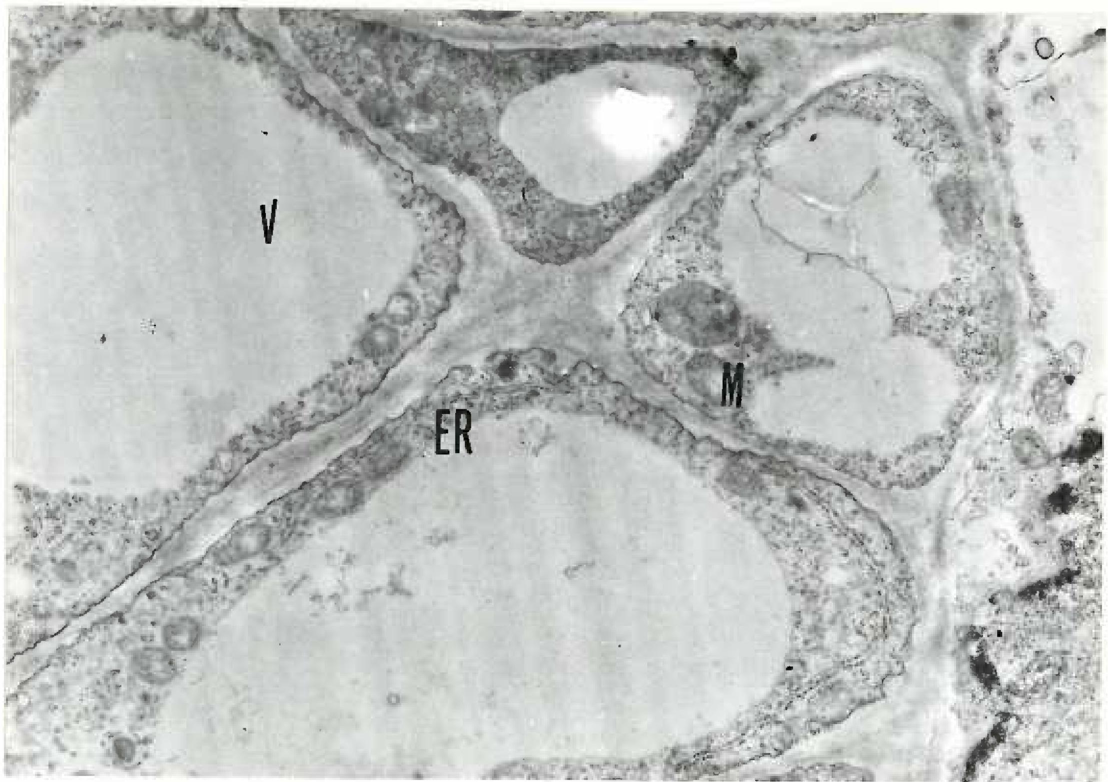
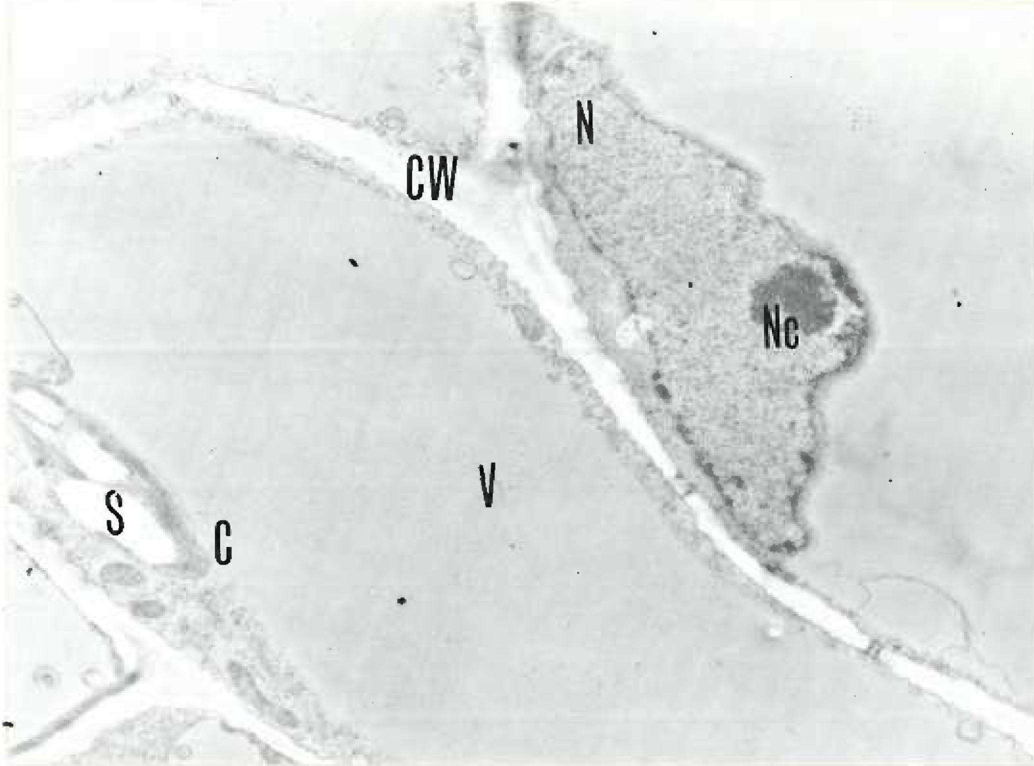

V
M
ER

Figure 8:

Differentiated normal tomato cells. Large central vacuoles (V), eccentrically placed nucleus (N), nucleolus (Nc), starch grains (S), cell wall (CW), and chloroplast (C). X12,000.

Figure 9:

Portion of a normal differentiated tomato cell with mitochondrion (M), and part of chloroplast (C) containing starch grain (S), osmiophilic plastoglobuli (Pg) and stromal lamellae (Sl). Cell wall (CW) and plasmalemma (Pl). X52,900.


N
CW
Nc
S
C
V

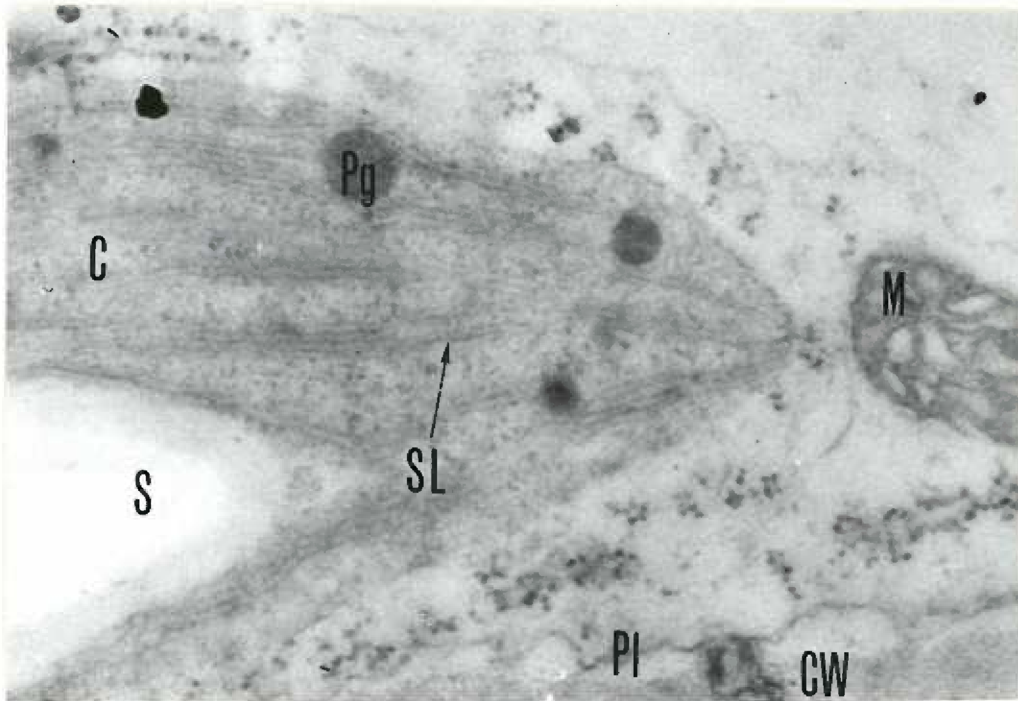

Pg
C
M
SL
S
Pl
CW

Figure 10:

Portion of a differentiated normal tomato cell showing a number of chloroplasts (C) with starch grains (S), stromal lamellae (SL), grana lamellae (GL), plastoglobuli (Pg), and peripheral reticulum (PR). X47,800.

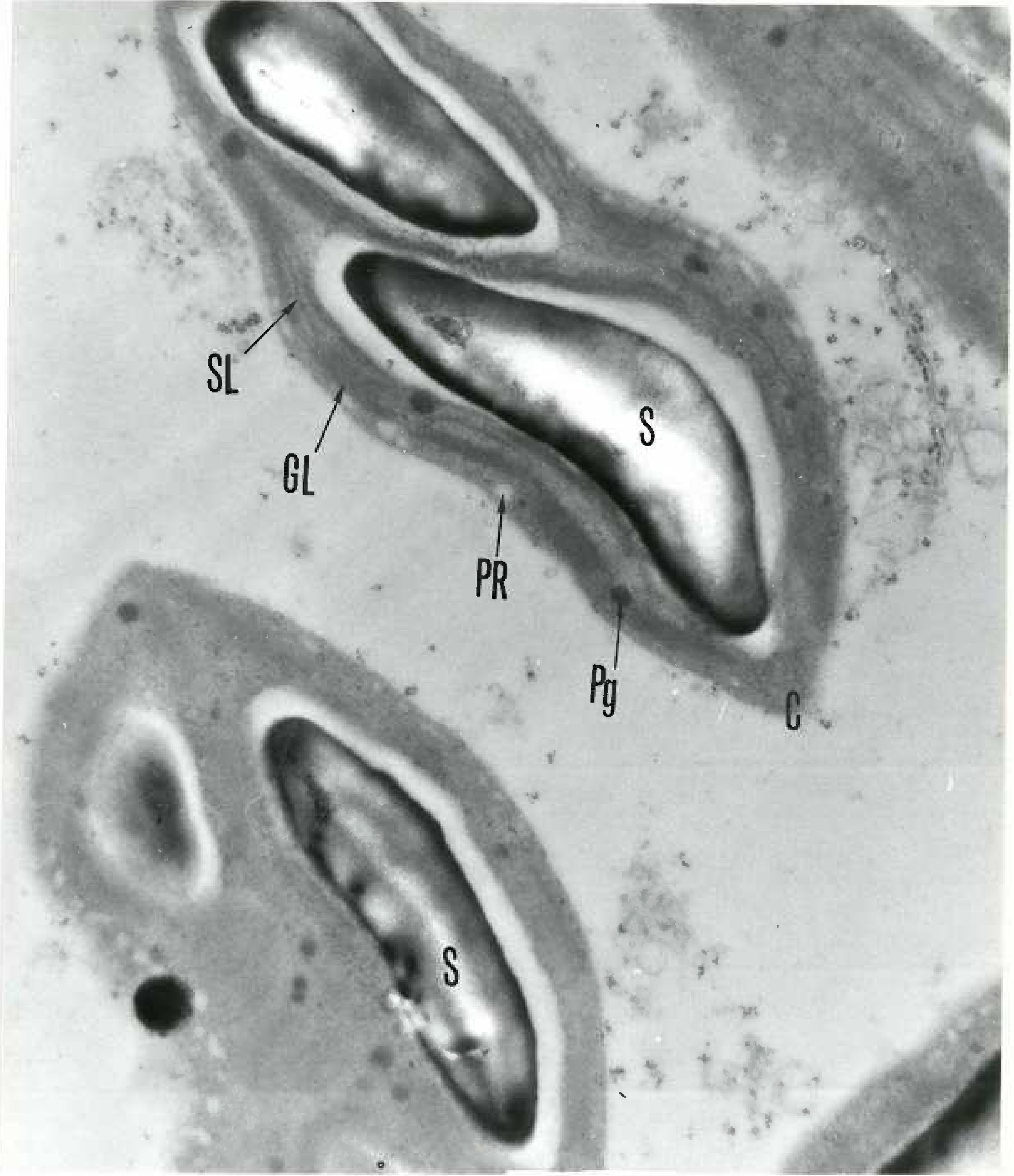
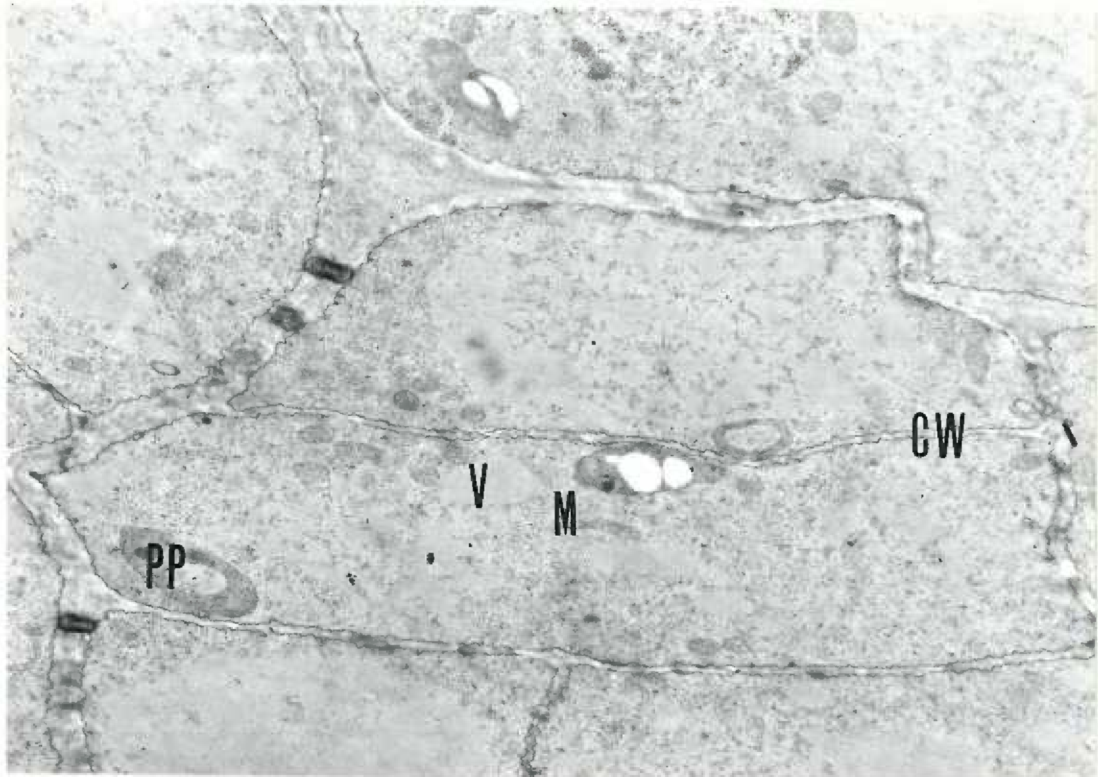

SL
S
GL
PR
Pg
C
S

Figure 11:

Normal-stimulated tomato cells grown in tissue culture. Note absence of lamellar systems in the structures interpreted as proplastids (PP). Thin, newly formed cell walls (CW). The cytoplasm appears homogeneous and has only small vacuoles (V). X7,250.

Figure 12:

Normal stimulated tomato cell grown in tissue culture. This differs from the smaller cells of Figure 11 in that vacuoles (V) are larger, and proplastids (PP) have prominent starch grains (S). X8,000.


CW
V
M
PP

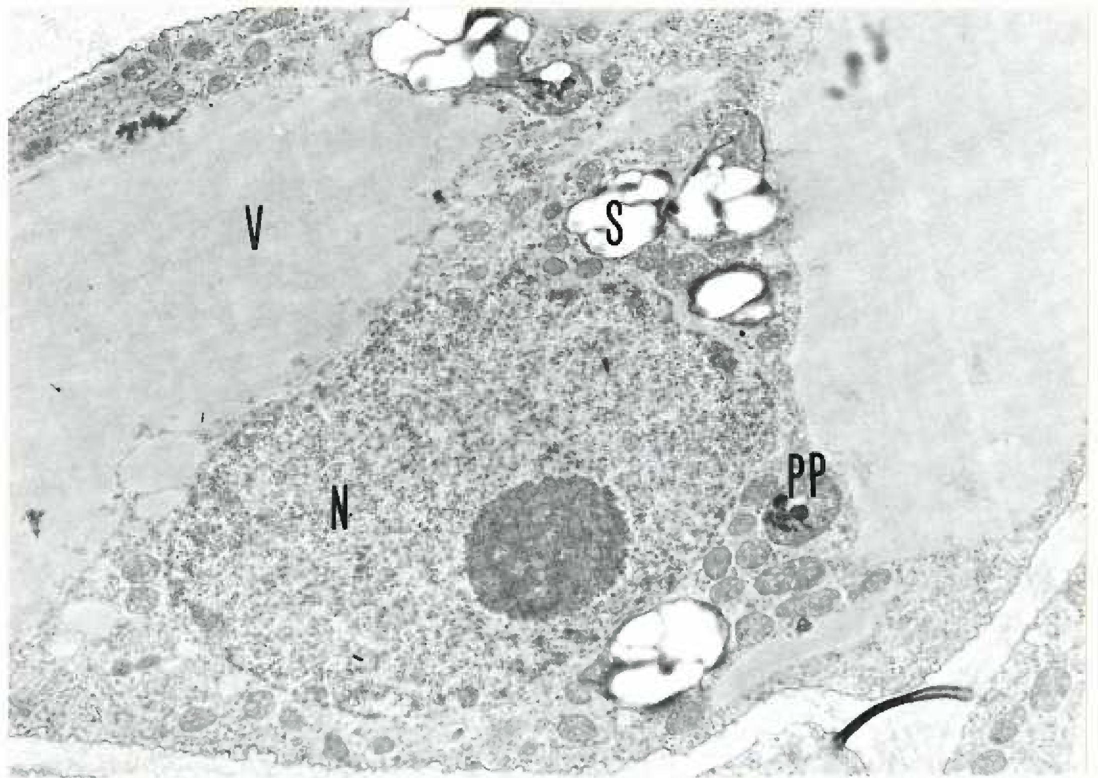

V
S
PP
N

Figure 13:

Portion of normal-stimulated tomato cell grown in tissue culture. The plastid has prominent starch grains (S), thus termed an amyloplast (A). Osmiophilic material (arrow) is seen in strands between starch grains and as poorly defined droplets in the matrix of the amyloplast. X18,450.

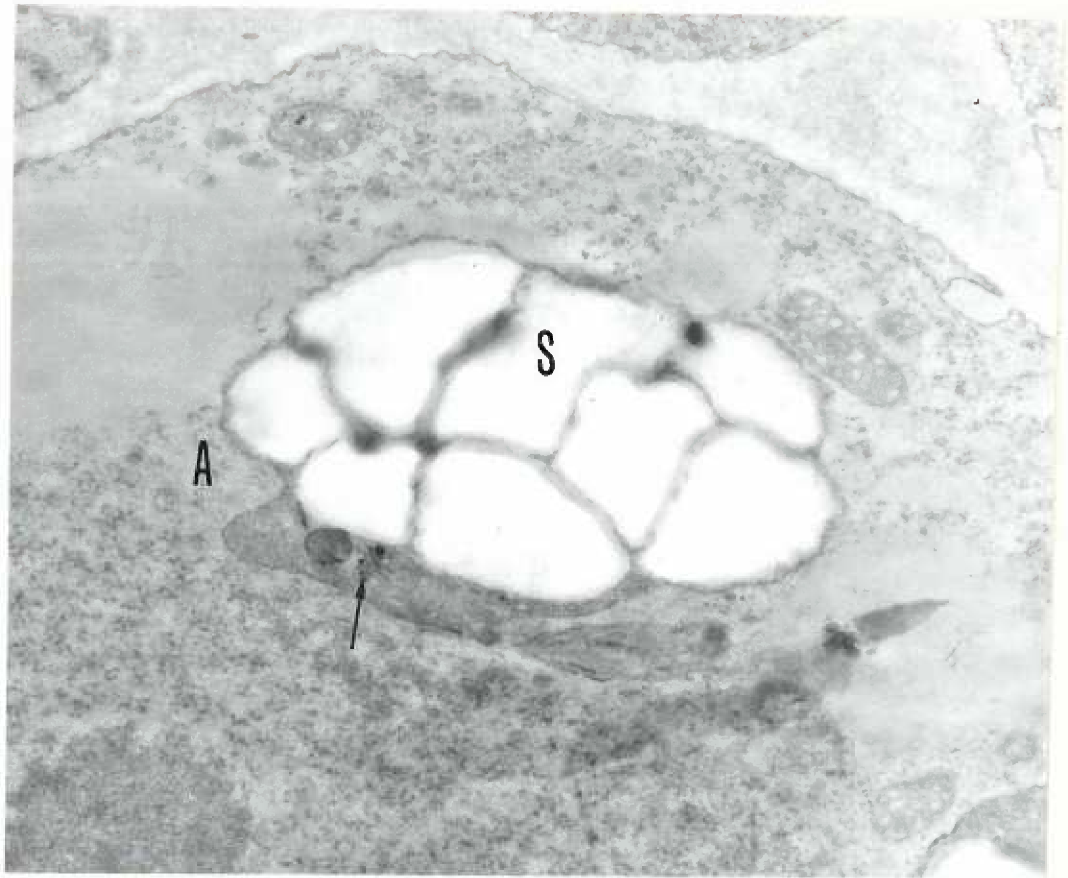

S
A

Figure 14:

Normal stimulated tomato cell from tissue culture. Nucleus (N), nucleolus (Nc). Amyloplasts (A) have prominent starch grains (S). One plastid shows osmiophilic material (arrow), interpreted as arrested lamellar development. X17,600.

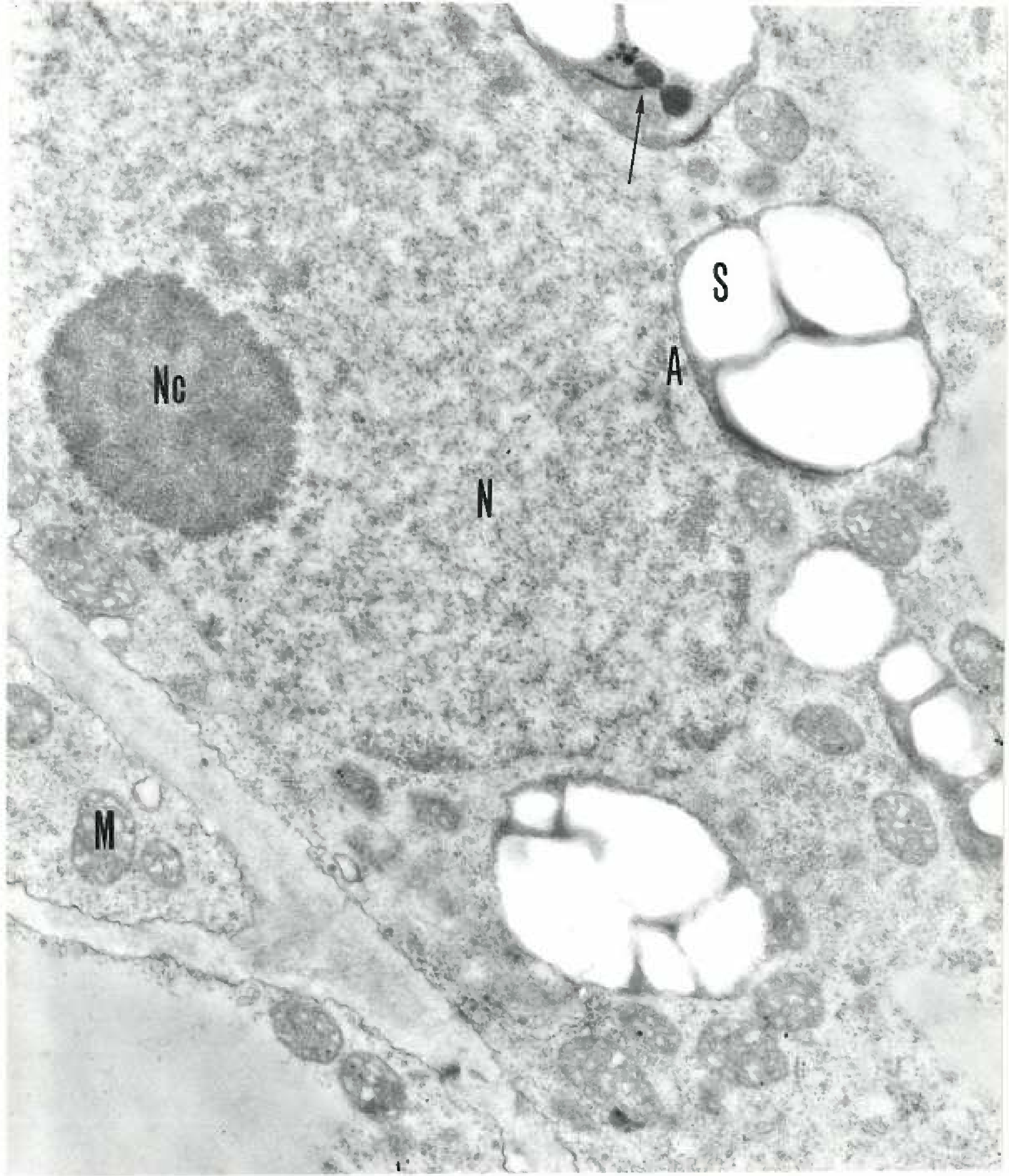

S
A
Nc
N
M

Figure 15:

Undifferentiated tumor cell showing nucleus (N), prominent nucleoli (Nc), mitochondria (M), cell wall (CW), dictyosomes (D), proplastids (PP), and endoplasmic reticulum (ER). Note osmiophilic granular material (G) of unknown nature. These granules are characteristic of tumor cells. X12,600.

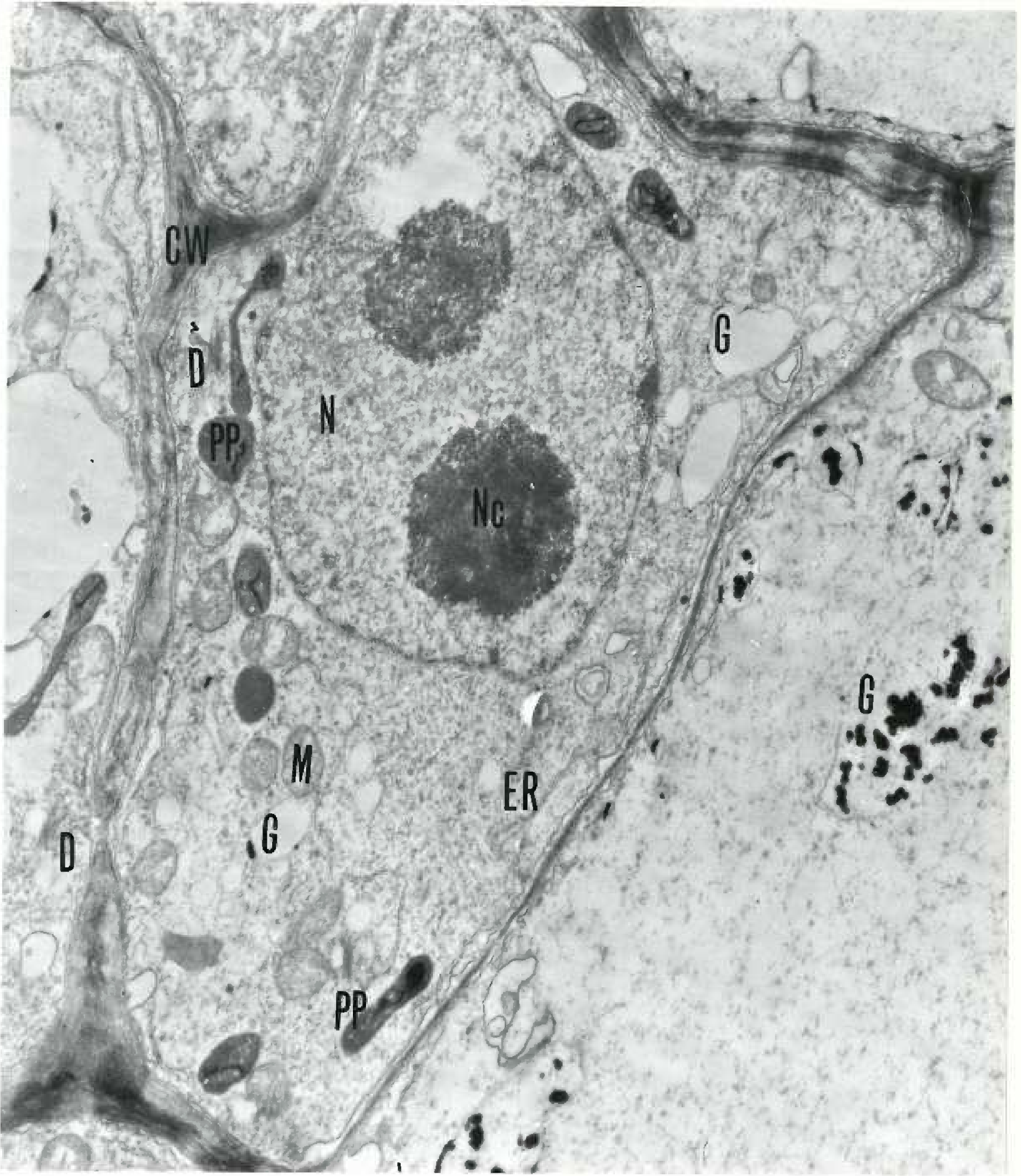

CW
D
PP
N
Nc
G
M
G
ER
G
D
PP

Figure 16:

Tumor cell. Nucleus (N), nucleoli (Nc), mitochondria (M), proplastids (PP) have bizarre patterns of internal osmiophilic material. Note prominent osmiophilic cytoplasmic granules (G) similar to those in Figure 15. A membrane-bound crystal (Cr) is also present. X17,400.

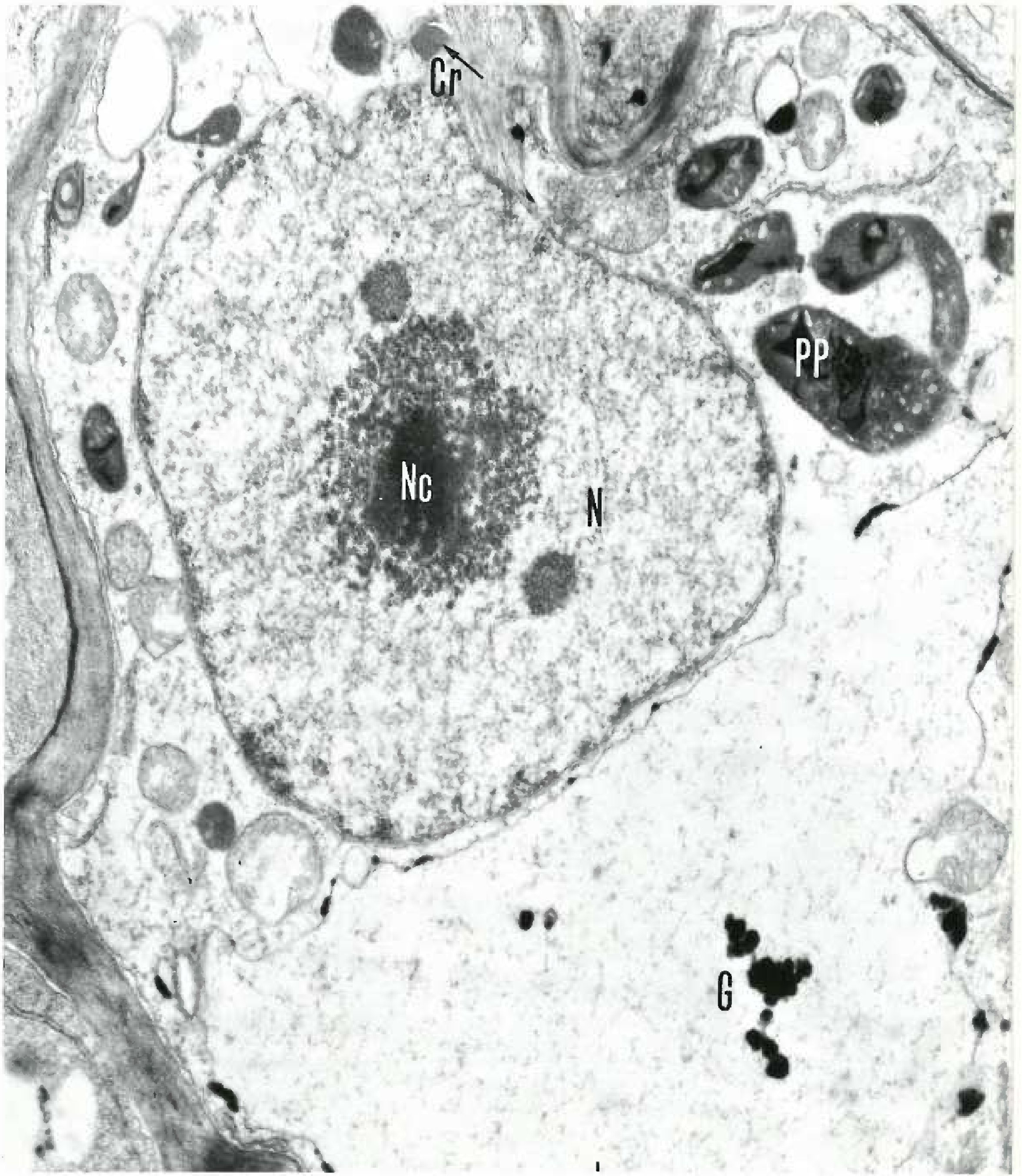

Cr
PP
Nc
N
G

Figure 17:

Higher power of cell in Figure 16. Plasmalemma (Pl), cell wall (CW), mitochondria (M), nuclear envelope (NE), granules (G), and proplastids (PP). X31,600.

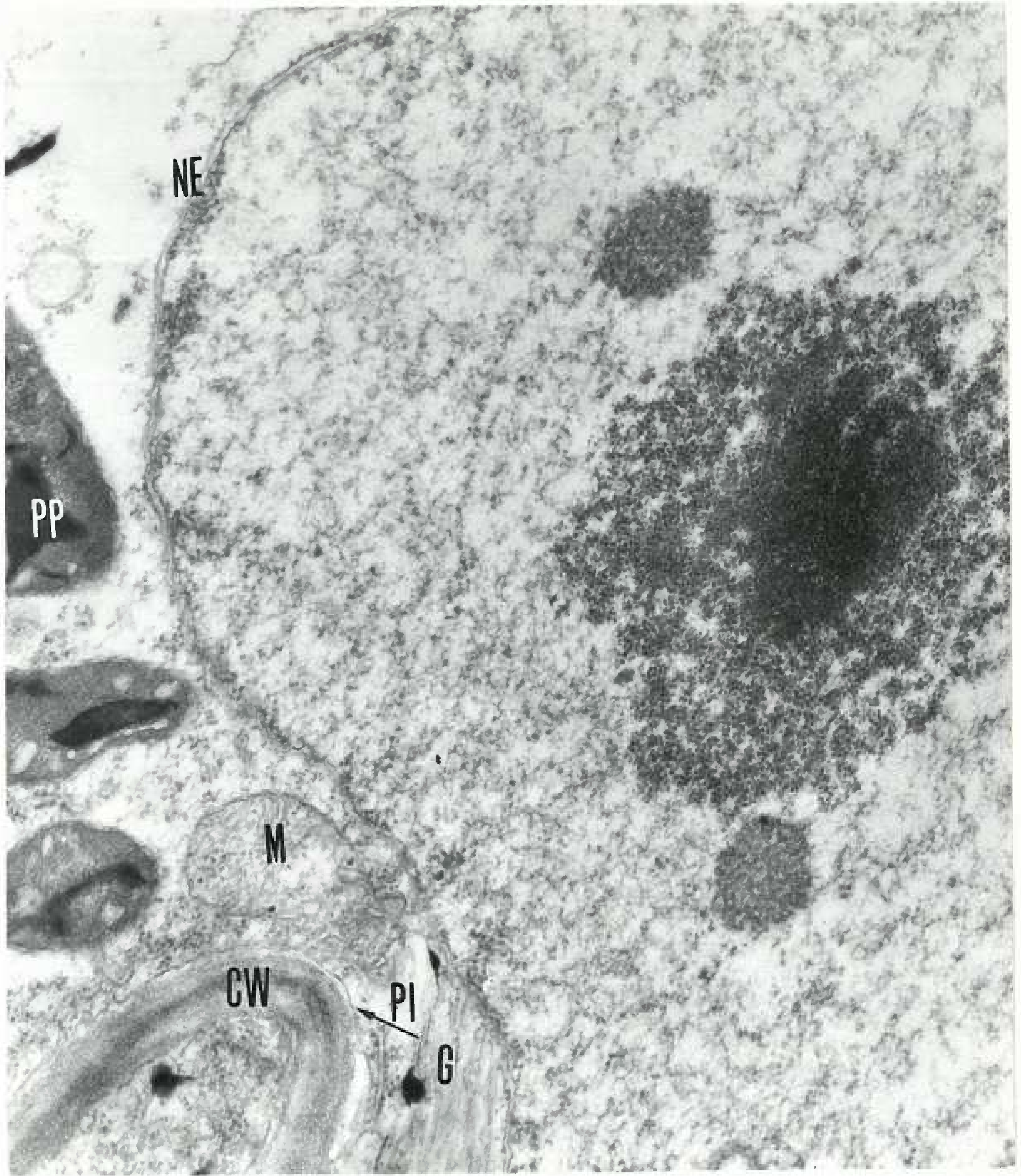

NE
PP
M
CW
PI
G

Figure 18:

Tumor cell. Note small vesicles (arrows) which appear to be located between the plasmalemma and the cell wall. Nucleus (N), nucleolus (Nc), plasmalemma (Pl), cell wall (CW), mitochondria (M), proplastids (PP), and osmiophilic granules (G). Note the close approximation between mitochondria and thick nuclear envelope. X18,450.

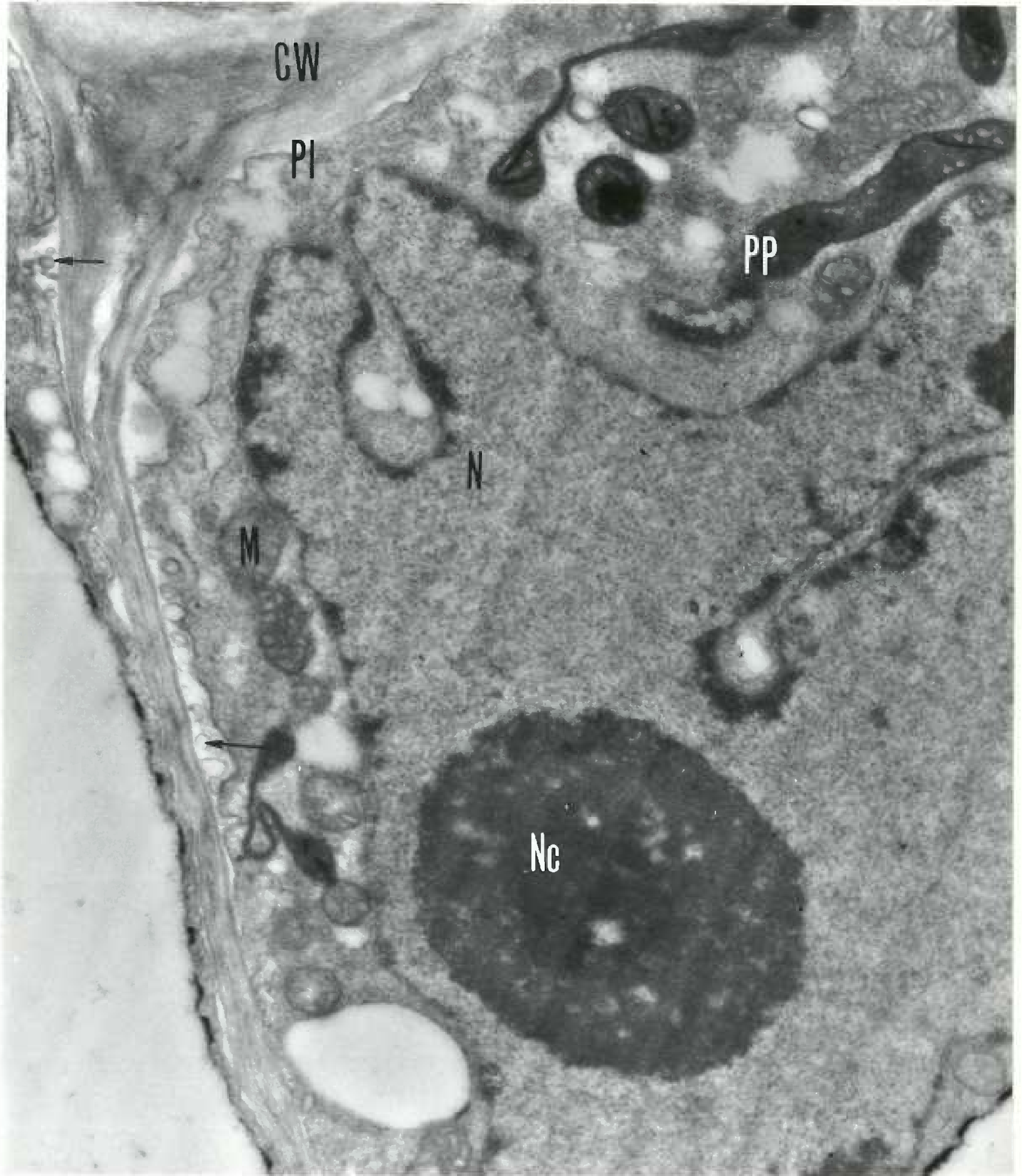

CW
Pl
PP
N
M
Nc

Figure 19:

Tumor tissue. Note irregular shape of the more differentiated cells which have central vacuoles (V). Nuclei (N), crystal (Cr), cell wall (CW). Note dividing cell at right. X8,400.

V
CW
N
N
V
Cr

Figure 20:

Higher power of dividing tumor cell shown in Figure 19. A row of vesicles represents the site of the forming cell wall between the daughter cells (arrows). Inasmuch as the dividing cell has a large vacuole (V), this is interpreted as a division occurring in a differentiated cell. Proplastid (PP). X17,800.

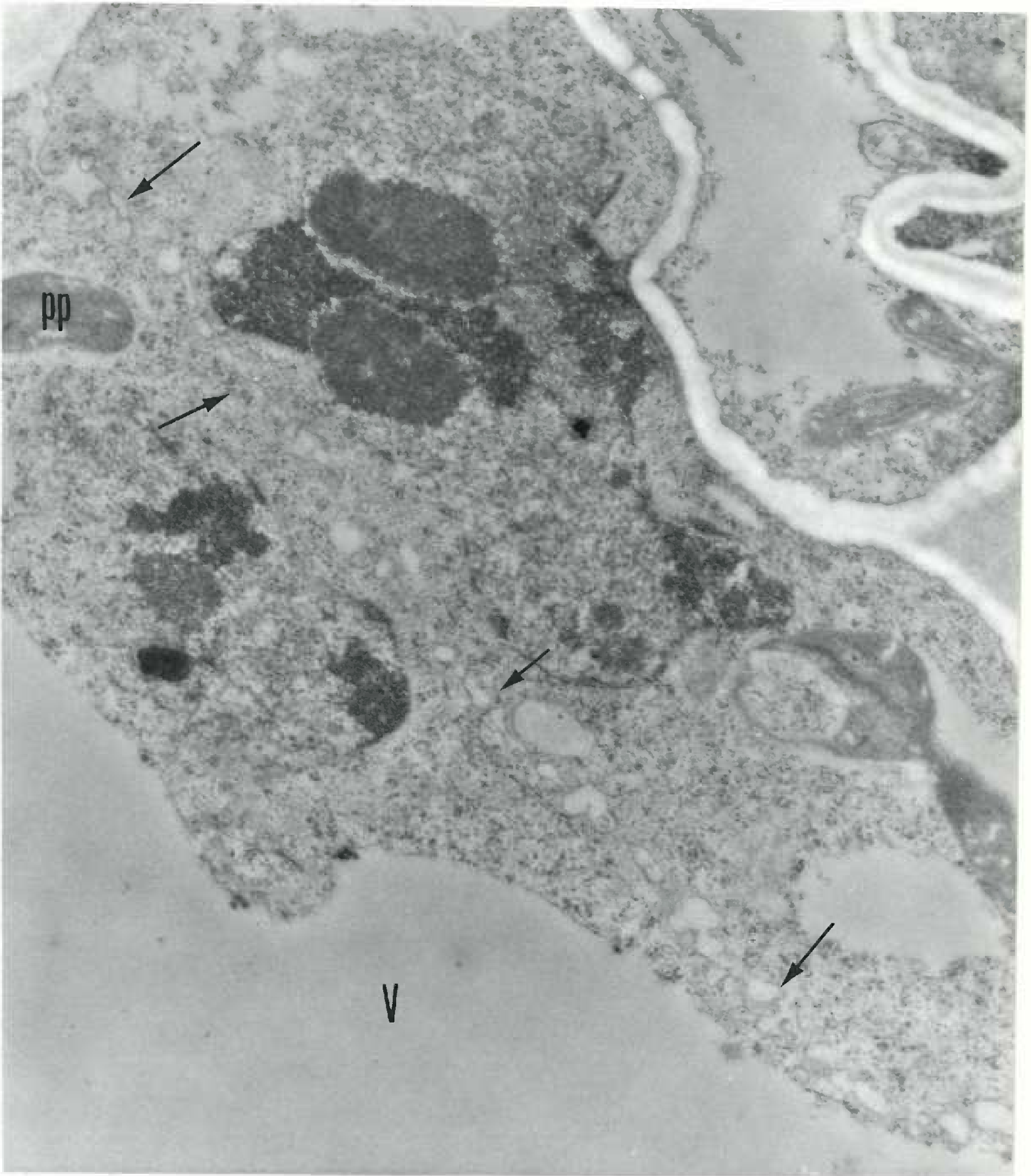

pp
V

Figure 21:

Crystalline lattice structure (Cr), commonly found within the cytoplasm of differentiated tomato tumor cells. X118,000.

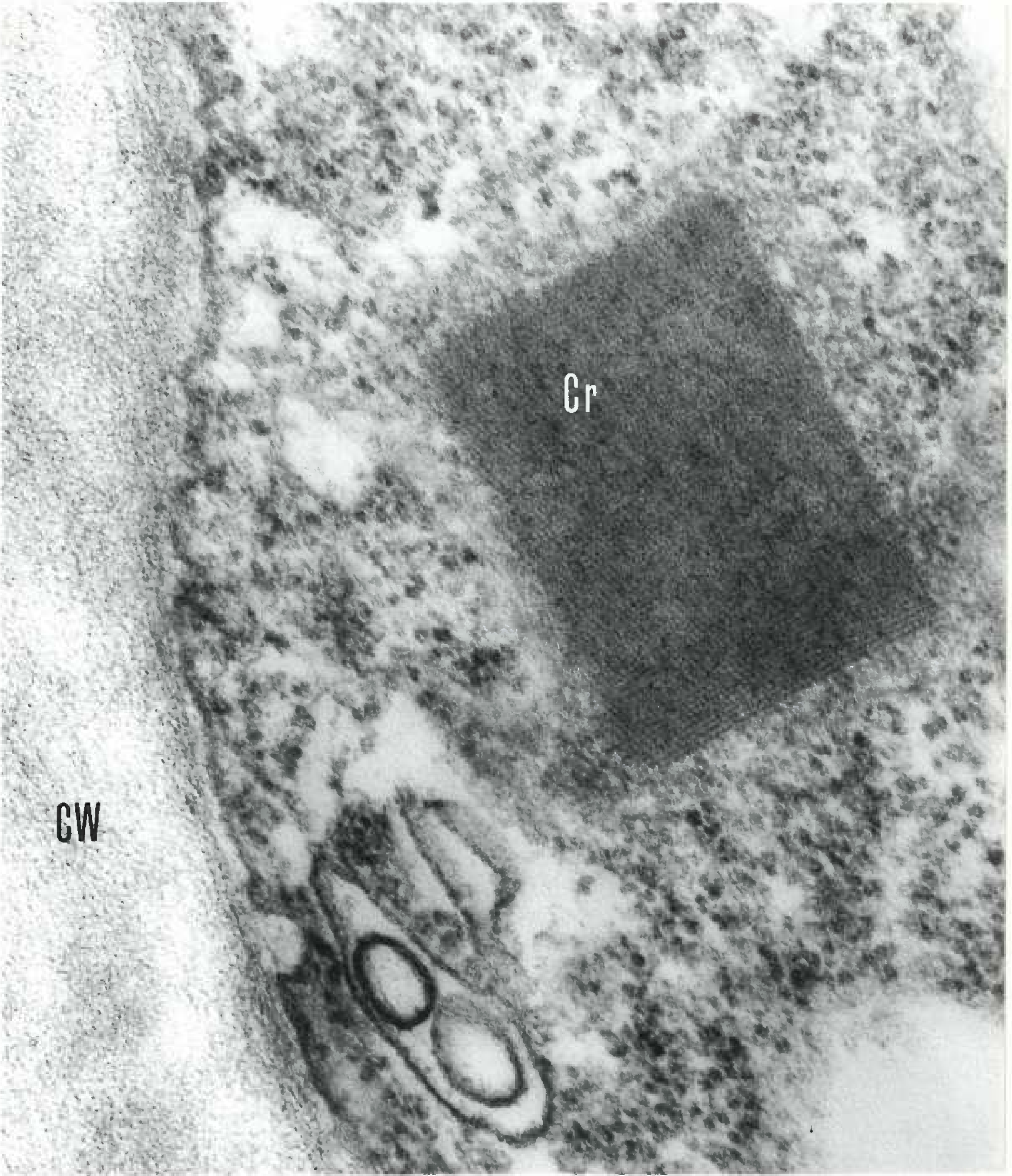
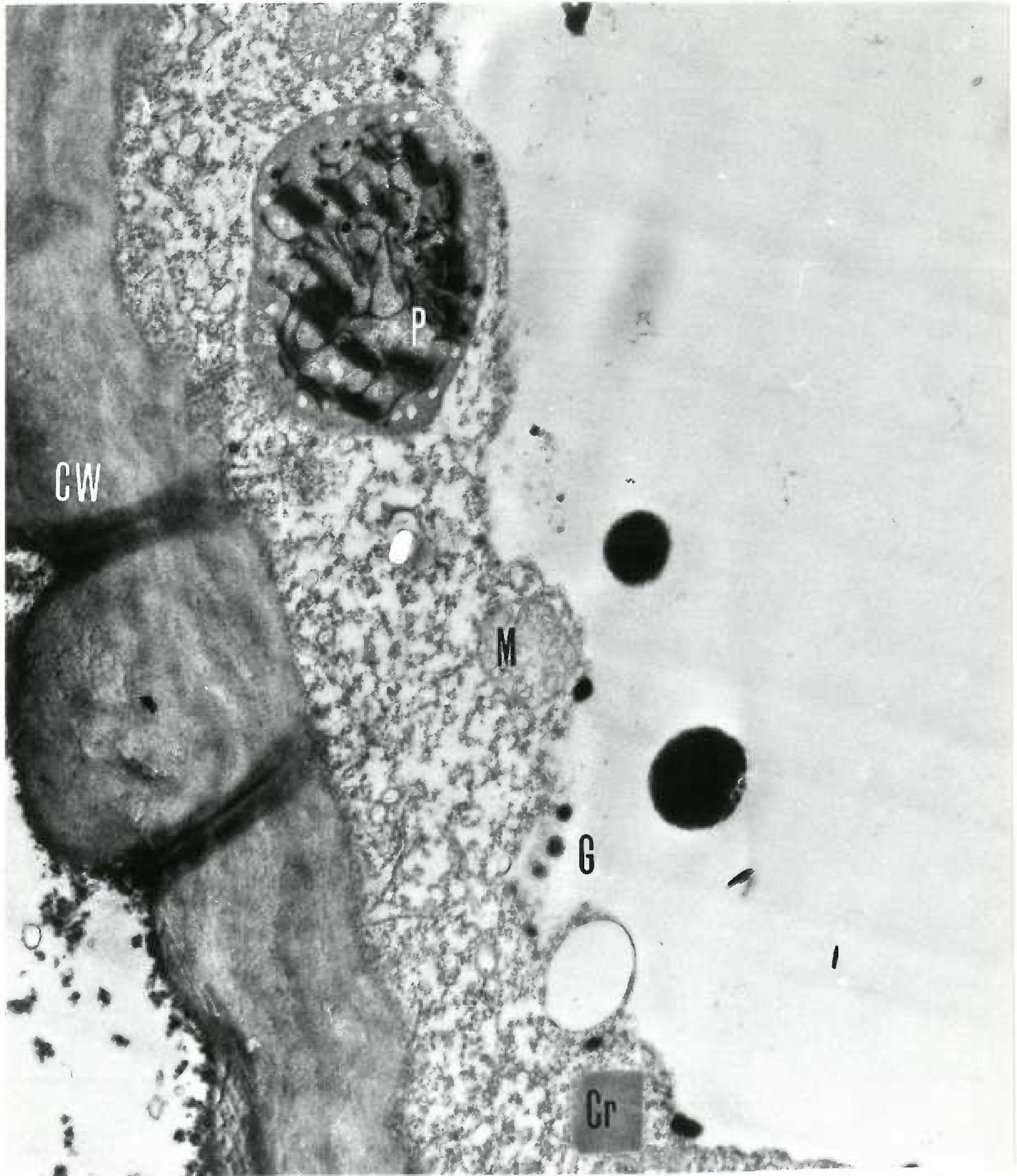

Cr
CW

Figure 22:

Tumor cell. Abnormal plastid (P), crystal (Cr), cell wall (CW), granules (G). X16,800.



P
CW
M
G
Cr

Figure 23:

Several tumor cells with vacuoles (V), lined with granules (G), nucleus (N), proplastids (PP). Large inclusions (arrow) in plastids. X10,000.

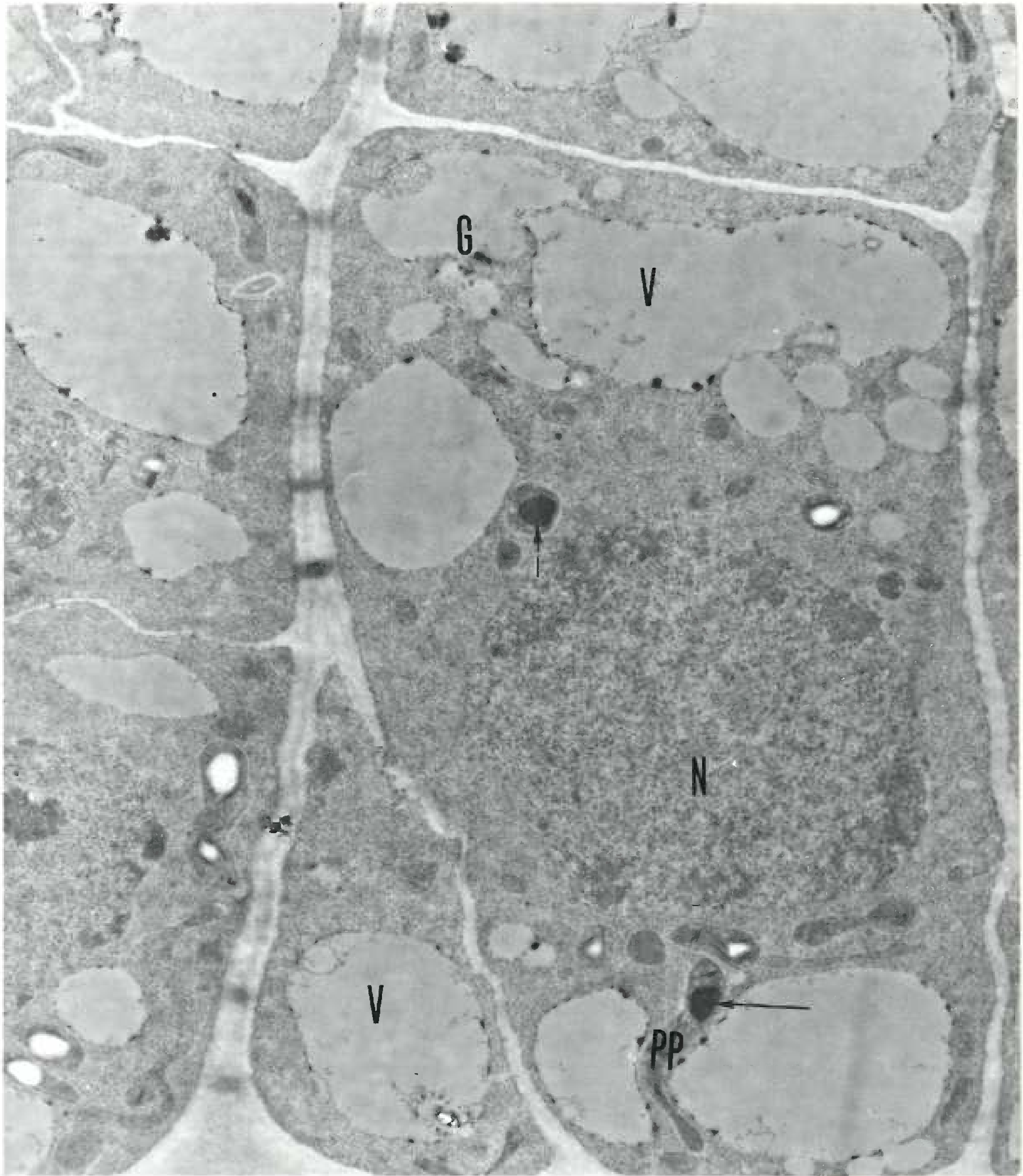
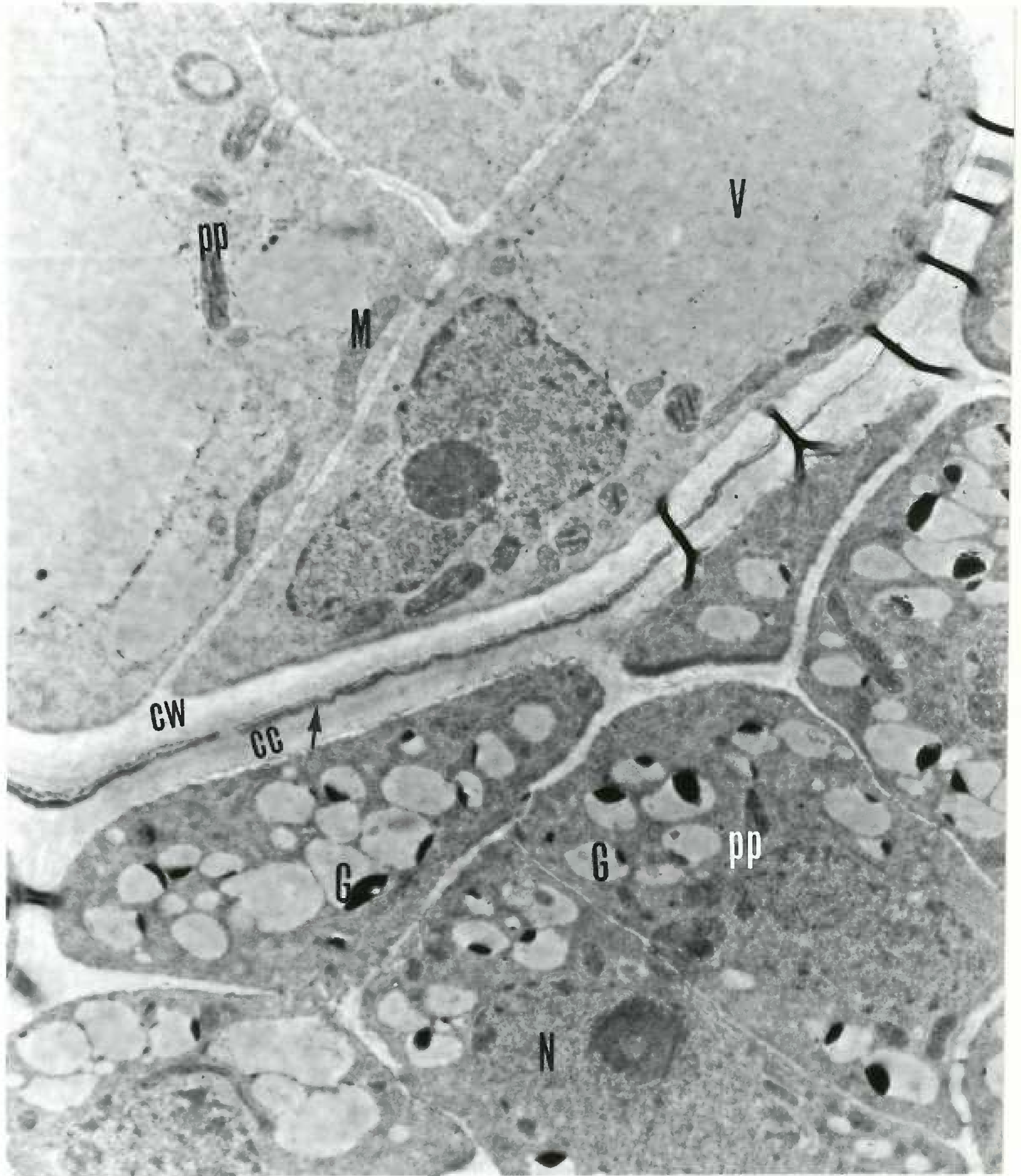

G
V
N
V
PP

Figure 24:

Tomato tumor cells. Note multiple small vacuoles with osmiophilic granules in the undifferentiated cells on the lower right, as compared to the large vacuoles (V), characterizing the differentiated cells above. Note central canal (CC) in cell wall (CW). Proplastids (PP) in undifferentiated cells on lower right have large dense internal inclusions. On the other hand, the plastids of the more differentiated cells above lack these inclusions. X5,800.


pp
V
M
CW
CC
G
G
pp
N

<u>Figure 25</u>:

Portion of differentiated normal tobacco cell. A chloroplast with starch granule (S), stromal lamellae (SL), grana lamellae (GL), plastglobuli (Pg), and cell wall (CW). X31,600.

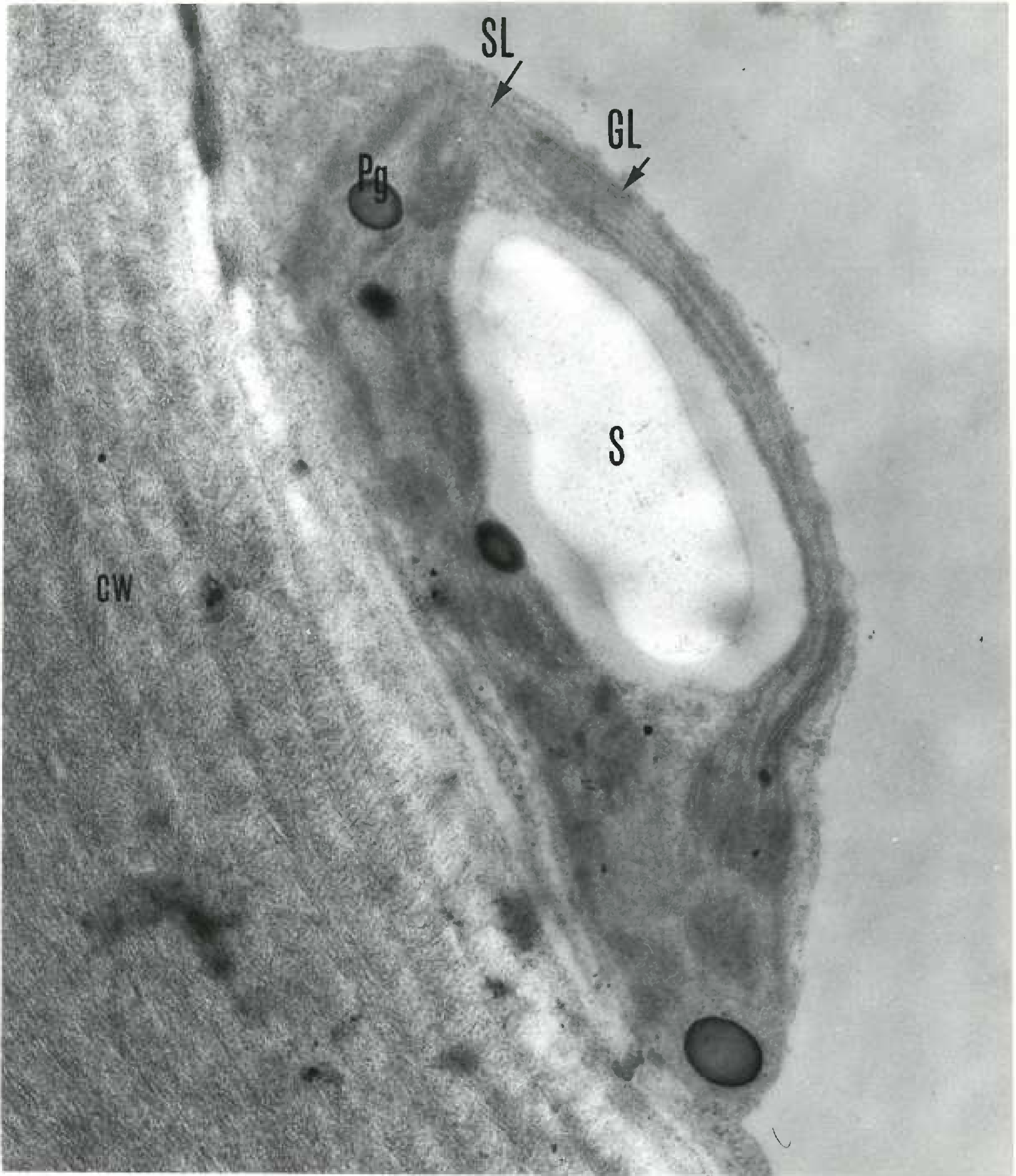

SL
GL
Pg
S
CW

Figure 26:

Portion of tobacco tumor cell containing one large chloroplast (C) demonstrating an extensive plastid vacuole filled with coarsely granular material. Grana lamellae (GL). X52,600.

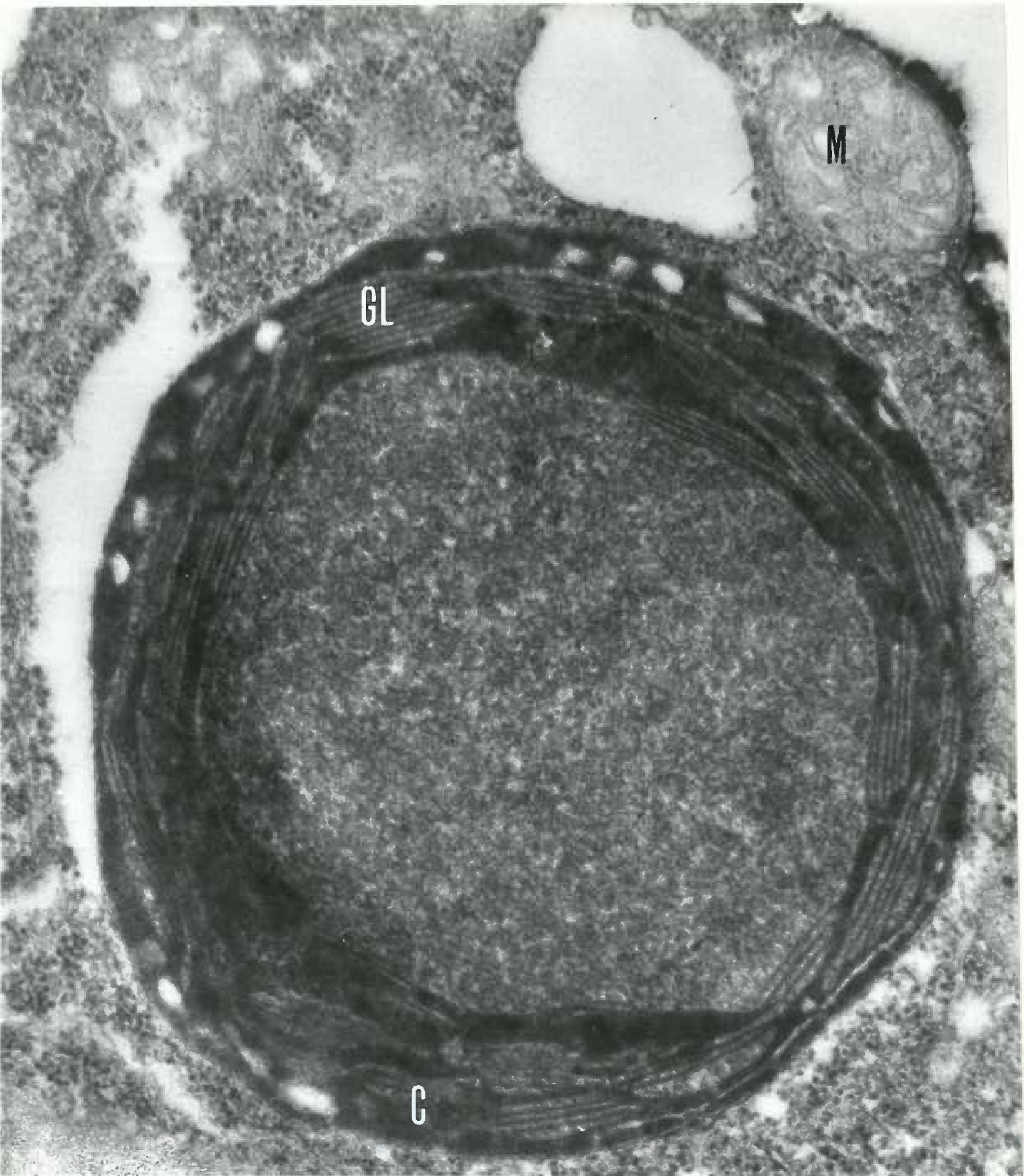
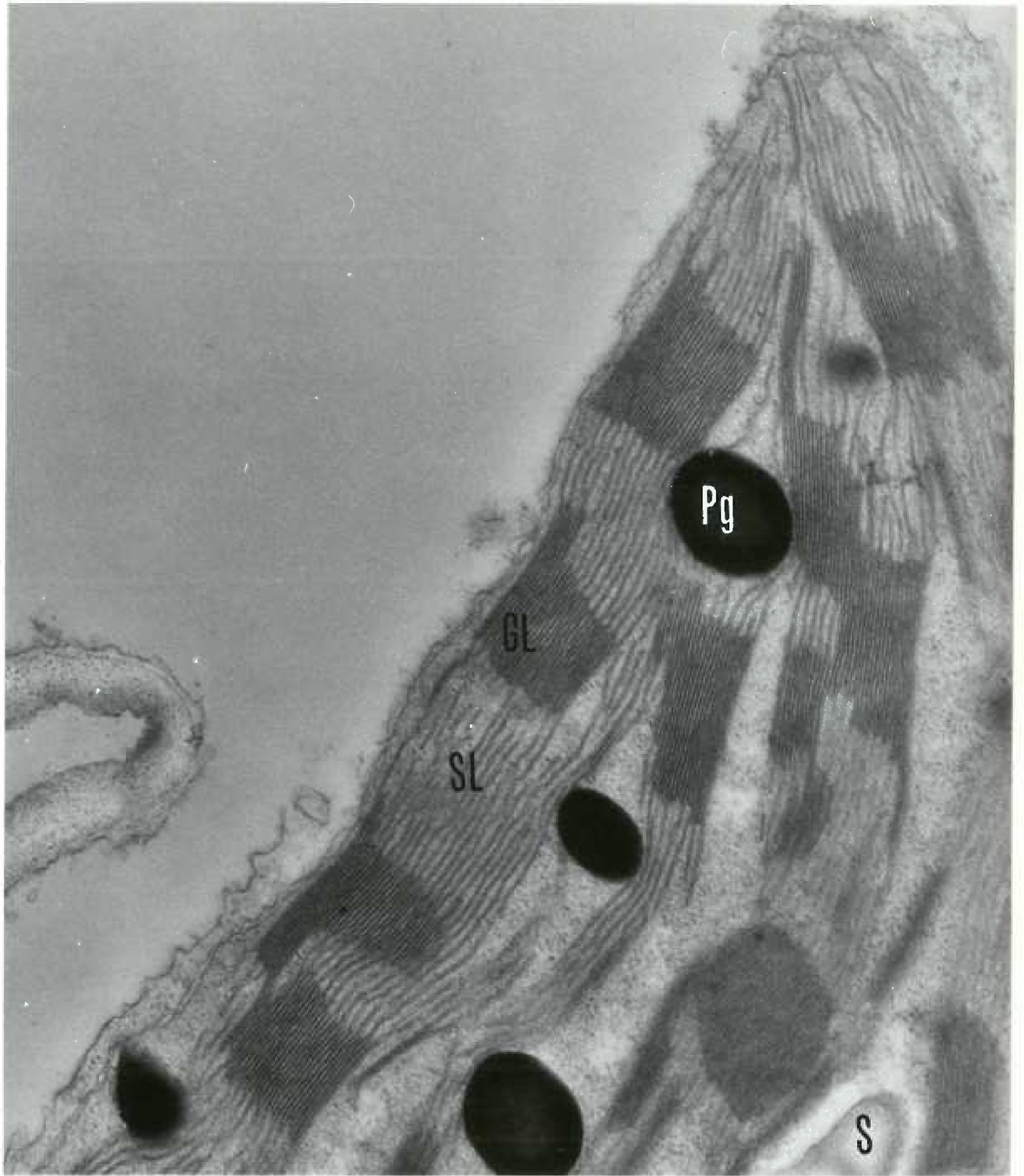

M
GL
C

Figure 27:

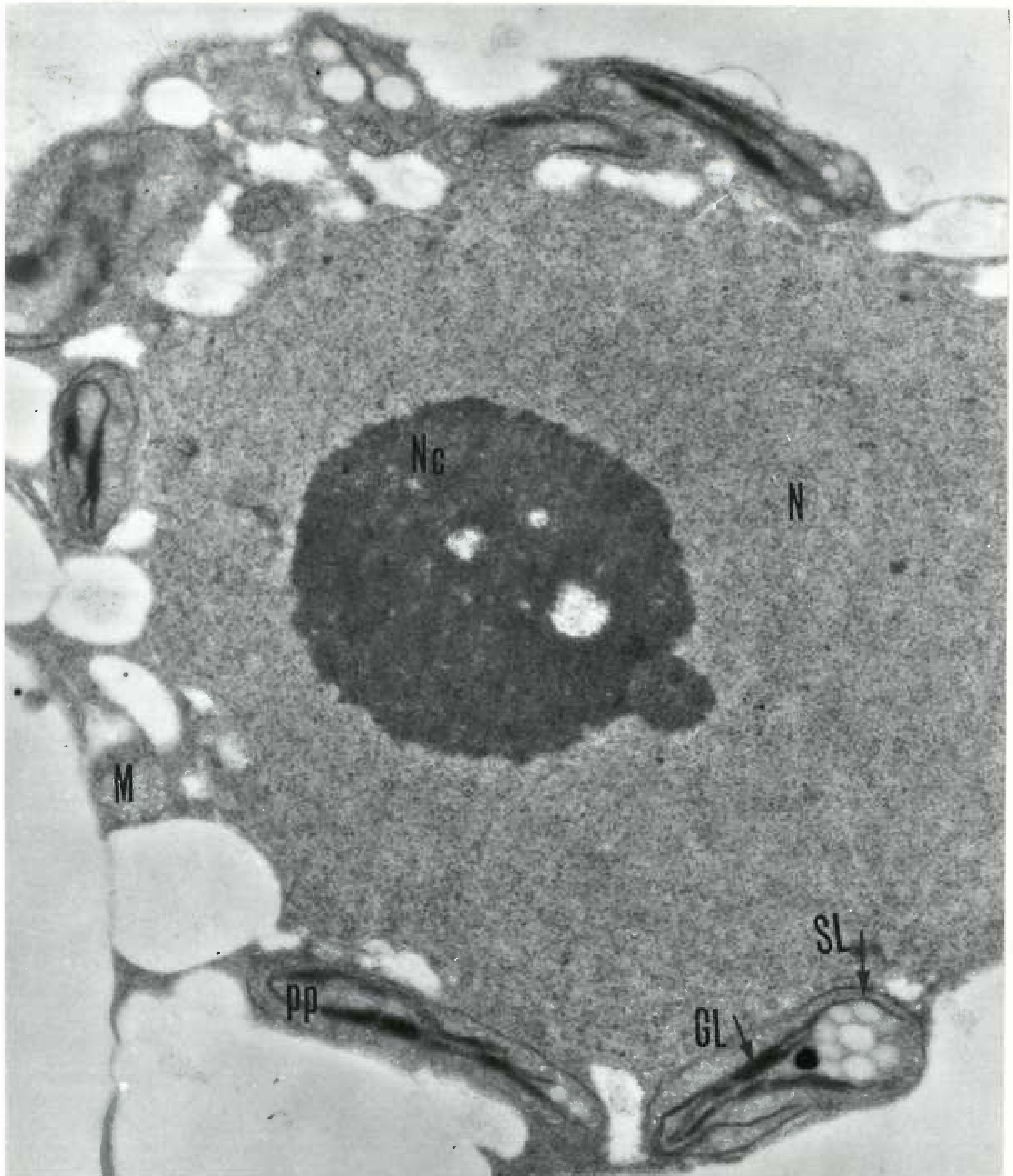
Chloroplast from normal differentiated *Bryophyllum* leaf. Starch (S), stromal lamellae (SL), grana lamellae (GL), plastoglobuli (Pg). X39,500.



Pg
GL
SL
S

Figure 28:

Bryophyllum tumor cell. Nucleus (N), nucleolus (Nc), mitochondrion (M). Proplastids (PP) containing vacuoles as well as stromal lamellae (SL) and grana lamellae (GL). X17,500.


Nc
N
M
SL
pp
GL