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The effects of ammonium nutrition on the structure and function of tomato leaf chloroplasts.

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THE EFFECTS OF AMMONIUM NUTRITION ON THE STRUCTURE AND FUNCTION OF TOMATO LEAF CHLOROPLASTS

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INTRODUCTION

A great deal of investigation has been undertaken on the physiological effects of ammonium on plants. A large part of this work, however, has been done "in vitro", and attempts have not been made to relate the changes in physiology with ammonium-induced morphological changes, such as the yellowing and necrosis of leaves. The symptoms of ammonium toxicity indicate that structural changes might occur in the chloroplast, especially since the chloroplast contains the chlorophyll and most of the leaf protein. The "in vitro" studies have shown that ammonium inhibits certain physiological processes associated with photosynthesis and subsequently causes structural modifications of the chloroplasts.

The purpose of this study was to study the effect of ammonium on the "in vivo" structure and function of chloroplasts.

The tomato plant was chosen for this study because of its growth characteristics on ammonium nutrition. With tomato, toxicity symptoms develop rapidly and sequentially. Further, the tomato plant has the ability to live under ammonium nutrition for prolonged periods.

LITERATURE REVIEW

In many higher plants, the application of ammonium as a source of nitrogen leads, over prolonged periods of time, to serious physiological and morphological disorders. These disorders are frequently observable as chlorosis of the leaves, restricted growth, necrotic spots and in some cases death (8, 9, 16, 54, 55). Such physical manifestations of ammonium nutrition are commonly termed ammonium toxicity and result from a series of physiological reactions initiated by the uptake of the ammonium ion.

Once within the plant, the ammonium ion is rapidly assimilated into the amides, glutamine and asparagine (30, 52). These compounds are synthesized by the amidation of glutamic and aspartic acids, respectively (62). The amides are generally considered to be harmless organic nitrogenous storage compounds (6, 7). They are synthesized very rapidly under ammonium nutrition and may account for as much as 90% of the soluble nitrogen compounds (64).

The increased synthesis of organic nitrogenous compounds effected by the ammonium ion leads to an increased utilization of the carboxylic acids (46). As ammonium treatment continues, the requirement for organic substrates becomes more demanding, resulting in a diminishing of the carbohydrate reserves (6, 16). In some cases, respiration is enhanced,

as shown by the experiments with nitrogen-starved Chlorella (45), while in others it is unaffected (6). The respiratory quotients of ammonium-treated plants are commonly around one (6, 45).

After prolonged ammonium nutrition, other aspects of the nitrogen metabolism of the plant begin to be affected. Yemm and Willis (64) noted that in ammonium-treated barley roots the synthesis of the insoluble nitrogen compounds, (primarily proteins and nucleic acids), leveled off and eventually showed a small decrease. Barker (8) was able to show a similar trend in pole bean leaves. Use of the stable isotope N^{15} revealed that soluble nitrogen compounds, especially the α-amino acids, were primarily derived from an endogenous source. Free ammonium shows a similar trend with approximately 60% having an endogenous origin. Large scale degradation of organic nitrogen compounds is, therefore, apparent with excessive ammonium nutrition.

The increase in free ammonium leads, in turn, to further upsets in the plant metabolism. The ammonium ion is a very effective uncoupler of photophosphorylation (3). In concentrations of 6×10^{-4}M, the ammonium ion inhibits ATP formation by 50%, while in concentrations of 4.7×10^{-3}M, it inhibits the production of ATP by 100% (27). This substantial reduction in chemical energy results in a reduction of carbon fixation within the chloroplast (48). Although in low concentrations the ammonium ion enhances electron transport in photophosphorylation through the act of uncoupling, at

concentrations of 6 x 10^{-3}M and greater it inhibits the reduction of ferricyanide in the Hill reaction (19). Similar experiments have also revealed that it inhibits the reduction of nicotinamide adeninedinucleotidephosphate, the high energy reducing product of non-cyclic photophosphorylation (50). Although these experiments involved "in vitro" systems, it has been determined that ammonium nutrition greatly restricts both the carbon dioxide consumption and oxygen evolution of intact leaves (Barker, unpublished data).

Recently, it was suggested that the ammonium ion may play a role in influencing the tertiary configuration of certain enzymes. It is well established that cations are involved in certain enzymatic systems (18, 62). It appears that these cations may be involved in the impartation of the tertiary structure to the enzymes (44). It is felt that a prolonged ammonium environment would interfere with the ionic-enzyme relationship. As the ammonium ion has a high hydrogen-bonding potential, it could affect either the formation or maintenance of the tertiary configuration of these enzymes. In such a way, the activity of the enzyme would be impaired and, in turn, made more susceptible to proteolysis.

Although intensive investigation has been undertaken on the physiological responses to ammonium nutrition, very little has been done to correlate them with morphological changes. One of the earliest signs of ammonium toxicity is the yellowing and necrosis of the leaves (16, 54). This chlorosis has been reported as occurring in conjunction with

the altered balance between the soluble and insoluble nitrogen compounds (8). Hence, it would appear that there is a strong relationship between the physical symptoms and metabolism of ammonium toxicity. Within the leaves of higher plants, the majority of proteins are chloroplastic. Zucker and Stinson (65) reported that 75% of the leaf protein in Oenothera leaves was contained in the chloroplast. Lyttleton and Ts'o (29) reported a similar concentration in the plastids of spinach. Chloroplastic protein exists in a state of fluctuation and is constantly being degraded and resynthesized as much as 20% per day (41). It would therefore seem logical that the morphological effects of ammonium toxicity would manifest themselves within these photosynthetic organelles. This is further indicated by the evident loss of chlorophyll from the leaves (55). In fact, it has been noted that chlorophyll synthesis is extremely dependent upon protein synthesis. Kirk and Allen (26) reported that chlorophyll synthesis could be efficiently blocked in etiolated beans with the use of the protein synthesis inhibitor, chloramphenicol. In view of the fact that the chloroplast plays a primary role in carbohydrate synthesis, energy production and reducing potential formation (13), it seems apparent that the degradation of this organelle would greatly upset the metabolic balance of the plant and lead to physiological disorders.

Examination of the plastid on the ultrastructural level reveals it to be a complex system of lamellar membranes and anastomosing channels (56). The basic unit of the

chloroplast is the grana, which consists of a united sequence of closed compartments (58). The compartments are linked through a series of channels termed frets and are thereby isolated from the proteinaceous matrix or stroma (57). The grana-fret network contains the chlorophyll molecules and the chemical constituents necessary for the formation of adenosine triphosphate and reduced NADP, (37). The stroma which surrounds the grana-fret network contains the necessary enzymes for the dark reaction of photosynthesis, carbon dioxide fixation (36). Surrounding the stroma, grana and frets is a limiting double membrane which provides for the osmotic regulation of the chloroplast (2).

The intricate organization of the chloroplast is directly dependent upon the cellular environment of the leaf. This is exemplified by the examination of plastids from nutritionally deficient plants (11, 33, 39, 47, 51). Manganese deficiency results in a reduction in granal number, extensive swelling of the compartments, and reduction of the fretwork system (33). Iron deficiency causes a similar type of disorganization with swollen grana and degraded frets (11). Disrupting the electron transport system in photosynthesis with an uncoupling agent, e.g, ammonium, can also upset the internal organization of the plastid (23). Fine structural analysis, therefore, may indicate physiological disorders. Such an ultrastructural examination of the plastids of ammonium-treated plants has not been undertaken as of yet. The apparently strong relationship between ammonium toxicity and the

structure and function of the leaf chloroplasts would support such a study.

MATERIALS AND METHODS

I. General

The plant used for this research was the tomato, Lycopersicon esculentum 'Heinz 1350', Mill. The seeds were sown in soil, and the seedlings were allowed to grow for five weeks. The young plants were subsequently transplanted into 8-inch plastic pots containing a 1:1 mixture of coarse and fine silica sand. The plants were immediately subjected to Hoagland's No. 1 nutrient solution (20) and treated for 15 days. At the end of the 15-day period, the plants were randomly divided into two groups, NH_4^+ and NO_3^-. The root medium of the ammonium-treated plants was washed with the ammonium solution. This ammonium solution was supplied in the form of a modified Hoagland's solution with all the nitrogen as ammonium. The other group was retained on the Hoagland No. 1 solution and was termed the nitrate or control group. The solutions were applied twice daily with 250 ml per pot. The minor element solution of Hoagland and Arnon (20) was used to supply micronutrients.

This procedure was carried out on a total of four different series in February, April, June and July. The first series was initiated on February 5, 1966, and

continued until February 26. The leaves of the plants from the end of this sequence were selected for fixation and embedding in preparation for an ultrastructural examination of the plastids. The plants in the second series were started on ammonium nutrition April 19, 1966, and were continually treated until May 17. Every second day within this series, leaf samples were selected and used for chlorophyll determinations and electron microscopy. At various periods after the initiation of ammonium treatment, 5 g of leaf tissue were selected for ammonium and amide determinations. The third series was started May 31, 1966, and continued until June 18. Similar to the April series, leaf samples were selected every other day, for electron microscopy, chlorophyll content, ammonium and amide content. In addition, manometric measurements and carbon dioxide fixation measurements were carried out. The latter two determinations were established on intact leaves beginning June 12. Previously, attempts had been made to use leaf discs for the manometric measurements in the Gilson respirometer. Unfortunately, due to some undetermined factor, a high degree of variability was obtained. The use of whole leaves gave constant, reproducible rates throughout the series. The fourth and final series was initiated June 27, 1966, and continued for 12 days. Chlorophyll samples and whole leaves for manometric measurements were selected every other day throughout the sequence.

II. Photosynthesis and Respiration

Analyses for photosynthesis and respiration were carried out on the June and July series. Small leaves were selected from the second largest branch from the top of both NH_4^+ and NO_3^- plants, and their fresh weight recorded. These leaves were each placed in a 15-ml Gilson reaction vessel, Model GME-140, in 5 ml of a 0.2M tris [2-amino-2-(hydroxymethyl)-1,3-propanediol] solution adjusted to pH 7.2 with 0.1N HCl. A constant carbon dioxide atmosphere was obtained by placing 0.6 ml of Pardee's buffer in the center well of the reaction vessel along with a filter paper wick (cf 49). Pardee's buffer was made up the night previous to the experimental run and consisted of 6 ml diethanolamine, 6.8 ml H_2O, 15 mg thiourea, 3 g $KHCO_3$, 2.2 ml 6N HCl and 21 microcuries of $KHC^{14}O_3$. The manometric measurements were determined on a Gilson Differential Respirometer, Model GRP 20. The oxygen evolved during photosynthesis was measured in microliters at 10 minute intervals over a 60 minute period. During this interval, the reaction vessels were shaken in a water bath at a constant temperature of 25°C and illuminated from below with a light intensity of 1000 foot-candles. Respiration, in terms of oxygen consumption, was measured after photosynthesis by turning off the light source and covering the respirometer with a black cloth. In all cases, the manometric determinations were carried out on five replicates of both NH_4^+ and NO_3^- plants.

III. Carbon Dioxide Fixation

The radioactive carbon was obtained from the Nuclear Chicago Company in the form of solid $Ba^{14}CO_3$. The encapsuled $Ba^{14}CO_3$ was placed in a small vial containing 1.0 ml of 0.04N KOH and 4 ml of CO_2-free water. This vial was stoppered with a syringe cap and $C^{14}O_2$ generated by injecting 0.5 cc of 40% perchloric acid into the $BaC^{14}O_3$ vial. The generated $C^{14}O_2$ was trapped as $KH^{14}CO_3$ in the KOH solution of the vial. The radioactive solution was then diluted to 10 ml with water, and 0.21 ml, containing 100 μc per ml, was added to 15 ml of the Pardee buffer. After measurement of gas evolution, the individual leaves were washed in distilled water and partitioned into small portions. These portions were placed on planchets and put in the freezer in order to disrupt the cell wall and allow for flattening of the leaf section to the planchet. The leaf portions were pressed to the planchet after removal from the freezer and were subsequently placed in an oven at 60°C for 12 hours. Radioactivity of the leaf sections was determined with a Nuclear Chicago gas flow detector (Model D47) and the time required for 2500 counts recorded using the digital counter (Model 8167).

IV. Ammonium and Amide

Ammonium and amide contents were determined for both the April and June series. The samples for analysis in the April series consisted of 5g of leaf tissue while in the June series the sample size was increased to 10g.

In both instances, the leaves were weighed immediately upon removal from the plant. The nitrogenous components were extracted by grinding the leaf tissue in 70% (v/v) ethanol in a Virtis 45 Homogenizer for 10 minutes. The leaf extract was suction-filtered through Whatman No. 1 filter paper and the cellular debris washed with additional 70% ethanol until the chlorophyll was removed. The ethanol extract was evaporated to dryness at room temperature in a 600 ml beaker and then transferred to Kjeldahl flasks. This transfer was performed by redissolving the dried extract with a series of chloroform, ethanol and water washes. The solution was evaporated to dryness in the flasks at room temperature using steady streams of air. The ammonium and amide content was determined by a modified Kjeldahl procedure (5).

V. Chlorophyll

Chlorophyll determinations were carried out on both leaf discs and whole leaves. When discs were used, 15 discs would be cut out from a single plant leaf using a No. 4(9mm) brass cork borer. These discs were weighed immediately and the chlorophyll extracted by grinding in 80% acetone in a Virtis 45 Homogenizer. The chlorophyll extract was filtered through Whatman No. 1 filter paper and broughtup to 50 ml in volume. The chlorophyll content was determined by the Arnon method (1) using a Beckman DU-2 spectrophotometer. This same procedure was utilized for the whole leaf samples.

VI. Electron Microscopy

Leaf sections cut 1 mm^2 for fixing were taken from the middle of the leaf sample and were fixed in unbuffered 2% $KMnO_4$ for 20 minutes at room temperature. The Luft (28) procedure for fixation and embedding was followed. This entailed dehydration through a graded series of acetone-water mixtures, staining with uranyl nitrate for 30 min and infiltrating with an epoxy resin. The resin consisted of a 1:1 mixture of Epon A to Epon B. Epon A consisted of 62 ml Epon 812 and 200 ml dodecenyl succinic anhydride, while Epon B was composed of 100 ml Epon 812 and 89 ml methyl nadic anhydride. After thoroughly infiltrating with the resin mixture, the small leaf sections were oriented in plastic capsules so that the flat surface of the leaf would be parallel to the diamond knife during sectioning. The capsules were filled with the 1:1 Epon mixture plus 1.5% of an accelerator, dimethylamino-methyl phenol (DMP-30) and placed in a 60°C oven overnight or longer for polymerizing. The polymerized block was trimmed down to expose the embedded leaf section and sectioned with a diamond knife on a Porter-Blum microtome (Servall MT-1). The sections were collected on 200-mesh formvar-coated copper grids and examined with a Carl Zeiss, EM-9, electron microscope.[1]

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[1]The electron microscope was made available through a grant from the National Science Foundation (Grant GB-1062 to J. R. Rowley).

RESULTS

I. Ultrastructure of Tomato Leaf Chloroplasts

The analysis included here represents the results of a detailed examination of over 150 micrographs of plastids from ammonium and nitrate treated plants. These micrographs cover three series, February, April and June, and represent a total of 21, 28 and 20 days on ammonium treatment, respectively. In order to best present the data obtained, the component parts of both the ammonium treated and control plastids will be discussed simultaneously. In this way, variations among the treated plastids can be described as well as the differences between treated (NH_4^+) and control (NO_3^-).

A. Appearance

1. Membrane

In all of the control plastids observed under adequate magnification, the chloroplast was bounded by a membrane comprised of two dense lines separated by a transparent region. This double limiting membrane was also evident in the treated plastids although it sometimes appeared broken (Fig. 2). In many treated plastids, the membrane appeared to form small protrusions (Fig. 3a & 3b).

The treated plastids clearly illustrated a continuity between the chloroplast membranes and the granal lamellar system (Fig. 4).

2. Position of the Chloroplast

The control plastids were situated around the periphery of the cell, appressed to the cell wall and tightly abutted to each other (Fig. 1a, 1b, 1c). After ammonium treatment, the plastids no longer retained a close relationship with each other and appeared to be "floating" within the cell (Figs. 5 & 6).

3. Shape

The shape of the chloroplasts was highly variable in both treated and control plants. The control plastids, due to their close allignment with the cell wall, generally appeared rectangular or spindle-shaped (Fig. 1b). The chloroplasts of the treated plants, on the other hand, frequently had a rounded conture (Fig. 7).

4. Number per Cell

It was extremely difficult to obtain numerical comparisons of plastid number as the micrographs usually showed portions of cells rather than entire cells. The wide spacing of the treated chloroplasts, however, suggested that a reduction in plastid number had occurred (Fig. 6).

5. Starch

With few exceptions, the plastids of both treated and control plants contained large starch grains evident as large white bodies in the micrographs. Frequently the starch grains had a peculiar marking of thick black lines (Fig. 5).

B. Internal Structure

For this discussion, the terminology suggested by Weier, et. al. (59), has been adopted.

1. Grana

(a) Outward Appearance

(1) Shape

In face view, the grana of control plastids appeared circular and gave evidence of parallel lines transversing their face (Fig. 1a). In side view, these grana were usually rectangular or square and clearly illustrated their compartmental composition (Fig. 1a, 1b, 1c). The grana of the treated plants, when present, were commonly distorted due to swollen compartments (Fig. 8).

(2) Length (Number of Compartments)

The control plastids contained well-defined grana consisting of 10 to 30 compartments (Fig. 1a). Treated plastids were marked by an absence of large grana

with only 1% of the plastids observed containing grana with greater than 20 compartments. The average number of compartments per granum in the treated plants was approximately five (Fig. 7).

(b) Internal Appearance

The compartments of the grana of both treated and control plastids consisted of an electron transparent loculus separated from the strona by margins and from other compartments by partitions. In many cases the loculi could be seen opening into frets (Fig. 9), however, they could not be observed opening into the stroma. The end compartments of the grana of nitrate plants frequently showed swelling (Fig. 1c). In only very isolated instances did this swelling affect the internal compartments. In contrast to this condition, the treated plants typically showed swelling of all compartments (Fig. 9). In more extreme cases, the grana completely lost their orientation within the plastid and appeared as a dispersed lamellar system (Fig. 4) or as various sized vesicles (Fig. 10). In some instances the plastid wall had ruptured and the plastid components were scattered

throughout the cell (Figs. 2 & 11).

2. Fretwork

In the control plants the fretwork system was well-developed, consisting of a series of anastomosing channels (Fig. 1a). These channels appeared either as short dashes or long lamellae (Fig. 1c). The fretwork system of the treated plants was greatly reduced and when present was typically swollen into vesicles and tubules (Fig. 8). In more extreme cases, the frets existed as very large vesicles and were pushed to the end of the plastid (Fig. 10). These vesicles were then released into the cell with the breaking of the plastid membrane (Fig. 2).

3. Stroma

The stroma in the treated plastids appeared similar to that of the control, although it may have increased in amount due to the disruption of the grana and frets.

4. Starbodies

Starbodies appeared as dark rod-like structures that had various degrees of branching. In some instances, they seemed to be connected to the fretwork system (Fig. 12). Although they appeared in the control plastids, they seemed more evident in the treated.

II. Chloroplastic Ultrastructure with Varying Time under NH_4^+ Treatment

In both the April and June series, the first evidence of ultrastructural modifications in the plastids appeared after approximately 10 days of ammonium treatment. Examination of micrographs before this time revealed plastids similar in position and structure to control plastids (Figs. 13 & 14). However, sections from 10-day ammonium treated plants, in the April series, showed the presence of vesicle-filled plastids interspersed among normal plastids (Figs. 16 & 17). These vesicles could be clearly seen to have developed from the fretwork (Fig. 18). The grana appeared to be relatively unaffected by this vesiculation, as they maintained their structural integrity (Figs. 16, 17, 18). Some of the plastids appeared to have been broken, as lamellae were scattered throughout the cell (Figs. 16 & 17). The variation in chloroplastic ultrastructure after 10 days of ammonium treatment was similarly shown in the June series. In these plastids, vesicles were not apparent although the plastids showed structural modifications such as swollen compartments, reduced grana and disrupted frets (Fig. 9). Micrographs from plants under ammonium treatment for longer than 10 days revealed further structural alterations. The plastids no longer appeared abutted, but were quite widely spaced (Fig. 6). The grana were reduced in compartmental number and had expanded loculi (Fig. 19).

After 20 days of ammonium nutrition, chloroplastic organization reached the stage described in detail in Section I of the Results.

III. Chlorophyll Content

A significant loss in the chlorophyll content of the leaves occurred soon after ammonium treatment. Both the time and extent of this loss varied with the three series tested, probably in reflection of the greenhouse environmental conditions at the time of treatment. In the April series a significant loss of 14% of the chlorophyll from the treated plants occurred on the 7th day after initiation of ammonium treatment (Table 1a). The chlorophyll content of these leaves then showed a constant decline, dropping to 37% of the control leaves at the end of the experimental sequence. A similar trend in rapid and consistent chlorophyll loss was shown by the June and July series, although the first significant difference occurred on the 4th day of treatment in the June series and on the 8th day in the July series (Table 1b and 1c). By the 12th day of ammonium treatment, the chlorophyll content of the July plants had dropped to 40% of the controls while the chlorophyll content of the June plants after 11 days on ammonium was 25% of the controls (Tables 1b and 1c). The higher chlorophyll content of the July series and more rapid decline in the June series may have been a consequence of the differences in ammonium accumulation resulting from the

summer environmental conditions within the greenhouse.

IV. Ammonium Content

The free ammonium content of the treated plants increased significantly in both the April and June series. Within the April series, a significant increase in ammonium concentration was noted after 10 days of ammonium treatment. At this time the ammonium content of the treated plants was 4 times that of the controls (Table 2a). The ammonium level continued to increase throughout the sequence becoming 5.9 times greater than the control after 35 days of treatment. The ammonium concentration of the June series followed a similar trend, although the concentration of ammonium on the 9th day, the first day of significant differences, was 8.2 times higher than the controls (Table 2b). The ammonium concentration in this series also showed an increase with time on treatment, however, the determinations from the 17th and 19th days revealed nonsignificant values (Table 2b). This discrepancy may have been due to the fact that some of the ammonium samples for these two days were contaminated during evaporation procedure and an immeasurable source of variability was introduced.

V. Amides, Glutamine and Asparagine

The concentration of amides in the leaves of ammonium treated plants increased significantly over that of the controls in both series tested. Significance was first noted in the April series after 17 days of

ammonium treatment and continued until the end of the sequence (Table 3a). The determinations from the June series revealed significance after 13 days of ammonium nutrition. Subsequent determinations on the 15th and 19th day after initiation of ammonium treatment also showed significance but none could be obtained from the 17th day (Table 3b). Similar to the ammonium data mentioned above, the leaf samples from the end of the June sequence were contaminated during the extraction process. This contamination, in all probability, resulted in excessive variability in the amide values obtained and affected a loss of significance.

VI. Photosynthetic Oxygen Evolution

The data revealed that ten days of ammonium nutrition caused a 29% inhibition in the oxygen evolving capacity of the leaves (Table 4a). It is evident from Table 4a and 4b that oxygen production per gram fresh weight of the treated leaves decreased substantially with increasing days on treatment. In fact, the mean of the apparent photosynthetic rate of leaves that were 17 days on treatment was -8µl O_2/10 min/g fr wt (Table 4b). This indicates that these leaves were operating below their compensation point, i.e. their respiratory rate exceeded their photosynthetic rate. Although the treated leaves revealed a significant reduction in photosynthetic rate when expressed in terms of fresh weight, very little differences were revealed in rates

of oxygen evolution in terms of chlorophyll content. This relationship is shown in Tables 5a and 5b where it can be seen that the photosynthetic rates of the treated plants were slightly higher than that of the controls in the June series but slightly lower in the July series. Unfortunately, the chlorophyll values used in the above comparisons were the mean values of the chlorophyll contents of ammonium-toxic leaves and differed from those used for the photosynthetic determinations. As a result, no statistical comparisons could be established.

VII. Photosynthetic Carbon Dioxide Fixation

Carbon dioxide fixation data was obtained from the latter half of the June series. It is evident from the data obtained (Table 7) that in terms of fresh weight, the leaves of ammonium treated plants were much less active in incorporating carbon dioxide than were the corresponding controls. After 19 days on treatment, the treated plants incorporated only around 25% as much carbon dioxide into organic products as did the controls. Although the leaves from plants 17 days on treatment were functioning below their compensation point (Table 4b), they still fixed a substantial amount of CO_2 (Table 7). When carbon dioxide fixation was expressed in terms of milligrams of chlorophyll, the treated plants proved to be more efficient in the fixation of CO_2. Table 8 reveals that the fixation ability of the treated leaves per milligram of chlorophyll was 3 times that of the control leaves after 19 days of

ammonium nutrition. As in the case of oxygen evolution, no statistical comparisons could be established on the basis of carbon dioxide fixation per unit chlorophyll as the chlorophyll contents were not determined on the leaves used for CO_2 fixation.

VIII. Respiratory Oxygen Consumption

Initially the leaves from the ammonium-treated plants showed a respiratory rate equal to that of the control plants. After 8 days on treatment, the oxygen consumption of the treated leaves increased 26% over the respiratory rate of the control plants (Table 6a). The rate of O_2 consumption then declined with further treatment and became 56% less than the rate of the control plants after 13 days of treatment. This rate subsequently remained essentially unchanged until the end of the sequence (Table 6b).

EXPLANATION OF FIGURES

Fig. 1a.-Chloroplasts from a control plant at the end of the April series. Grana are evident in both face and side view and are connected by a well developed granal system. (4.6 x 8000) x 36,800.

Fig. 1b.-Chloroplast from the mesophyll cells of a control plant. Note the spindle-shape of the chloroplasts and their close appressement to the cell wall. (3.1 x 8000) x 24,800.

Fig. 1c.-Chloroplasts from a control plant showing well developed grana with swollen end compartments. (3.9 x 8000) x 31,200.

Fig. 2.—A chloroplast from an ammonium-treated plant (28 days on treatment) showing a ruptured plastid membrane. Grana and fret components are vesiculated and are dispersed throughout the cytoplasm. (3.6 x 8000) x 28,800.

Fig. 3a.-Chloroplast from the cells of an ammonium-treated plant (28 days on treatment) with a protrusion extending from the right hand portion of the plastid membrane. (4.5 x 8000) x 36,000.

Fig. 3b.-Mitochondrial-like protrusion extending from the plastid membrane of a chloroplast from an ammonium-treated plant (21 days on treatment). (4.7 x 3,600) x 16,900.

Fig. 4.-Chloroplast from an ammonium-treated plant (28 days on treatment) showing absence of granal organization. The plastid membrane appears continuous with the granal lamellar system. (3.6 x 8,000) x 28,800.

Fig. 5.-Floating chloroplasts from a plant 28 days on ammonium treatment. Starch grains show peculiar black markings. (3.4 x 2,800) x 27,200.

Fig. 6.-Plastids from the mesophyll cells of a 17 day ammonium-treated plant. The chloroplasts are rounded in conture and appear widely spaced from each other. (2.8 x 1,800) x 5,000.

Fig. 7.-Chloroplast from a plant under ammonium treatment (21 days on treatment) showing small numbers of compartments per granum. Note the rounded conture of the plastid. (2.9 x 8,000) x 23,200.

Fig. 8.-Chloroplasts from ammonium-treated plants (28 days on treatment). The granal compartments are swollen and the frets are evident as tubules and vesicles. Normal appearing mitochondria surround the plastids. (3.9 x 8,000) x 31,200.

Fig. 9.-Detail of a chloroplast from a 10-day ammonium-treated plant in the June series. Compartments are greatly swollen and loculi can be seen opening into frets. (3.6 x [illegible],000) x 75,600.

Fig. 10.-Chloroplast from an ammonium-treated plant (28 days on treatment). The grana-fret network is completely disrupted and vesiculated. (3.6 x 8,000) x 26,800.

Fig. 11.-Released granal and fret components from a disrupted chloroplast of a 28-day ammonium-treated plant. (4.4 x 21,000) x 35,[illegible]00.

Fig. 12.-Chloroplasts from the mesophyll cells of ammonium-treated plant (21 days on treatment). Starbodies are quite evident and appear to be continuous with the fretwork system. (3.1 x 8,000) x 24,800.

Fig. 13.-Chloroplasts from plants 3 days on ammonium treatment. Plastids appear similar in position and structure to control plastids. (3.0 x 3,800) x 24,000.

Fig. 14.-Chloroplasts from plants with 7 days of ammonium treatment. Plastids resemble control plastids in all respects. (4.3 x 3,700) x 15,900.

Fig. 15.-Dividing chloroplast from a plant 7 days on ammonium treatment. Ultrastructure of the plastid is the same as the control. (3.6 x 8,000) x 28,800.

Fig. 16.-Chloroplasts from 10-day ammonium-treated plants. Note the occurrence of vesicle-filled plastids among the normal plastids. Appearance of grana-fret components within the cytoplasm shows evidence of plastid disruption. (3.4 x 1,800) x 6,100.

Fig. 17.-Higher magnification of vesicle-filled plastids from 10-day ammonium-treated plants. Grana still maintain structural integrity. (3.0 x 5,000) x 15,000.

Fig. 18.-Details of chloroplasts in Fig. 17, showing formation of vesicles from the fretwork. (2.9 x 8,000) x 23,200.

Fig. 19.-Chloroplast from the mesophyll cells of an ammonium-treated plant (20 days on treatment), showing reduction in granal number and swollen compartment number. (5.0 x 8,000) x 40,000.

Figure 1a

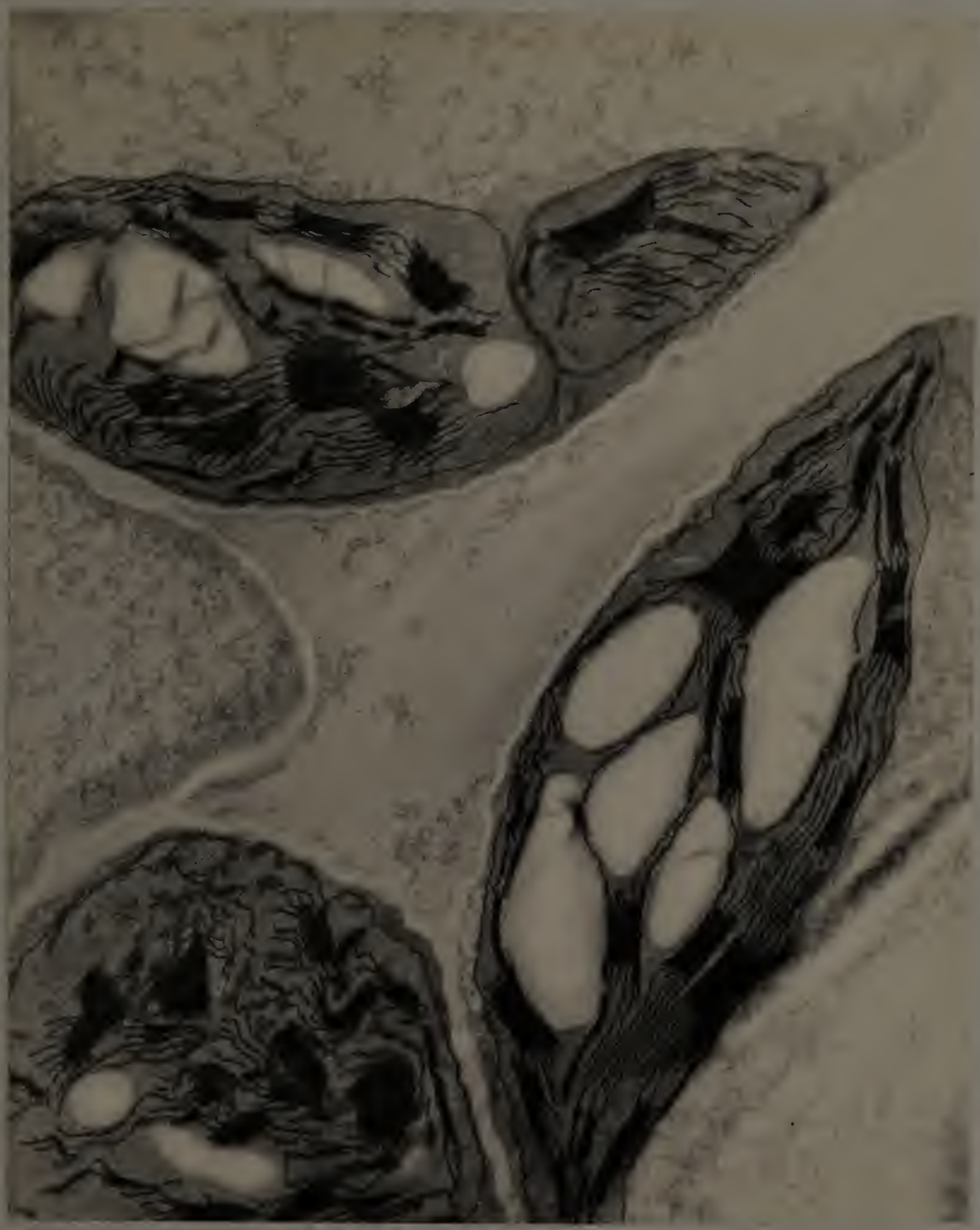


Figure 16

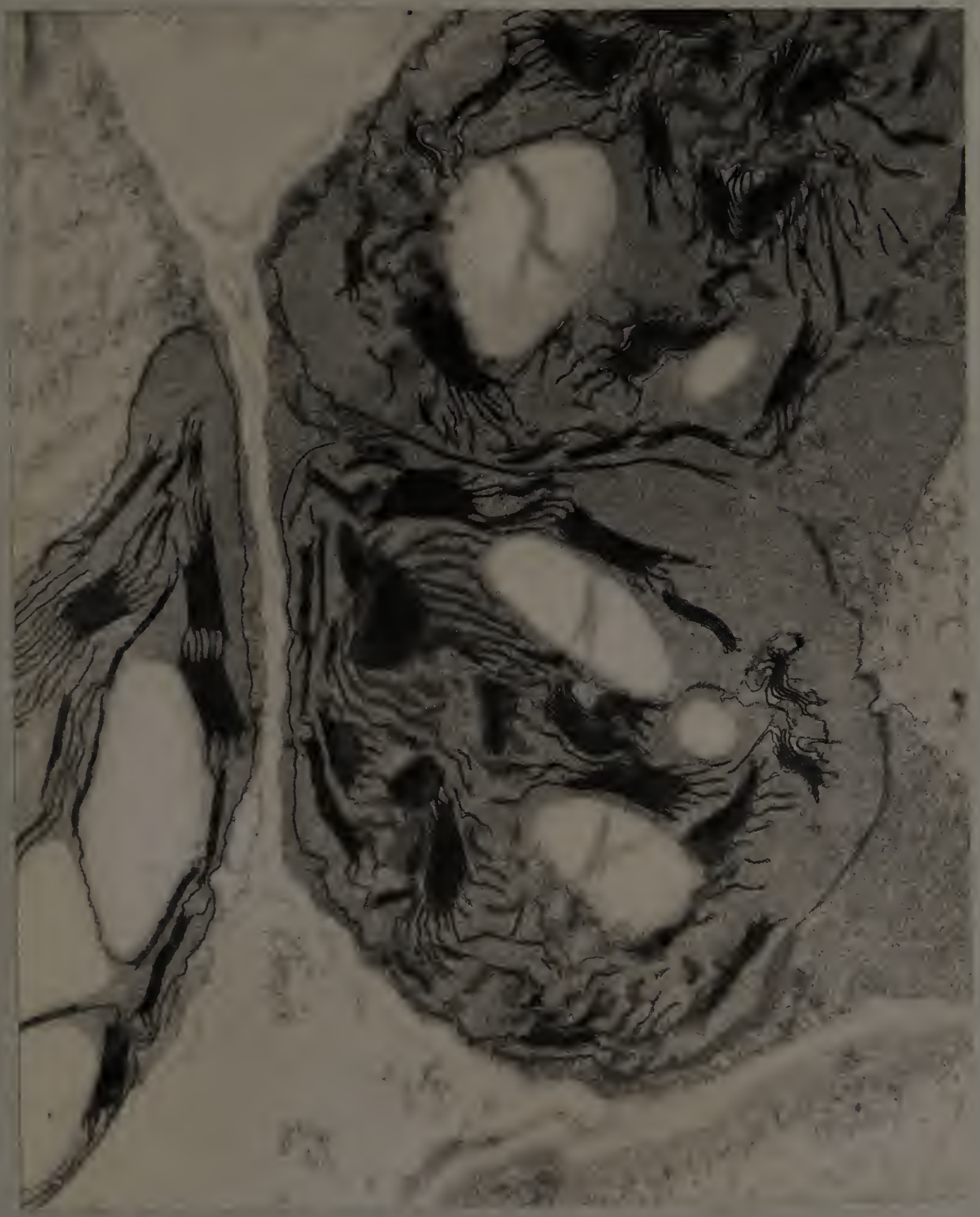


Figure 1c

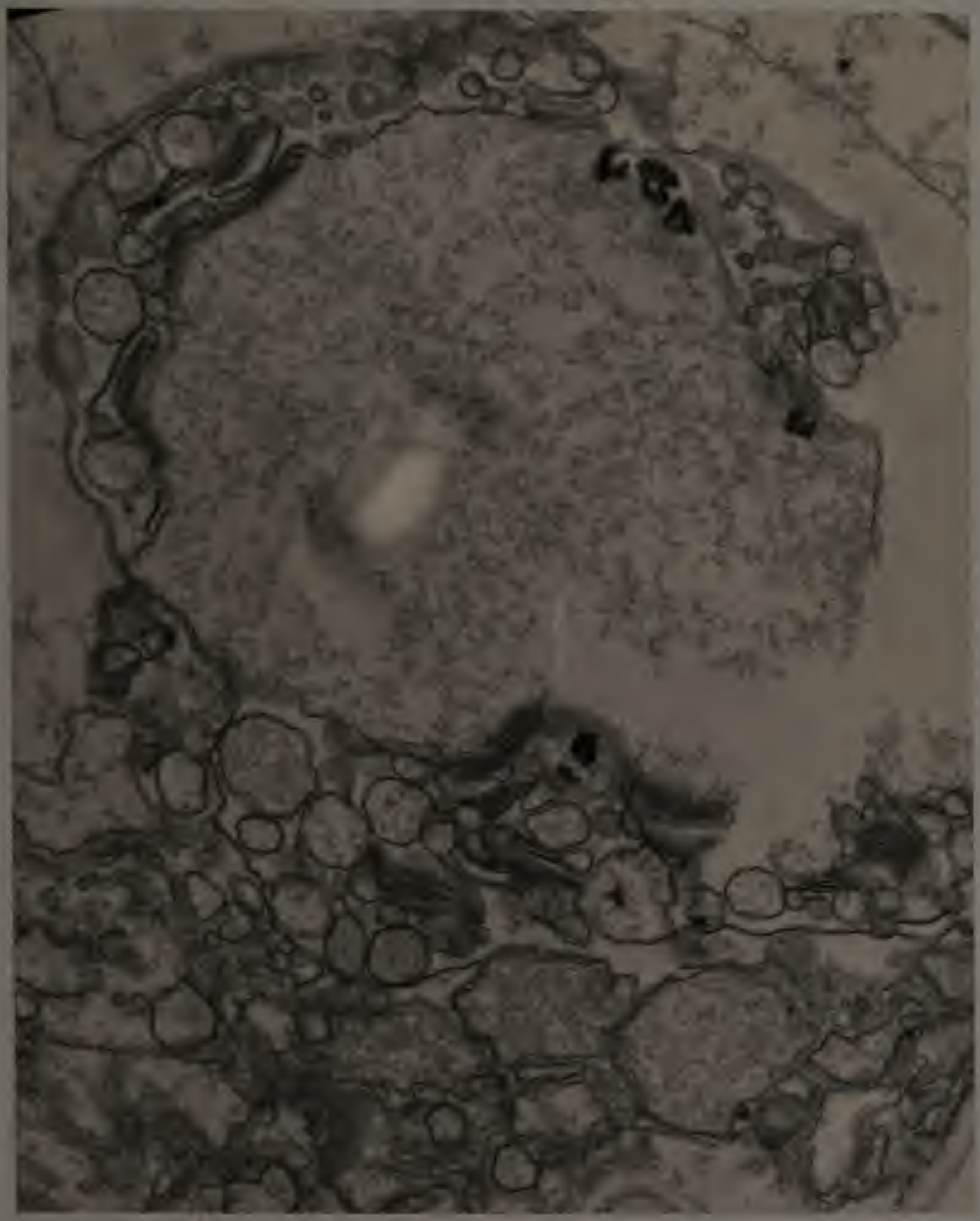


Figure 2

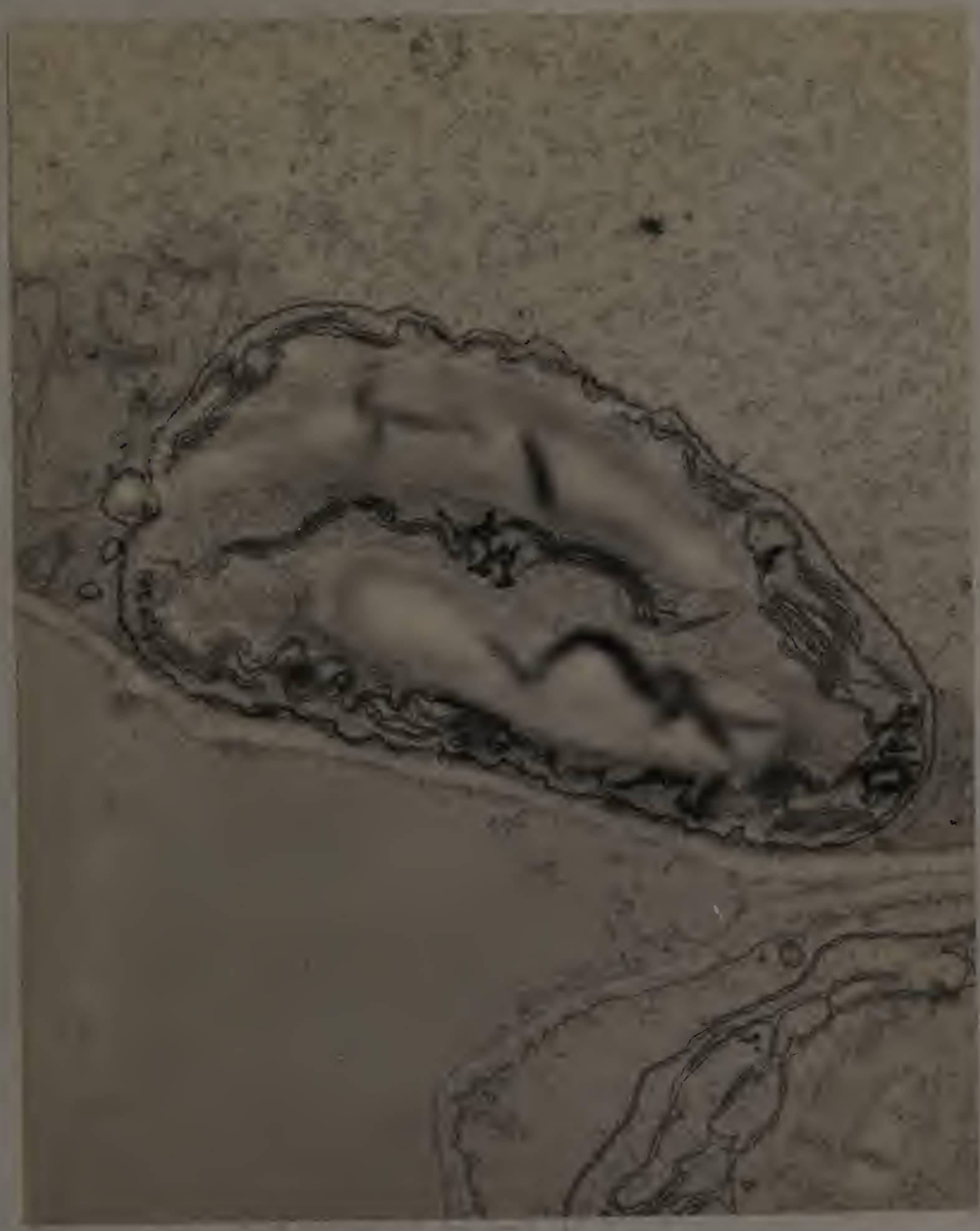
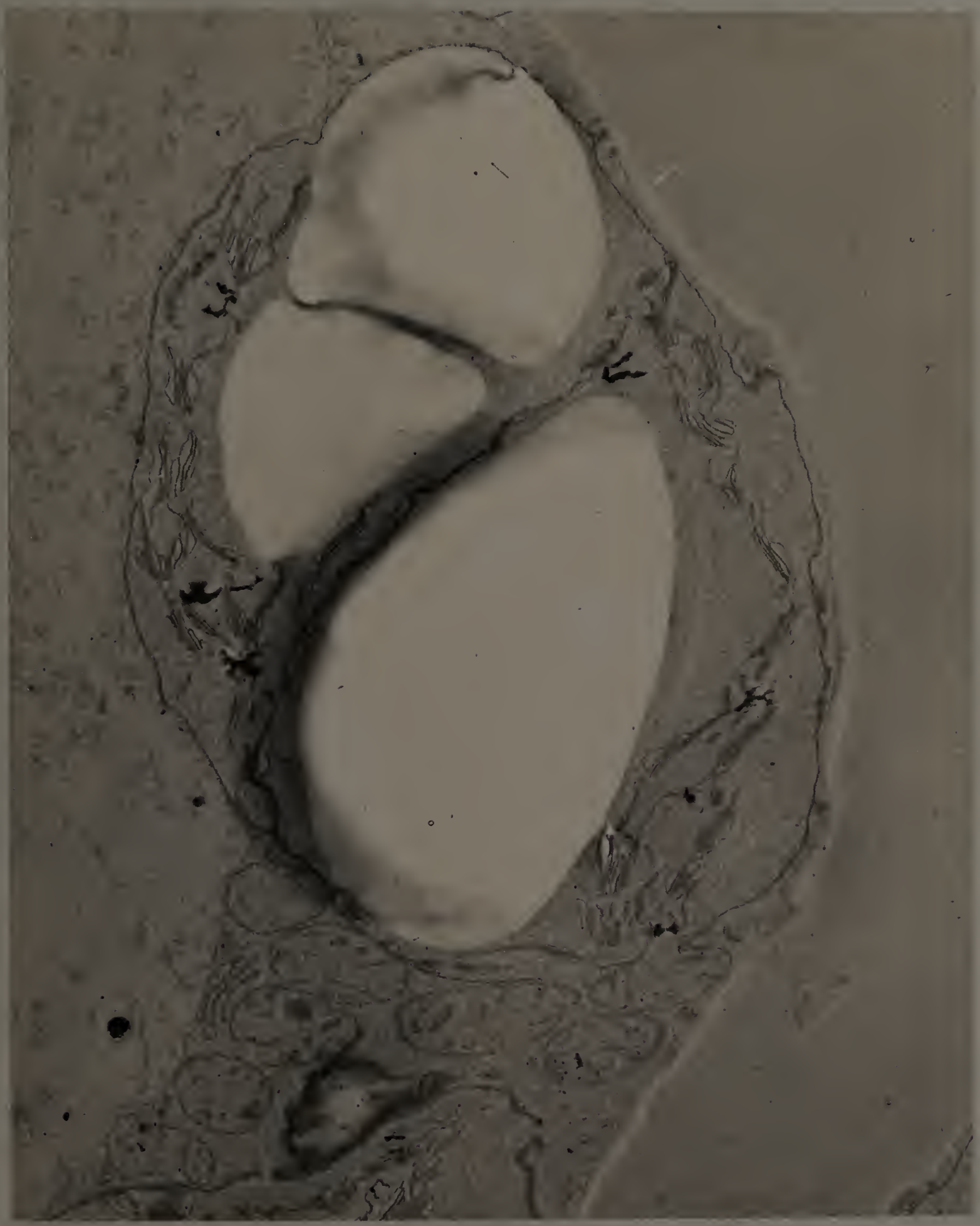


Figure 3a



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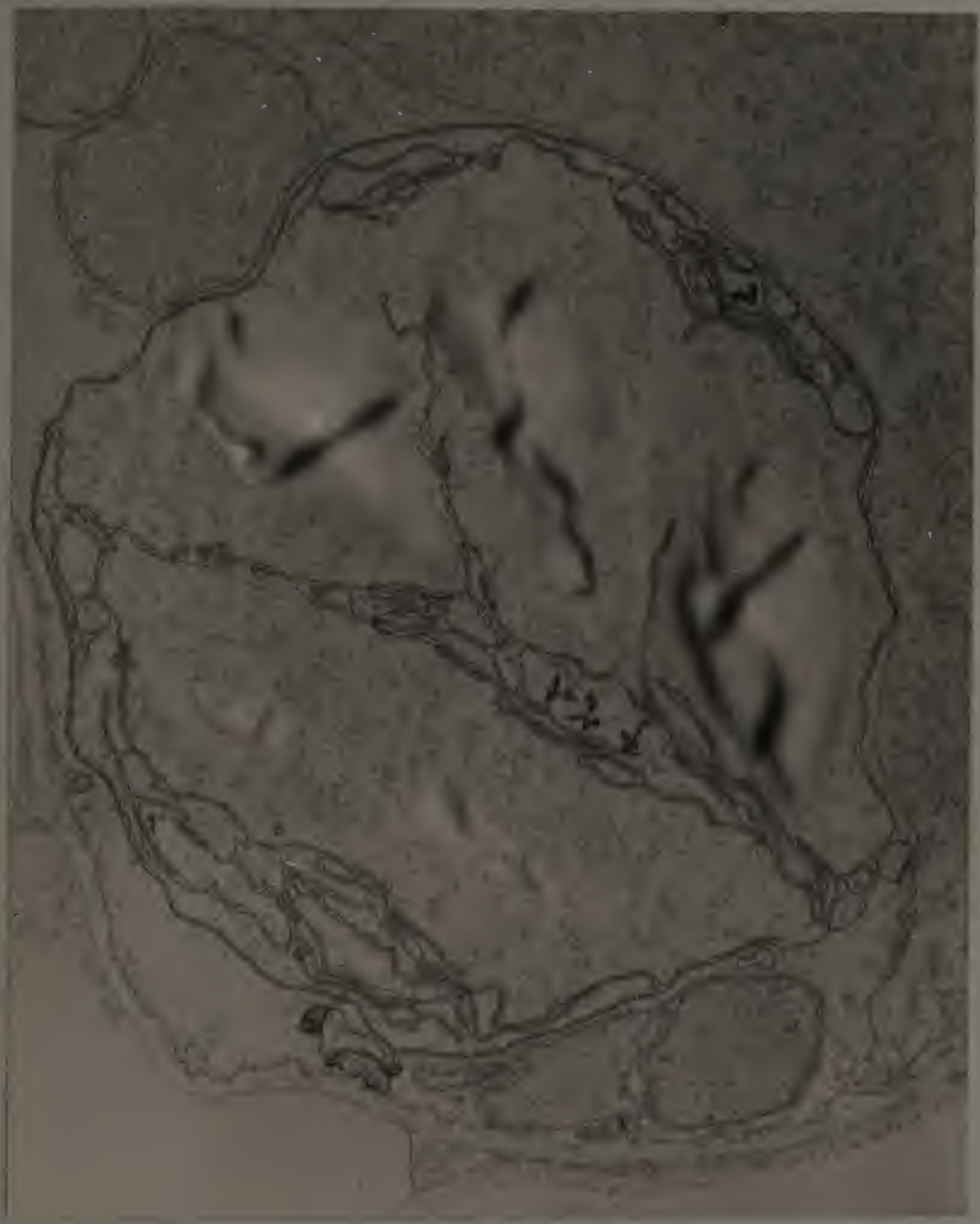

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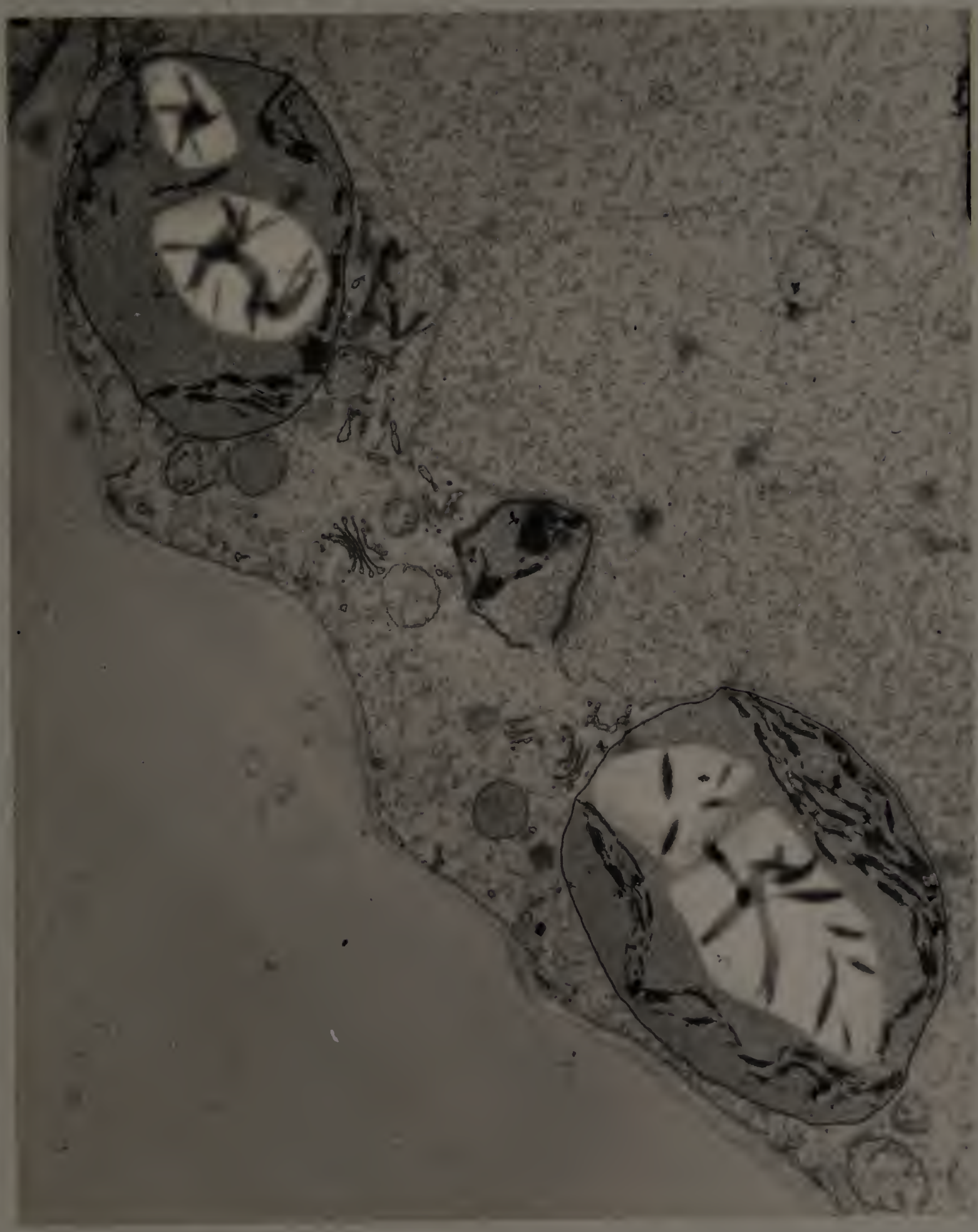

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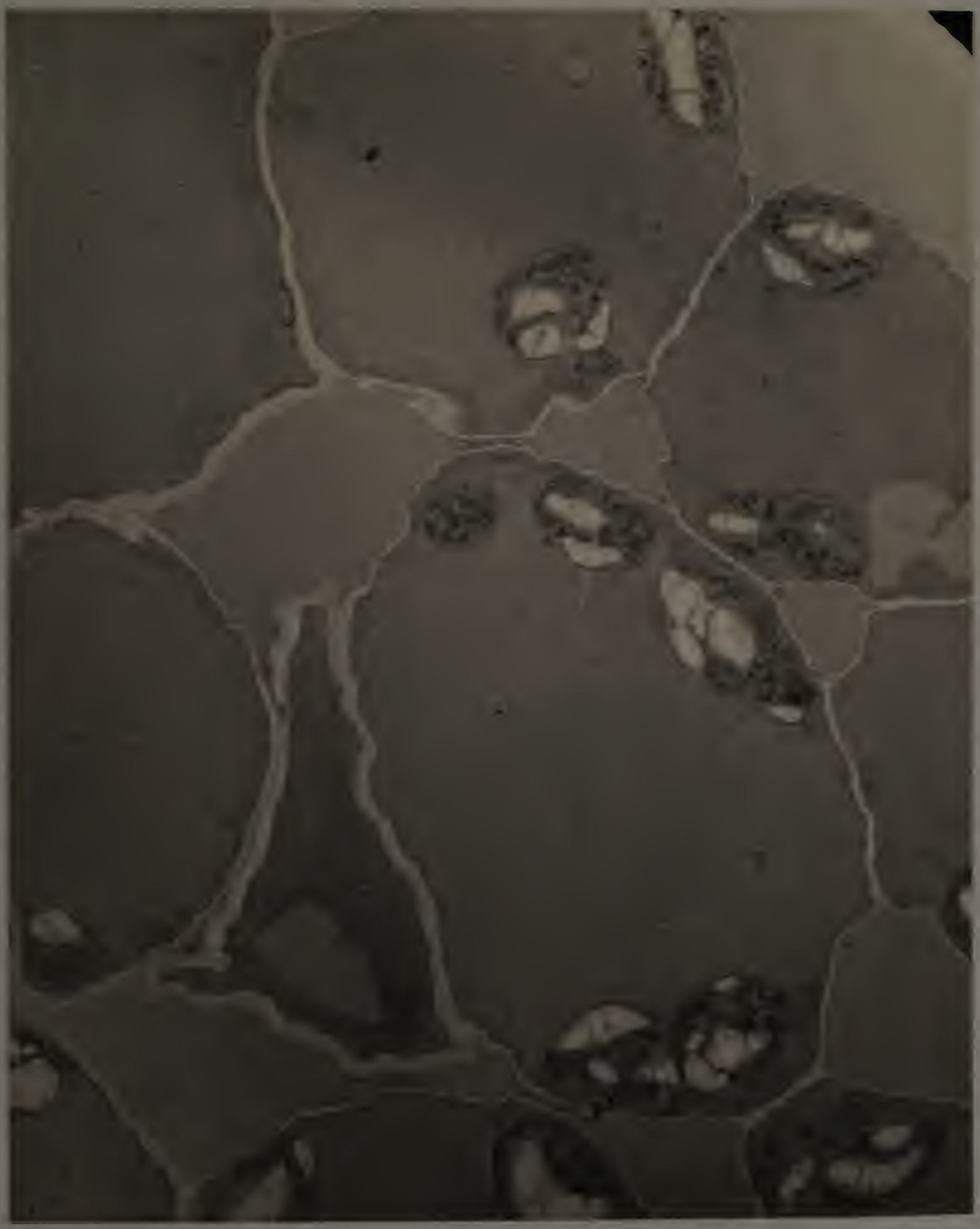
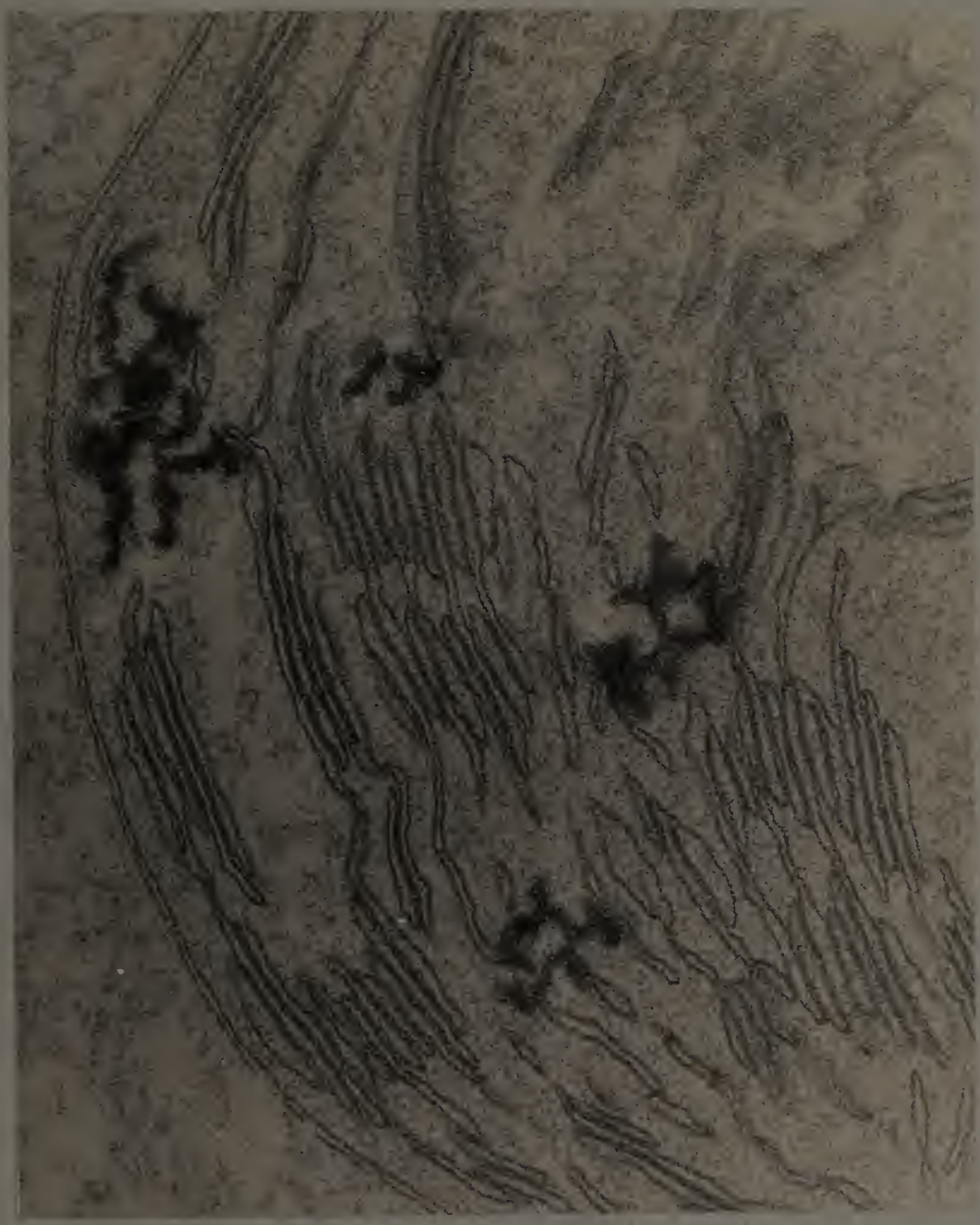


Figure 6

Figure 7

Figure 8



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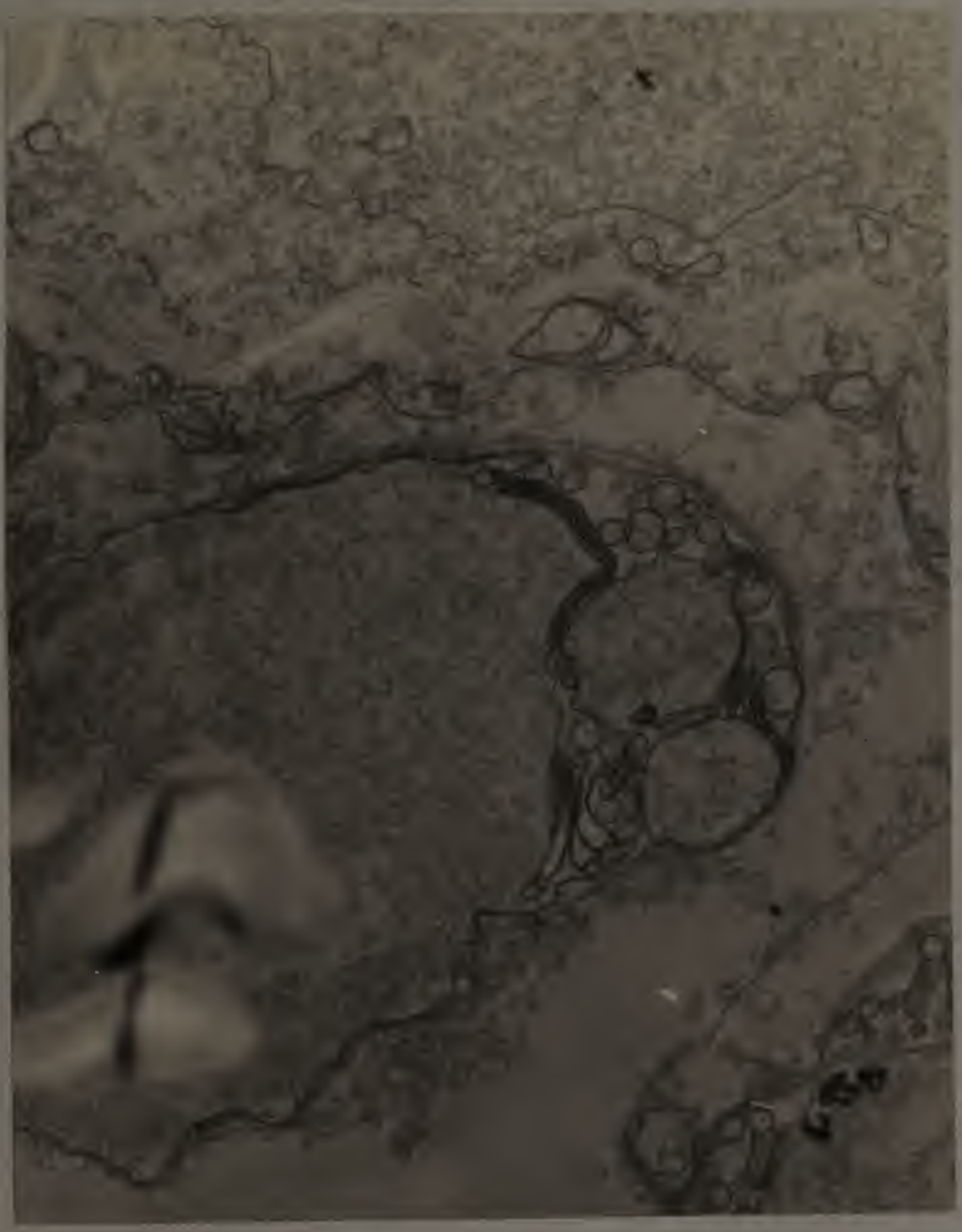

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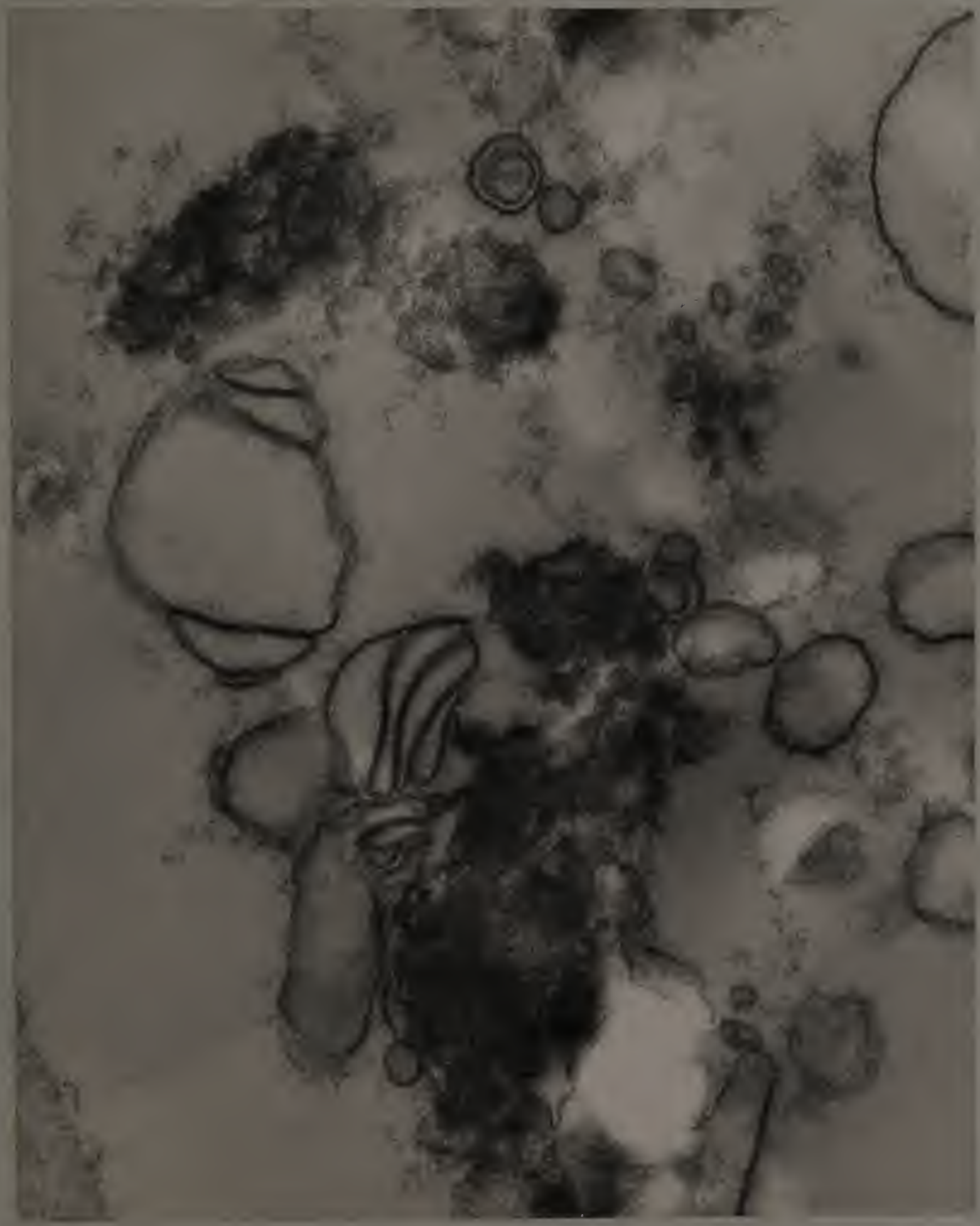

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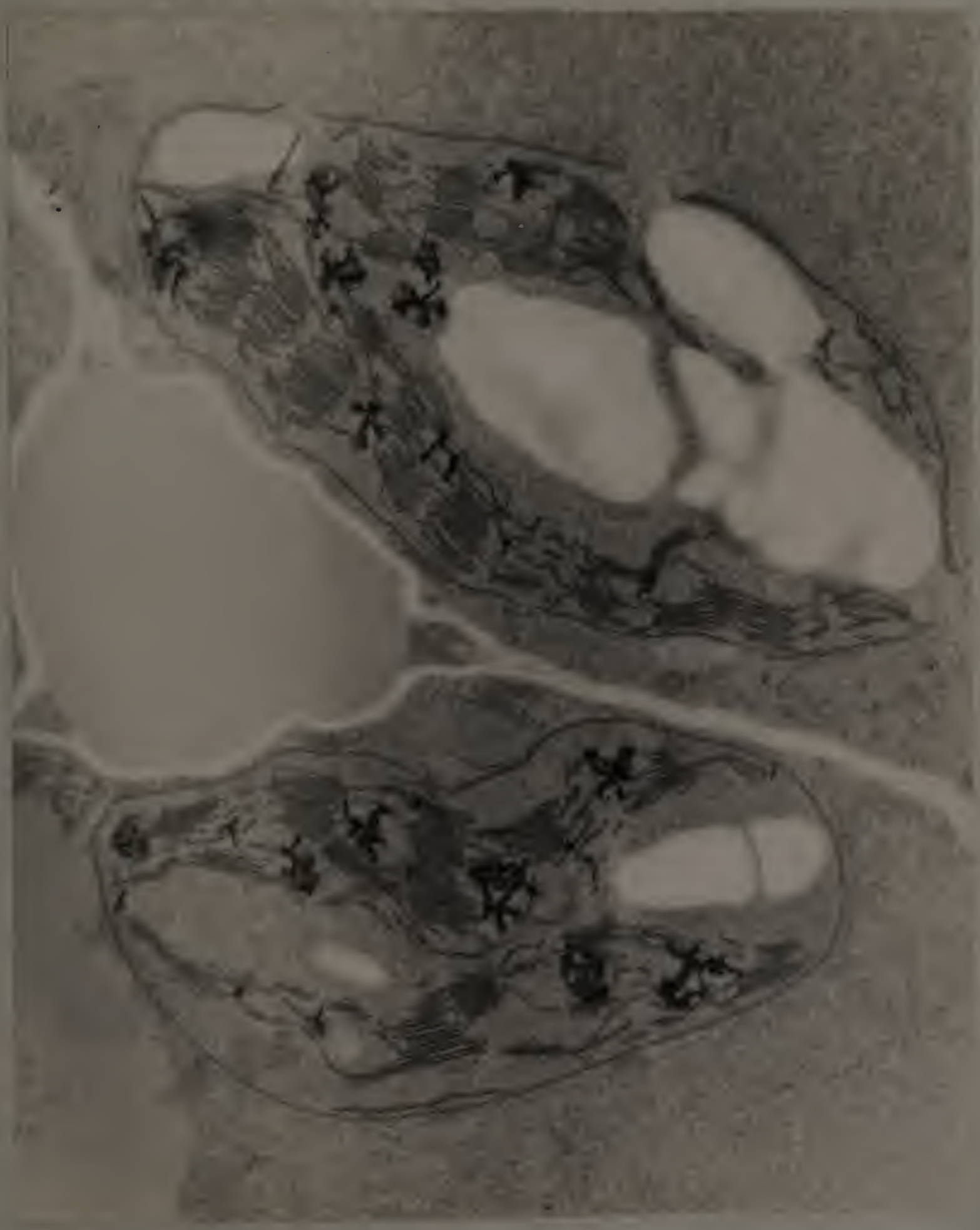

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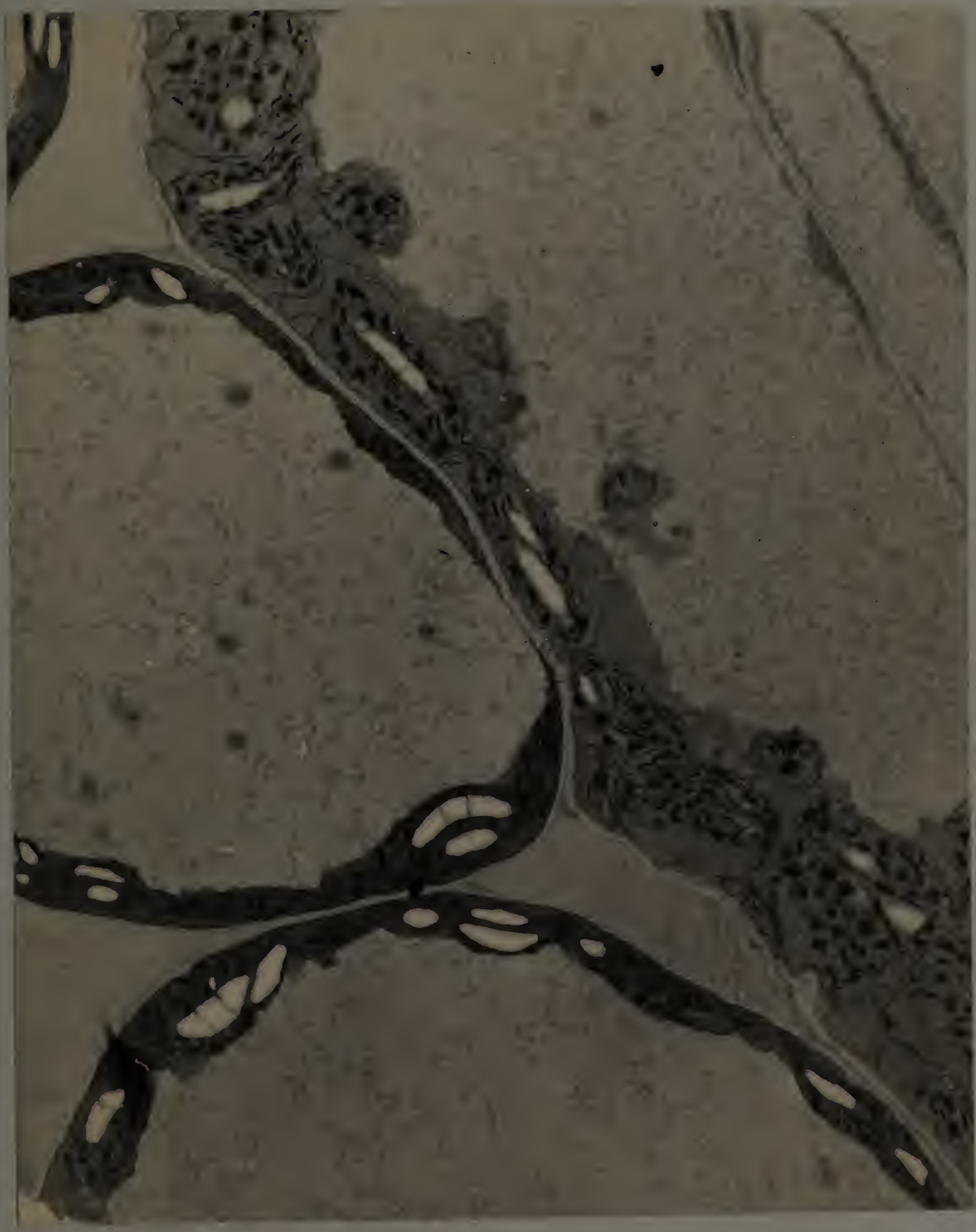

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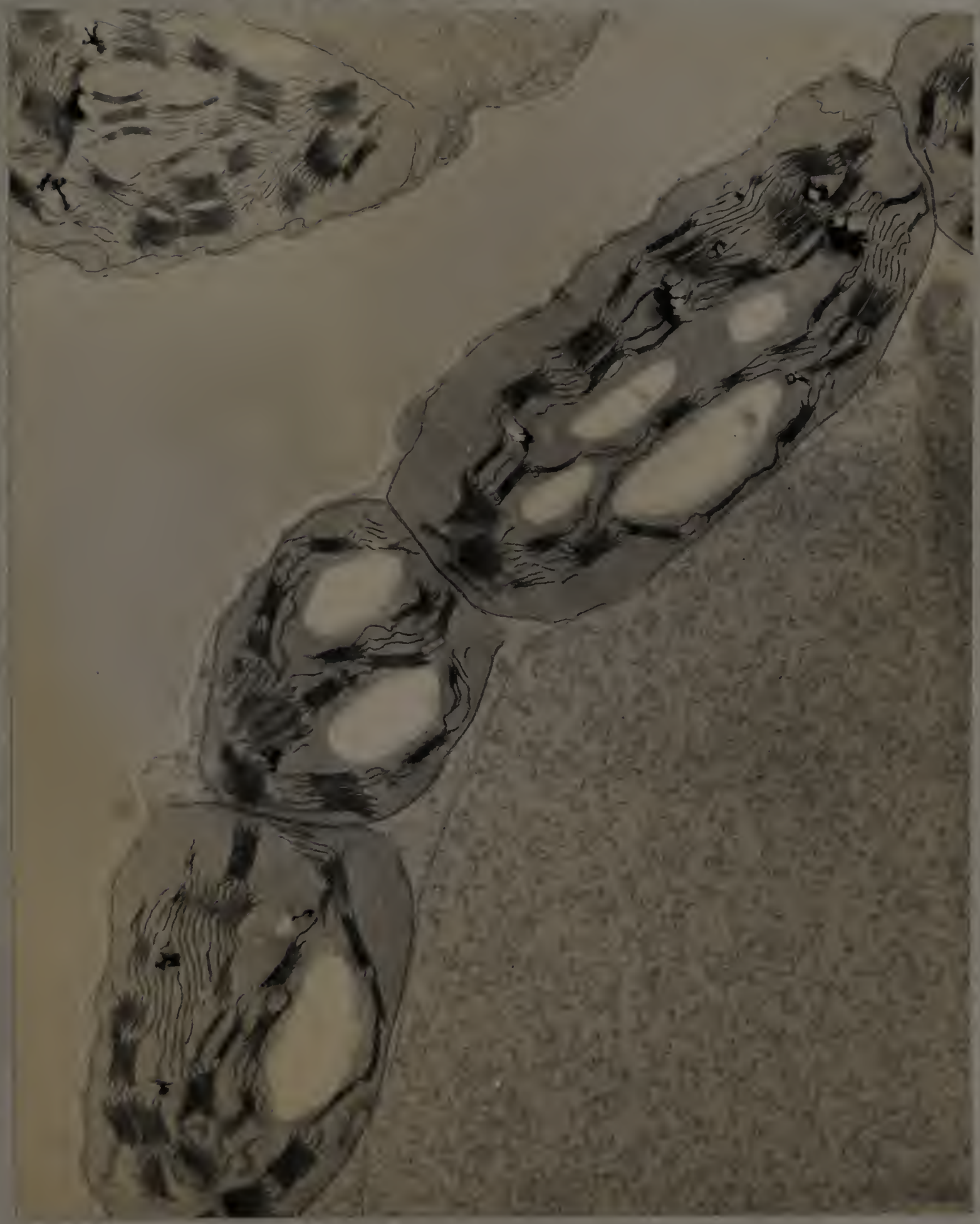

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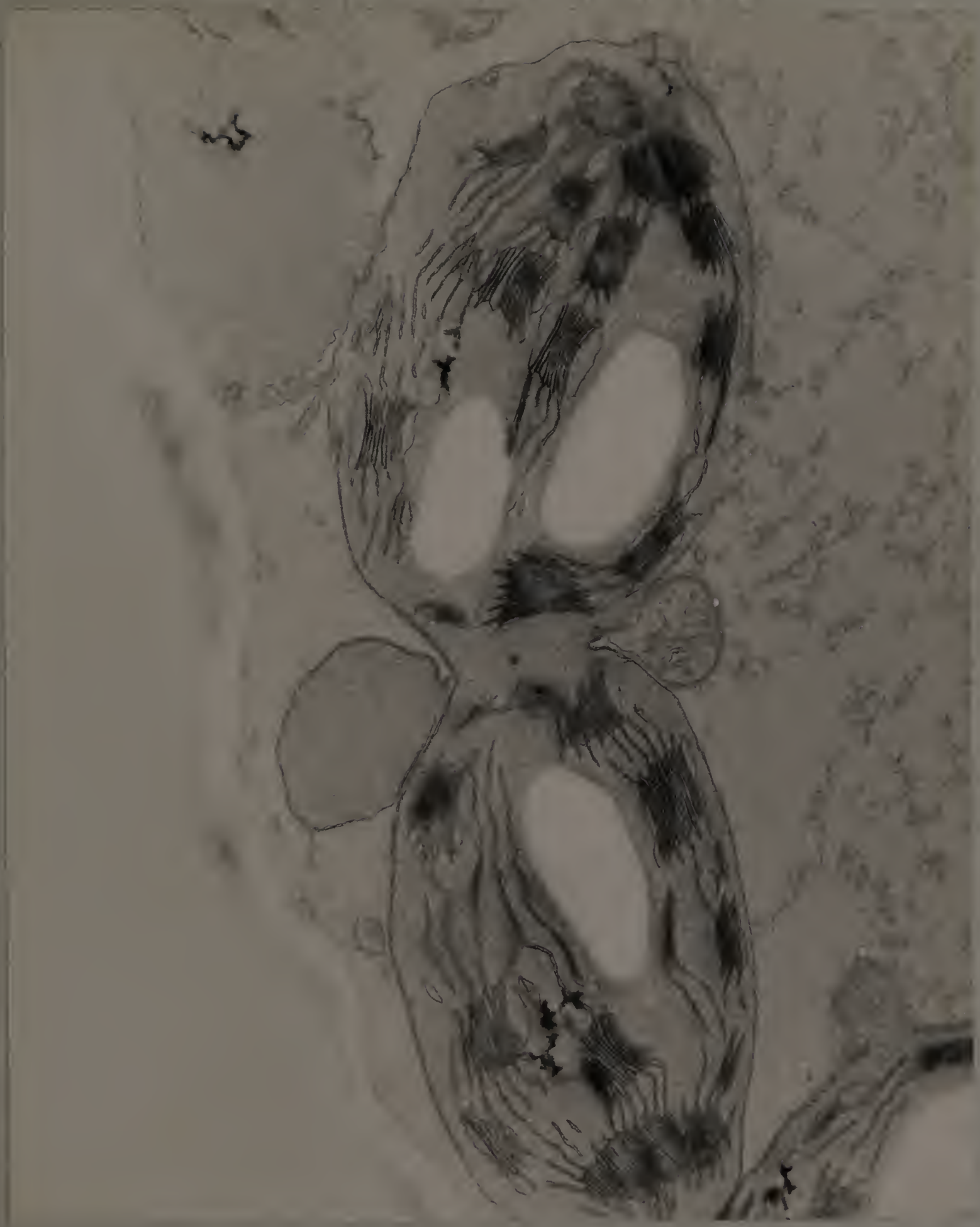

Figure 15

Figure 16

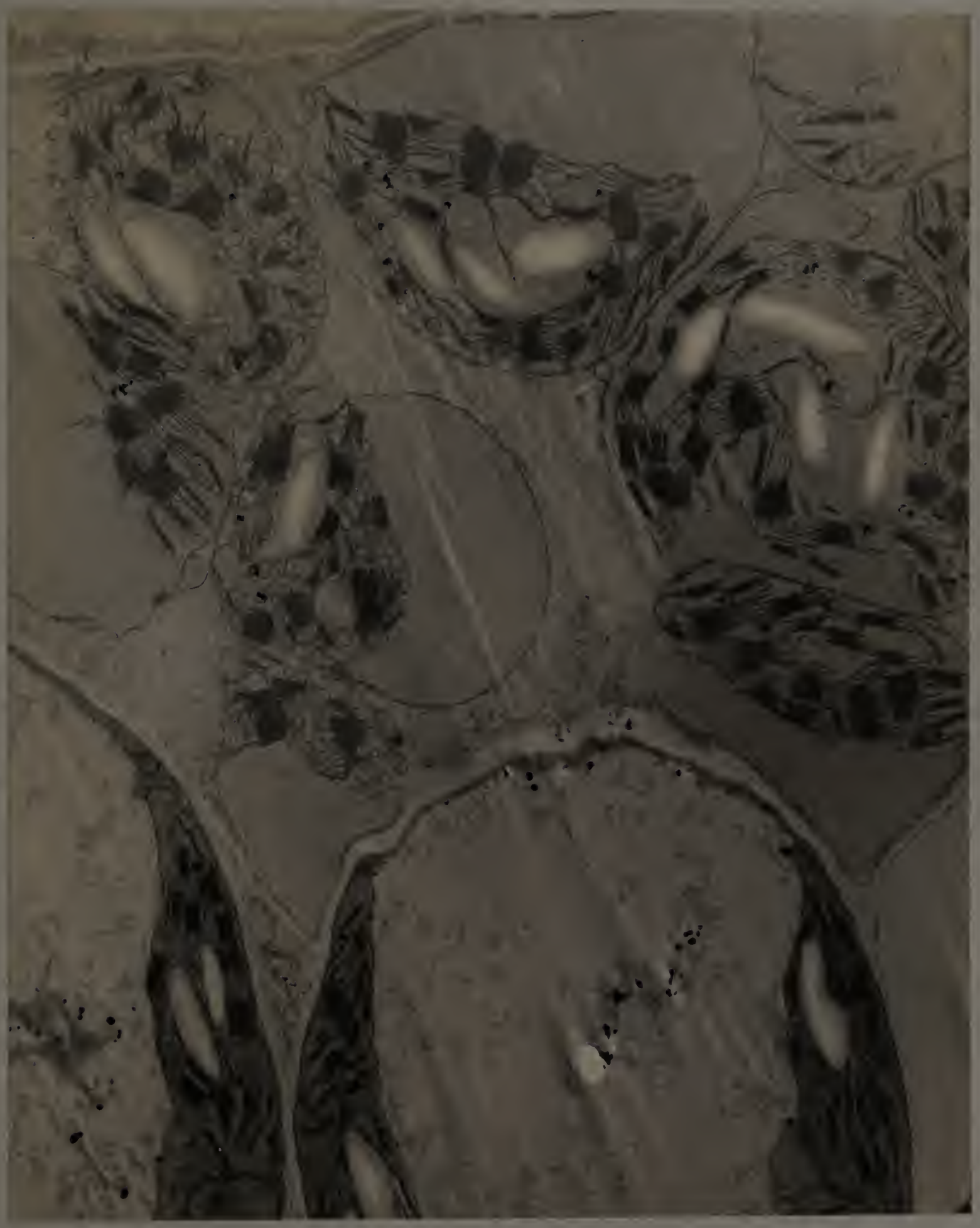

Figure 17

Figure 18

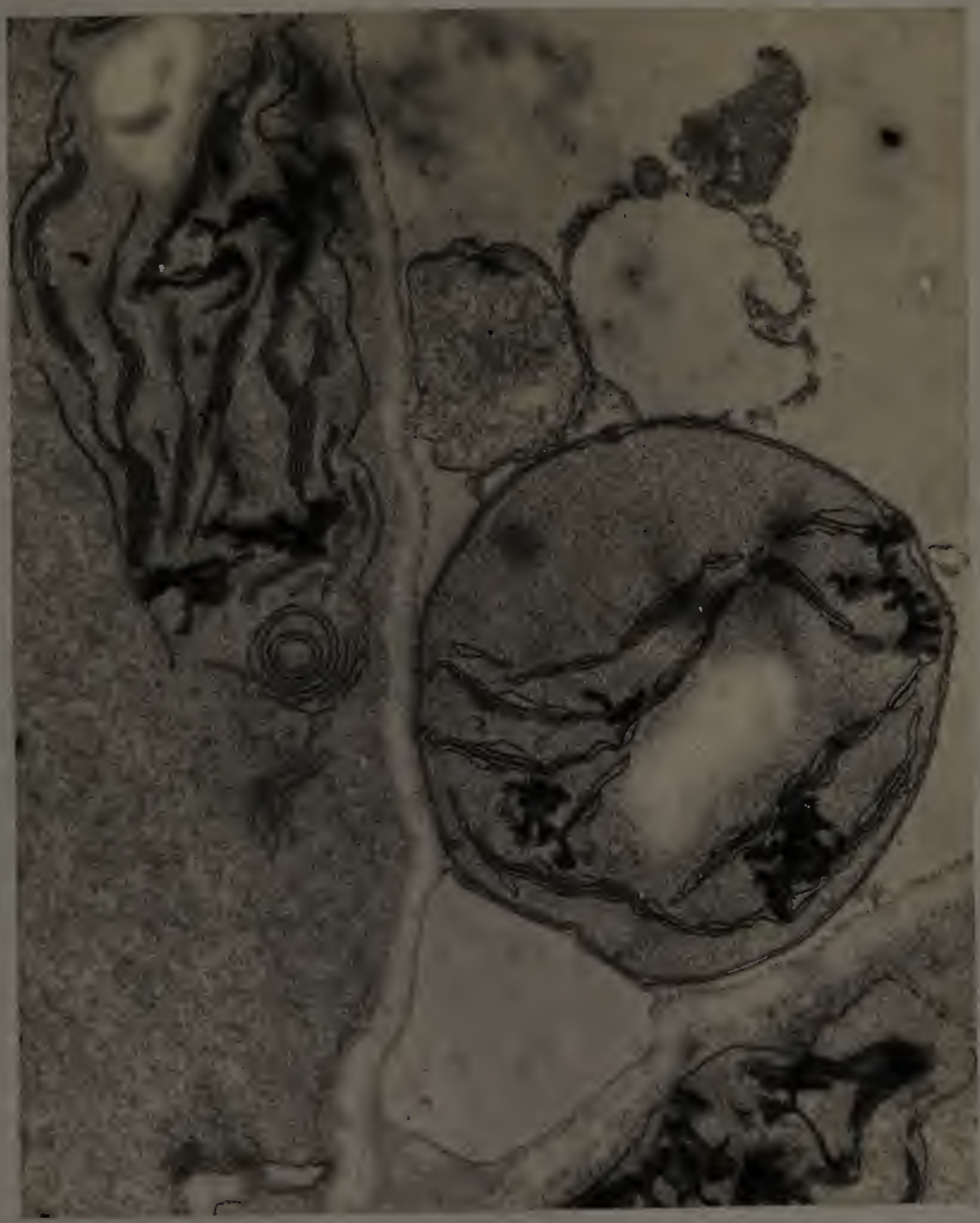

Figure 19

Table 1. The Effects of Ammonium and Nitrate Nutrition on the Chlorophyll Content of Tomato Leaves (mg chlorophyll/g fr wt)

(a) April series

Treatment	Days After Initiation of Ammonium Treatment													
	1	3	5	7	9	11	13	15	17	19	21	23	25	29
NH_4^+	1.96 cf	1.91 ef	2.44 f	2.75** fg	1.74* de	1.49** cd	1.52** cd	1.63** cde	1.31** bc	1.50** cd	1.54** cd	0.86** a	1.09** ab	0.95** a
NO_3^-	1.83 a	2.03 ab	2.52 cf	2.61 cf	2.17 bc	2.23 bcd	2.25 bcd	2.46 de	2.62 cf	2.40 cde	2.75 f	2.12 ab	2.06 ab	2.58 cf

(b) June Series

Treatment	Days After Initiation of Ammonium Treatment									
	1	4	6	7	9	11	13	15	17	19
NH_4^+	1.93 d	2.01* d	1.32** c	1.57** c	0.81** b	0.73** b	0.94** b	0.74** b	0.43** a	0.28** a
NO_3^-	1.66 a	2.52 b	2.75 b	2.59 b	2.66 b	2.90 b	2.91 b	2.59 b	2.76 b	2.63 b

(c) July series

Treatment	Days after Initiation of Ammonium Treatment				
	2	5	8	10	12
NH_4^+	2.37 b	2.51 b	2.51* b	1.47** a	1.40** a
NO_3^-	2.54 a	2.48 a	3.54 b	3.37 b	3.50 b

* Significant at 5% probability level
** Significant at 1% probability level

Within rows, means not followed by the same letter are significantly different at the 5% probability level.

Table 2. The Effects of Ammonium and Nitrate Nutrition on the Concentration of Free Ammonium in the Tomato leaves
(mg N/g fr wt)

(a) April Series	Days after Initiation of Ammonium Treatment					
Treatment	5	10	13	17	22	35
NH_4^+	0.08a	0.25*b	0.23b	0.36**bc	0.31**bc	0.40*c
NO_3^-	0.05ab	0.06ab	0.07bc	0.03a	0.10c	0.07c

(b) June Series	Days after Initiation of Ammonium Treatment									
Treatment	1	4	6	7	9	11	13	15	17	19
NH_4^+	0.09a	0.13ab	0.21abcd	0.20abcd	0.33*d	0.17*abc	0.33*d	0.23*bcd	0.26bcd	0.27cd
NO_3^-	0.08d	0.05bc	0.03a	0.06c	0.04ab	0.06c	0.06c	0.03a	0.05bc	0.03a

* Significant at 5% probability level
** Significant at 1% probability level

Within rows, means not followed by the same letter are significantly different at the 5% probability level.

Table 3. The Effects of Ammonium and Nitrate Nutrition on the Concentration of Amides in the Tomato Leaves
(mg N/g fr wt)

(a) April Series

Treatment	Days after Initiation of Ammonium Treatment					
	5	10	13	17	22	35
NH_4^+	0.06a	0.10b	0.11b	0.14**cd	0.15*d	0.12*bc
NO_3^-	0.09a	0.06a	0.07a	0.05a	0.06a	0.06a

(b) June Series

Treatment	Days after Initiation of Ammonium Treatment									
	1	4	6	7	9	11	13	15	17	19
NH_4^+	0.11a	0.20abc	0.19abc	0.15ab	0.24bc	0.19abc	0.16*ab	0.28*c	0.24 bc	0.18**abc
NO_3^-	0.08b	0.12c	0.07b	0.08b	0.07b	0.07b	0.08b	0.06a	0.06a	0.09b

* Significant at 5% probability level
** Significant at 1% probability level

Within rows, means not followed by the same letter are significantly different at the 5% probability level.

Table 4. The Effects of Ammonium and Nitrate Nutrition on the Oxygen Evolving Capacity of Tomato Leaves ($\mu l\ O_2$/10 min/g fr wt)

(a) July Series

Treatment	Days after Initiation of Ammonium Treatment				
	2	5	8	10	12
NH_4^+	106a	112a	134a	75*a	109*a
NO_3^-	141a	132a	209ab	259b	263b

(b) June Series

Treatment	Days after Initiation of Ammonium Treatment			
	13	15	17	19
NH_4^+	86**b	63**b	-3**a	15**a
NO_3^-	163ab	189b	173ab	149a

* Significant at 5% probability level
** Significant at 1% probability level

Within rows, means not followed by the same letter are significantly different at the 5% probability level.

Table 5. The Effects of Ammonium and Nitrate Nutrition on the Oxygen Evolving Capacity of Tomato Leaves ($\mu l\ O_2$/10 min/mg chlorophyll)

(a) July Series

Treatment	Days after Initiation of Ammonium Treatment				
	2	5	8	10	12
NH_4^+	45	45	53	51	78
NO_3^-	56	53	82	77	75

(b) June Series

Treatment	Days after Initiation of Ammonium Treatment			
	13	15	17	19
NH_4^+	92	85	-19	54
NO_3^-	56	73	63	57

Table 6. The Effects of Ammonium Nutrition on the Oxygen Consumption Capacity of Tomato Leaves ($\mu l\ O_2$/10 min/g fr wt)

(a) July

Treatment	Days after Initiation of Ammonium Treatment				
	2	5	8	10	12
NH_4^+	70a	64a	123*c	103bc	83ab
NO_3^-	73a	70a	97b	99b	92b

(b) June

Treatment	Days after Initiation of Ammonium Treatment			
	13	15	17	19
NH_4^+	59*a	62*a	70a	55*a
NO_3^-	106b	88a	89a	100b

* Significant at 5% probability level

Within rows, means not followed by the same letter are significantly different at the 5% probability level.

Table 7. The Effects of Ammonium Nutrition on the Carbon Dioxide Fixation Capacity of Tomato Leaves (cpm/g fr wt)

June Series

Treatment	Days after Initiation of Ammonium Treatment			
	13	15	17	19
NH_4^+	6,009**c	3,246**b	1,242**a	1,743**ab
NO_3^-	9,584c	9,517c	5,348a	7,411b

** Significant at 1% probability level

Within rows, means not followed by the same letter are significantly different at the 5% probability level.

Table 8. The Effects of Ammonium Nutrition on the Carbon Dioxide Fixation Capacity of Tomato leaves (cpm/g chlorophyll)

June Series

Treatment	Days after Initiation of Ammonium Treatment			
	13	15	17	19
NH_4^+	6,392	4,387	2,888	6,225
NO_3^-	3,293	3,675	1,938	2,813

DISCUSSION

From the results obtained, it is evident that the ammonium nutrition altered the physiological mechanisms of the tomato leaf, impaired its metabolic functioning and disrupted its morphological organization. The sequential study of the effects of ammonium nutrition undertaken allows for an evaluation between the physiological disorders and the morphological alterations and permits a correlation of both of these factors with the functional capacity of the leaf.

One of the characteristic signs of ammonium toxicity is the yellowing of the leaves (9, 16, 25, 55). Although this chlorosis has been noted by many workers, no attempts have been made for a quantitative determination. The results of the chlorophyll determinations in this investigation indicate that the chlorophyll content of the leaves was reduced very early after the initiation of ammonium treatment and continued to decline with increasing days on ammonium nutrition. The rapid chlorophyll loss may be explained in terms of the effect of ammonium toxicity on the biosynthesis of chlorophyll.

The substrates for chlorophyll biosynthesis consist of glycine and an intermediate of the citric acid cycle,

succinyl coenzyme A (62). These two compounds react in the presence of the enzyme aminolevulinic acid synthetase, (ALA synthetase), and pyridoxal phosphate to form δ-aminolevulinic acid, the initial precursor in chlorophyll biosynthesis (10, 14). Bogorad (10) reported that ALA synthetase serves as the primary control factor in the synthesis of the chlorophylls. He further stated that regulation of chlorophyll synthesis could be accomplished by controlling the concentration of this enzyme or the availability of succinyl CoA and glycine. Recently work with inhibitors of protein synthesis have indicated that ALA synthetase undergoes a rapid turnover rate (26). In view of these facts, ammonium nutrition could effect a chlorophyll loss in two possible ways. First, the increased requirement for carboxylic acids of the citric acid cycle, caused by the ammonium-stimulated amino acid and amide synthesis (64), may result in a reduction in the amount of available succinyl CoA. Secondly, reduction of net protein synthesis that may result from ammonium nutrition (8), coupled with the rapid turnover rate of ALA synthetase, could result in a decreased concentration of this enzyme. As the chlorophyll content of the leaves undergoes a continuous diurnal rhythm of synthesis and degradation (63), reduction in the rate of synthesis of chlorophyll would quickly be expressed as yellowing of the leaves, similar to that observed in this experiment. Further duration of ammonium treatment would magnify this inhibition and result in even greater losses in chlorophyll content.

Although the leaves showed a significant loss in chlorophyll under ammonium treatment (Table 1c), they did not show a simultaneous decrease in their oxygen evolving capacity (Table 4a). It would, therefore, appear that the photosynthetic capacity of the leaves was not directly dependent upon chlorophyll concentration. This type of relationship was first determined in 1918 by Willstättler and Stoll (cf 40) when they established that the assimilation number (g CO_2 consumed per hour per g of chlorophyll) of etiolated bean plants was more than ten times higher than that of normal green plants. Rabinowitch (40), in his review on the influences of chlorophyll content on photosynthesis concluded that chlorophyll was not the rate-limiting component of the photosynthetic mechanism. It would appear, therefore, that such is the case for the ammonium-induced chlorosis in the tomato leaf in that initial chlorophyll loss did not affect the photosynthetic rate.

The initial loss of chlorophyll in the treated leaves also appeared to exert little effect on the chloroplastic ultrastructure although, as will be explained later, it may have done so after further loss. In any case, a severe disruption of the morphological placement and internal organization of the chloroplasts occurred under ammonium treatment. The chloroplasts from the treated plants typically showed a lack of orientation in relation to the cell and appeared to be floating (Fig. 6). In contrast, the chloroplasts of the control cells were closely appressed to

the cell wall and tightly abutted to each other (Fig. 1c). The ammonium-toxic chloroplasts were often round in cross section and frequently had small vesicle-like protrusions extending from the plastid membrane (Fig. 3a and 3b). Possingham et al. (39) reported similar protrusions on the chloroplasts of manganese-deficient spinach. These workers attributed the appearance of the protrusions to the reduction of the intergranal lamellar system which helps maintain the structural integrity of the plastid. It is quite possible that the protrusions observed here could be explained along similar lines as the intergranal lamellae or frets are markedly disrupted and reduced as early as 10 days after treatment (Figs. 16, 17, 18, 19). The granal component of the chloroplast was likewise modified by the ammonium nutrition. The number of compartments per granum was greatly reduced in the treated plastids, and in certain cases the grana were entirely absent (Fig. 4). In more extreme instances, actual breakdown of the chloroplast was observable with vesiculation of both granal and fret components and disruption of the plastid membrane (Fig. 2). This type of breakdown could perhaps result in a large increase in soluble nitrogen compounds such as was observed by Barker (6) in ammonium-toxic pole beans.

It would appear that the observable alterations in chloroplastic ultrastructure in the leaves of ammonium-treated plants could be accomplished by three different routes. First, the ammonium ion increase which occurs

under ammonium treatment may reach concentrations high enough to uncouple photophosphorylation (27) and thus lead to direct structural modifications within the chloroplast. Secondly, the constant loss of chlorophyll may disrupt the structural integrity of the chloroplastic membranes, causing internal disorganization. Finally the decreased protein synthesis coupled with the increased protein breakdown that occurs under ammonium toxicity (8) may seriously affect the functioning of certain chloroplastic enzymes thereby causing a breakdown of both chloroplast structure and function.

The first of these possibilities is rather interesting in that the alteration in chloroplastic ultrastructure correlates with both the ammonium content and photosynthetic capacity of the leaves. The first significant decrease in photosynthesis (Table 4a) occurred at the same time on ammonium treatment as the increase in free ammonium (Table 2) and the change in plastid structure (Figs. 16, 17, 18, 19). As the ammonium ion acts as an efficient uncoupler of photophosphorylation and inhibitor of the reduction of nicotinamide adeninedinucleotide (NADP), it would restrict both the oxygen evolving and carbon dioxide fixation capacity of the leaves. The uncoupling of electron transport in photosynthesis, in turn, may also have resulted in the disruption of the substructural organization of the plastid.

This latter relationship, i.e., between electron transport and chloroplast configuration, has recently been the subject of intensive investigation (21, 34, 35). It has

been determined that the electron transfer reactions in both cyclic and non-cyclic photophosphorylation are responsible for mechanochemical changes in the chloroplastic membranes (35). Measurements of the scattering of actinic red light by isolated, intact chloroplasts have shown that conditions of electron transport, such as induced by light or addition of ATP, cause a shrinking of the plastid (34). If the phosphorylation sequence is interrupted with an uncoupler, e.g. ammonium, or with removal of the light source, the chloroplast undergoes a swelling. These volume changes fluctuate as much as 50% to 80% (35). Extension of the physiological investigations to ultrastructural analysis revealed that the volume changes were the result of distinct alterations in plastid morphology. Under conditions of rapid electron transport and energy production, the plastids appeared as long spindles or crescents (21). When photophosphorylation ceased, the plastids underwent an axial ratio change from 2.34 to 1.96 and appeared ellipsoidal in shape. A more detailed examination of this relationship between electron transport and structural modification of the chloroplasts showed that not only the shape of the chloroplast was altered by conditions of photophosphorylation but the internal structure as well. In this respect, it was established (23) that the uncoupling of the electron transport system led to an intensive swelling and disruption of the grana-fret network.

It appears that in some respects the structural modifications of the "in vitro" uncoupled chloroplasts resembled

those of the "in vivo" ammonium-toxic chloroplasts. This is particularly evident in respect to the ellipsoidal chloroplasts and the swollen grana (Fig. 6). As a result, it seems possible to assume that both of these phenomenon may be caused by the same factor, i.e., uncoupling of photophosphorylation. This possibility, however, has one serious drawback and that is the observation that ammonium-treated plants were more efficient than the controls in the fixation of carbon dioxide when expressed in milligrams of chlorophyll (Table 8). As a result, it would appear that some other factors were more important than uncoupling in altering "in vivo" the structure and function of the plastids. If this is the case, then the act of uncoupling may be relatively unimportant in ammonium toxicity. On the other hand, there is the possibility that the treated plants received enough energy for carbon dioxide fixation from the respiratory cycle. In this way, uncoupling could have occurred but been undetected by the carbon dioxide fixation determinations. The uncoupling, in turn, would have accounted in part for the great reduction in oxygen evolution that resulted in the treated plants (Tables 4a and 4b). In fact, there is evidence in the micrographs for a preponderance of apparently normal mitochondria (Fig. 8). However, until further information is available on the interchange of energy between the two main phosphorylation mechanisms of the plant, photosynthesis and respiration, the actual role of uncoupling in ammonium toxicity cannot clearly be established.

The second possible mechanism of ultrastructural breakdown of the chloroplast under ammonium nutrition is the disruption of membrane structure through constant loss of chlorophyll. The chlorophyll molecules are a component part of both the grana and the fretwork of the chloroplast. The molecules are situated within the lipid component of the lipoprotein layers of the membranes (36). In view of the fact that the chlorophyll molecules are intimately linked with the membranous component of the grana-fret network, it would seem logical that under conditions of constant chlorophyll loss, the chloroplastic ultrastructure would be disrupted. Unfortunately, the actual effect of chlorophyll content on the fine structure of the chloroplast could not be clearly determined from the data obtained in this investigation.

The third and favored cause of the structural modifications of ammonium-toxic chloroplasts is the upset in the protein metabolism of the plant. Work on pole beans has established that ammonium nutrition causes a reduction in the synthesis of insoluble nitrogen compounds in the leafy parts of the plant (8). Use of the stable isotope N^{15} also revealed that the ammonium treatment causes a large increase in the amino acids at the expense of an endogenous source (8). Hence, it appeared that ammonium treatment of these plants not only prevented normal protein synthesis but enhanced its breakdown. As the chloroplasts contain as much as 70% of the leaf protein (65) and as chloroplastic protein

undergoes a very rapid turnover rate, approximately 0.9% per hour (41), it would appear that the altered protein metabolism would exert its main effect on the chloroplast. Since the chloroplast contains all the enzymes for carbon dioxide fixation and chlorophyll biosynthesis as well as the components for photophosphorylation and energy production, it is evident that disrupting the chloroplast would lead to widespread physiological and morphological disorders. It has already been proposed in this discussion that the loss of chlorophyll in the ammonium-treated plants could be attributed to an altered protein metabolism. It is likewise possible to make the same proposal in terms of chloroplastic ultrastructure and photosynthesis. For instance, the high turnover rate of chloroplastic proteins described above coupled with decreased protein synthesis would easily manifest an alteration in structure and function on the leaf chloroplasts of treated plants. Continued degradation without synthesis would, in turn, lead to intensive structural breakdown of the plastids and complete cessation of their physiological processes.

It is quite likely that respiration is similarly affected by the altered protein metabolism. As ammonium nutrition is initiated, an enhanced accumulation of the α-amino acids (16) and amides (Table 3) occurs. These nitrogen compounds are synthesized from components of the citric acid cycle, especially α-ketoglutaric and oxaloacetic acids. This increased requirement for organic substrates

could explain the enhanced respiratory rate of the treated plants after 8 days of ammonium nutrition (Table 6a). Later as ammonium treatment continues, the altered protein metabolism described above may affect the enzymes of the respiratory cycle resulting in a decreased respiration rate (Table 6b).

SUMMARY

Experiments were conducted to determine the effect of ammonium nutrition on the structure and function of the tomato leaf chloroplasts. The following results were obtained:

1.) Within 4 to 8 days after initiation of ammonium treatment, a significant loss of chlorophyll occurred in the leaves of the treated plants. The loss of chlorophyll increased as the treatment continued.

2.) Free ammonium and amide contents increased in the plants under ammonium nutrition. The amount of free ammonium increased substantially over the controls after 9 days of treatment, while the amide concentration reached significant values after 13 to 17 days.

3.) On a fresh weight basis, the leaves from plants with greater than 10 days of ammonium nutrition were significantly reduced in photosynthetic capacity in terms of both rate of oxygen evolution and amount of carbon dioxide fixation. When these two parameters of photosynthesis were expressed in relation to chlorophyll content, the treated leaves were equivalent to the controls in the rate of oxygen evolution and greater than the controls in the amount of carbon dioxide fixation.

4.) The respiration rate of the treated leaves increased significantly above the rate of the control leaves on the 8th day of ammonium treatment. The rate of oxygen uptake in the treated plants subsequently decreased and became significantly less than the controls 13 days after the initiation of the ammonium treatment.

5.) Chloroplastic fine structure and orientation within the cells were disrupted in plants that were under ammonium nutrition. The first apparent signs of the alteration in plastid morphology occurred around the 10th day of ammonium treatment.

6.) It is proposed that the disruption of the chloroplastic fine structure was a direct consequence of the altered physiological processes effected by the ammonium nutrition. Possible mechanisms by which the breakdown of chloroplastic ultrastructure were accomplished are discussed.

Conclusions.

The initial loss in chlorophyll did not reduce the photosynthetic efficiency of the leaves, thus confirming the findings of other workers (40) that efficiency of photosynthesis is not strictly dependent upon the concentration of chlorophyll. Structural alteration in the chloroplasts of ammonium-treated plants coincided with an increase in the free ammonium concentration and loss in the photosynthetic capacity of the treated leaves.

Three mechanisms were presented relating the alteration

in chloroplastic fine structure to the ammonium-induced physiological processes. First, it was suggested that the increase in free ammonium resulted in an uncoupling of photophosphorylation, which in turn caused a disruption of the plastid-fine structure and a subsequent reduction in its functional capacity. The actual importance of the uncoupling factor was questioned as the supposedly "uncoupled" chloroplasts fixed carbon dioxide with greater efficiency, per unit chlorophyll, than the control. Secondly, it was envisioned that the constant chlorophyll loss which resulted from the ammonium treatment may have destroyed the membranous substructural organization of the plastid. Finally, it has been suggested (8) that ammonium nutrition results in a reduction of protein synthesis and an increase in protein degradation. It was felt that as the majority of leaf protein is located in the chloroplast (65), and as the chloroplastic protein undergoes a high turnover rate (41), protein degradation without synthesis would result in a disruption of the photosynthetic organelles in terms of both structure and function. It was also proposed that this altered protein metabolism was the cause of the chlorophyll loss in the leaves from ammonium-treated plants.

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