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FINAL REPORT

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Technical Proposal No. BB32-79-217P  
Contract NAS9-12671

Submitted To

National Aeronautics and Space Administration  
Manned Spacecraft Center  
Houston, Texas 77058

Cytological Studies of Lunar  
Treated Tissue Cultures

Submitted By

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- Section II --- Letter explaining additional work carried out which was not provided for in the original contract
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Baur, P. S., C. H. Walkinshaw, R. S. Halliwell and V. E. Scholes, 1973, Morphology of Nicotiana tabacum cells grown in contact with lunar material. Canadian Journal of Botany 51:151-156.

The following summarizes the progress on contract NAS9-12671 for the period March 1 through May 31, 1972.

The following specimens were dehydrated, embedded in epoxy resin, stained, thin sectioned and mounted on grids for electron microscopy.

Specimens:

23-7	28-26
23-1	24-28
24-25	27-7
22-46	24-37
22-29	22-10
23-22	26-28
23-21	29-15
28-1	26-20
28-11	
28-31	

The mounted specimens were submitted to the NASA monitor for examination.

The following summarizes the progress on contract NAS9-12671 for the period June 1 through August 31, 1972.

The following specimens were dehydrated, embedded in epoxy resin, stained, thin sectioned and mounted on grids for electron microscopy.

Specimens:

24-10	15-23
23-35	16-14
23-28	15-20
25-5	14-14
25-1	16-11
29-6	14-19
23-14	16-7
26-20	13-25
21-25	13-13
14-11	15-7
13-7	12-33

The mounted specimens were submitted to the NASA monitor for examination.

This terminates the objectives outlined in the original proposal. However, by agreement (letter, July 31, 1972; copy appended) with the project monitor, the objective of this project will include the analysis and evaluation of the previously proposed specimens.

July 31, 1972

Mr. George Huff/BB32 (79)  
NASA Manned Spacecraft Center  
Facility and Laboratory Support Branch  
Houston, Texas 71058.

Dear Mr. Huff:

The work outlined in the objectives of NAS 9-12671 contract was finished more rapidly than anticipated. The technician doing this work has been terminated on this contract.

The project monitor, Dr. Charles Walkinshaw recommends the hiring of a qualified research associate, Dr. Paul Baur, who has the expertise to further evaluate the specimens prepared under this contract. Dr. Baur will be retained at his current salary (\$6.35/hr.) and his tenure shall be determined by existing contract funds (approximately 4 3/4 months).

This is not a request for additional support, but a re-programming of personnel and salary schedule to meet the expertise required by the proposed additional duties.

Thank you.

Sincerely,

Robert S. Halliwell  
Associate Professor

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APPROVED:

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Dr. David W. Rosberg  
Professor and Head  
Department of Plant Sciences

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Dr. H. O. Kunkel  
Dean and Acting Director  
Texas Agricultural Experiment Station

PINE TISSUE CULTURES USED DURING  
APOLLO 11 & 12 QUARANTINE STUDIES

Characterization of Control Tissues

Apollo 11 Pinus palustris (control)

1. Dumbbell organelles present.
2. Chloroplasts well developed - good grana organization and evenly staining stroma. No crystals seen in these organelles. Occasional small areas of fibrillar material found within some plastids.
3. Endoplasmic reticulum is prominent and is most frequently rough. Frequent plasmadesmata were observed. Very little localized hypertrophy of E.R.
4. Very little vesicular activity observed in the cytoplasm of this tissue. Paramural bodies are frequent.
5. Spherosomes are evenly distributed throughout the cytoplasm and are in concentrations considered normal for callus tissue.
6. Nucleus was routinely found in a centralized location. Normal membrane and pore structures.
7. Mitochondria appeared normal in appearance and numbers.
8. Little or no starch present in the chloroplasts.
9. Vacuoles (large) were usually devoid (95%) of any ergastic material.

Apollo 11 Pinus lambertiana (control)

These comments are from observations alone. They can be documented if need be although may not be necessary. This tissue (those cells considered alive) was almost identical to A-11 Palustris, with the exception of a slightly higher degree of ergastic material in the vacuoles and an increase in the starch granules found in the chloroplasts. A great many cells were ghost types, with cellular debris and intact spherosomes all that remained of the cytoplasm. About 90% of the cells in both specimens (TC-1199 and TC-1195) were dead. Those cells that were considered viable are described by the comments above.

Apollo 11 Pinus palustris (Lunar and Sterile Lunar)

1. Dumbbells present.
2. Chloroplasts well developed with grana often stacked as observed in mesophyll cells. Few, if any, crystals noticed. Membranes of these plastids were well developed. No fibrillar material was observed in any of the chloroplasts studied.
3. E.R. was highly developed and usually (85-90%) of this organelle was considered to be involved in apparent protein synthesis (rough). Localized activity (hypertrophy and/or vesicular formation) was the outstanding feature in this cell.
4. Cytoplasm was generally filled with vesicles of various sizes (could be sectioning problem) origin of these was in doubt but golgi activity was often high in addition to the E.R. activity.
5. Some spherosomes were noted in the cytoplasm but the number of bodies per cell was very low.
6. Large nuclei appeared normal and were centrally located. Cells often had much smaller central vacuoles than did controls.
7. Mitochondria appeared normal in size and distribution.
8. Few starch grains were noticed in plastids in this treatment.
9. Ergastic material (dense and in spherical arrays) were noted in the central vacuoles.
10. Due to poor sectioning, few specimens were examined by me and these notes are observations made during scanning operations. Small membrane complexes reported by Dr. Paul G. Mahlberg that seemed to be involved in ergastic material synthesis were not observed but as the number of cells observed was small their presence cannot be discounted.

Apollo 11 Pinus lambertiana (Lunar and Sterile Lunar)

1. Dumbbells present.
2. Chloroplasts were well developed with no crystals or fibrillar material seen within the stroma. Occasional lipid droplets seen in these plastids. Membranes were all well developed.
3. Endoplasmic reticulum was greater in distribution than observed in the controls. Degree of ribosomes/unit length of E.R. was not as great as in controls but level of protein synthesis may have been similar. Many plasmodesmata were noted.
4. Vesicular activity was extremely high in this tissue. Golgi bodies undoubtedly producing these vesicles. Paramural bodies were abundant in nearly every cell.
5. Spherosomes were exceptionally rare in these tissues.
6. Nucleus was centrally located and normal in appearance. Chromatin content was lower than observed in the control samples.
7. Mitochondria were unchanged with perhaps a hint of increased ground substance density.
8. Few starch grains noted.
9. Large vacuoles were frequently filled with the ergastic material but this usually in the peripheral areas of each culture. Frequency of spherical aggrications is not high.
10. Membrane arrays probably involved in ergastic manufacture were often noted.

Apollo 12 Pinus palustris (Control - First Collection)

Of all the control tissues, these appeared to be best. Denser cytoplasm, more indication of mitotic activity, wall formation, etc.

1. Dumbbells present.
2. Chloroplasts were generally poorly developed (in spite of above comment) crystals were often present within stroma. Very little membrane activity noticed in these tissues. Very little grana observed at all. Fibrillar material often seen in these chloroplasts.
3. Endoplasmic Reticulum probably at maintenance levels. Always rough with good polyribosomal arrays often observed. Few vesicles were seen to pinch off of E.R. strands.
4. Vesicular activity somewhat greater than A-11 Palustris material. Golgi very active when observed and vesicular activity in these areas was high.
5. No spherosomes observed in this tissue.
6. Nucleus normally found in centralized locations and most were spherical. Some nuclear involutions were noted, however, and probably caused by closely appressed plastids.
7. Mitochondria were normal in appearance and number. No unusual features observed in these organelles.
8. Great numbers of starch grains noticed in chloroplasts of this species. As many as perhaps 35 individual grains could be counted in one individual plastid alone. Indicates an ability to take up the sucrose (carbohydrate source) from the media but

either its inability to materialize it or an excess in storage behavior on the part of the cell.

9. Large vacuoles were usually empty with only suggestion of ergastic material being stored.
10. Developmental stages in chloroplast formation, or degredation, were noted.
11. Nuclear pores and nuclear membranes were very classical.

Apollo 12 Pinus palustris (Lunar and Sterile Lunar - First Collection)

Of all the tissues, these appeared the very best cytologically. Cytoplasm density, index of metabolism, wall formation, etc. were clues to this observation.

1. Dumbbells were present.
2. Chloroplasts, while not having grana lamellae as found in A-11 specimens was far and again better than observed in the controls. Crystals and fibrillar material was not observed. Outer plastid membranes well developed. Unusual pro-grana bodies were present as coiled arrays. Plastid lipid content was very common.
3. E.R. level was higher than controls and nearly always covered with ribosomes. Intricate E.R. complexes were often observed. Localized E.R. hypertrophy and vesicular formation was the exception rather than the rule.
4. Vesicular activity was, in many cells, almost non-existent while in others the level was uncommonly high. Golgi bodies in the latter cells were very active. Number of paramural bodies about same as control.
5. Spherosomes were apparent as opposed to the complete absence of same in controls. Degree of abundance is hard to determine but appears to be about same for differentiated plant cells.
6. Nucleii were centrally located - often had several nucleoli. Membrane complexes appeared to join with or be formed from the nuclear membrane. Amount of chromatin probably same as in control tissues.

7. Mitochondria normal in appearance and number.
8. Number of starch grains did appear to be reduced in lunar treated than the quantity observed in controls. No massive depositions were noted in these as was observed in control tissues.
9. Large vacuoles with large amounts of ergastic material were common. Membrane structures were definitely involved in its deposit and/or manufacture.
10. Microtubules were very common and located in close proximity to the plasma membrane.
11. Ergastic material often appeared in those areas of the cells having massive membranes complexes.

Observations On Second Collection

Apollo 12 Pinus palustris (Control - Second Collection)

The control cells were generally dead. Cellular debris was commonly found within the cell walls. Those that did appear to be alive had small cytoplasmic layers closely appressed to the cell walls. Mitochondria appeared normal and in numbers in relation to amount of cytoplasm. No vesicular inclusions were noted. Nuclei were dense and were also closely appressed to cell walls. Paramural bodies were not observed. E.R. was scarce and seldom rough. No differentiated wall structures were noted. No microtubulus were seen. Golgi bodies and cytoplasmic vesicles were greatly reduced in numbers when compared to the earlier control samples. These cells were in generally poor condition.

Observations On A-12 Second Collection

Apollo 12 Pinus palustris (Lunar and Sterile Lunar - Second Collection)

Large vacuoles are predominant in the tissues examined. Incorporated in these vacuoles were ergastic materials stored in what appeared to be 3 ways:

The immature pine tissue culture cell characteristically has a large and distinct nucleus, many small vacuoles separated by transvacuolar strands and cytoplasm rich in organelles. The mature pine tissue culture cell maintains the large nucleus but becomes filled with large vacuoles that frequently contain ergastic material.

The mechanism of ergastic material deposition appears to follow three distinct patterns, each resulting in a deposition product that is easily recognizable on the histological level. These three types of deposition are termed froth, macrospherules, and microspherules. Cells containing the material within the vacuolar space normally display only one type of deposition but in some cases more than one type has been noted. In the cytological studies, within the same vacuole.

The ergastic material observed in these tissue culture cells has staining qualities similar to that reported for tannin products.\*

The "froth" type of deposition appears to involve microvesicles produced at the mature face of the golgi bodies. These vesicles migrate through the cytoplasm and accumulate at the tonoplast. By some mechanism

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\* M. C. Ledbetter and K. R. Porter, 1970. Introduction to the Fine Structure of Plant Cells. Springer-Verlog, New York.

these aggregates are transposed from the cytoplasmic side of the tonoplast to the vacuolar space. These vesicles may carry tannin precursors. Synthesis of osmiophilic material appears to occur within the vacuole at the margin of each microvesicle. In cells containing vacuoles completely filled with the froth type of deposition product the residual membranous structures of the microvesicles are no longer apparent.

In the "microspherule" type of deposition an osmiophilic material appears scattered diffusely throughout the cytoplasm of each cell involved in tannin synthesis. Often this material may be observed within small vesicles delimited by single membranes. These vesicles do not appear to evolve from the golgi complex. A concentration gradient of the osmiophilic material is observed with the highest concentration of the material adjacent to the tonoplast. By some, as yet undefined, mechanism the material is transported across the tonoplast to the vacuole. The resultant deposition, appearing as an aggregation of tiny granules, is peripherally distributed within the vacuole. This material later aggregates into microspherules.

The "macrospherule" type of deposition appears to involve the endoplasmic reticulum. Osmiophilic material may be observed within the cisternae of the endoplasmic reticulum. These deposits enlarge due to continued synthesis, endoplasmic reticular connections with the vacuole are frequently observed, thus allowing a physical pathway for vacuolar deposition of the synthesized material. The tannin material later becomes coalesced into macrospherules. In cells containing vacuoles filled with one or more

macrospherules. The vital characteristics of the cell are often inapparent with the cytoplasm clumped and disorganized.

Summary of Apollo 11 & 12 Pine Study

The Effect of Lunar Material

1. Deleterious effects were not observed when the lunar material was added to the tissue cultures.
2. Increase in vesicular activity could be correlated with Halls paper (Nature Vol. 226 #5252, June 27, 1970) on "Pinocytutin vesicles and ion transport in Plant Cells". This publication suggests all cationic uptake is initiated by pinocytosis by which extracellular solutions are taken into the cell by the vesicles and those cations not required for metabolism are actively expelled thru the plasmalemma.
3. Studies of other pine tissues grown on substandard and enriched media demonstrate an enhancement in appearance of cells on E.M. level after lunar material was added.
4. Number of Mitochondria did not change between control and treated cells as might have been expected.
5. Plastid structure and composition (grana) might be correlated with culture observations by NASA personnel and found in the quarantine reports.
6. Second collection samples showed Lunars and Sterile Lunar treated tissues better, cytologically than controls as would be expected if nutrition played a role in the response.
7. E.R. activity might also be correlated with cellular condition.

8. Tannin deposition certainly enhanced by the L & SL treatments.  
Mechanism so involved is still obscure.
9. Lunar material enhanced the cells growth and metabolism.
10. Tannin appeared to be produced from membrane structures.
11. Response did not seem to be species, specific.

Cytological Investigations of Botanical Materials  
Since June 2, 1970

Occurrence of Virus Like Inclusions  
Within Pine Tissue Culture Cells  
Treated with Lunar Material

Prepared By

Dr. Paul S. Baur, Jr.

## INTRODUCTION

During a routine ultrastructural re-examination of pine tissue culture cells collected during the Apollo 12 quarantine mission, notice was made of unusual structures within several of the lunar treated cells. These bodies, as yet unidentified, bear resemblance to virus particles observed within infected plant cells. Although, the size and shape of the structures are comparable to rod shaped virus particles such as Tobacco mosaic, the numerical distribution, the affinity for stains, and the intercellular location are quite different. Cytological characteristics of virus inclusions are well documented and readily available for comparative studies. We feel that the unique circumstances surrounding the collection and fixation of this tissue, and the lack of any reports in the scientific literature of similar cellular inclusions make this report necessary.

## METHODS AND MATERIALS

The tissues used in this study were originally collected during the Apollo 12 quarantine tests. The procedures used for their preparation were outlined in the Lunar Receiving Laboratory - Biological Laboratory Operations Protocol (Apollo 11) Report number: MSC 00030 and are as follows:

Preparation of botanical specimens for examination by electron microscopy.-

a. Essential solutions, equipment, and supplies

(1) Solutions

(a) Acetone series

Stock	1	2	3	4	5	6
	30	50	70	90	95	100
Acetone	30	50	70	90	95	100
Distilled water	70	50	30	10	5	---
To make	100	100	100	100	100	100

(b) Dodecetyl succinic anhydride (DDSA)

(c) 2, 4, 6-tri (dimethylaminomethyl) phenol (DMP)

(d) Epon 812

(e) Glutaraldehyde

(f) Reynolds lead citrate

Lead nitrate - 1.33 g

Sodium citrate - 1.76 g

Distilled water - 30 ml

Shake suspension 1 minute and intermittently thereafter during a 30-minute period.

Add 8 ml of freshly prepared 1N sodium hydroxide. Dilute the volume of the mixture to 50 ml with distilled water and mix. The pH should be 12. Storage life is 6 months.

(g) Methyl nadicanhydride (MNA)

(h) Osmium tetroxide

Place the vial in brown-glass stoppered bottle. Break the vial with a glass rod.

Add 25 ml of distilled water (2 percent stock).

For use as a 1 percent postfixative, add one volume of osmic acid (using a marked Pasteur pipette) to one volume of cacodylate buffer.

Add this mixture to plant tissue during processing at the appropriate time.

(i) Propylene oxide

(j) Sodium cacodylate

(k) Sodium hydroxide, 1N

(l) Uranyl acetate, 1 percent aqueous

b. Tissue Processing

(1) Preparing tissue segments

After the vials have been passed through the dunk tank, check the material for size. At this time the material must be cut into pieces  $1 \text{ mm}^3$  or smaller. The outside of each vial must be labeled for contents.

(2) Poststaining and dehydration with acetone

(a) Decant the vial to remove glutaraldehyde, leaving the tissue in the bottom of the vial.

If there is any danger of the tissue being decanted, it may be necessary to pipette the solution off.

- (b) Rinse in two changes of buffer, 10 minutes each.
- (c) Postfix in 1 percent buffered osmium tetroxide (15 to 30 minutes only).
- (d) Rinse in three changes of distilled water (10 minutes each).
- (e) Stain in 1 percent aqueous uranyl acetate for 12 to 16 hours. Use only the clear undisturbed uranyl acetate. Never go from buffer to uranyl acetate because a slight precipitate occurs.
- (f) Rinse in two changes of distilled water, 10 minutes each.
- (g) Dehydrate in 30, 50, 70, 90, 95, and 100 percent acetone (15 minutes in each concentration).
- (h) Place in two changes of propylene oxide (15 minutes each).

### (3) Resin infiltrations

- (a) Prepare the following Epon 812 mixtures (designated A and B) in advance of filtration. Keep these mixtures in separate beakers and store in the freezer (stable for 6 months). Cover the beakers with Parafilm (ref. 13).

Mixture A

Mixture B

Epon 812 - 52 ml

Epon 812 - 100 ml

DDSA - 100 ml

MNA - 89 ml

Stir the components of mixture A for approximately 5 minutes with an electric stirrer. Clean the blade, and stir mixture B thoroughly.

- (b) Embedding mixture is to be prepared immediately (a few hours) before use. The mixture includes an accelerator, DMP. The proportions are as follows.

Mixture A = 3 ml

Mixture B = 7 ml

DMP =  $\frac{0.18 \text{ ml}}{10.18 \text{ ml}}$  final mix

Increase quantities proportionally as necessary.

It is absolutely essential that the final mixture be stirred thoroughly - at least 5 minutes at slow speed.

- (c) Infiltrate as follows

Propylene oxide: resin final mixture  
(3 to 1) - 1 hour

Propylene oxide: resin final mixture  
(1 to 1) - 1 hour

Propylene oxide: resin final mixture  
(1 to 3) - 1 hour

Resin final mixture - 1 hour

Resin final mixture - 1 hour

(4) Embedding

Place a small drop of resin in a Beem capsule. Remove trapped air bubble with needle. Pipette one tissue piece into a capsule, and then carefully fill the capsule with additional resin. Center the tissue piece with a needle so that the piece drops into the tip of the capsule. Repeat this process for each piece of tissue. Insert a code label for the tissue in each capsule.

Place the open capsules in an oven at 45°C overnight. Then reposition any tissue to assure its dropping to the tip of the capsule.

Close the capsules and place in the oven at 65°C for 40 hours. (This time period is important for uniformity.)

(5) Microtoming and mounting material

Follow the instrument manufacturer's directions in mounting and cutting the resin blocks.

Cut the ribbons into double-distilled, (millipore) filtered water in the knife trough. Pick up the thin sections by touching them with a grid that is lowered down upon them. Dry the grid in grid holder.

(6) Staining

Lead citrate and uranyl acetate provide excellent contrast of the wall, membrane, and ribosome content of the cell.

Place a drop of stain on a piece of Parafilm, located on moistened filter paper in a Petri dish. Place the ribboned surface of the grid on a drop of the stain for a 3-minute period (time this closely). Remove and place sequentially on three drops of water for rinsing. Drain carefully.

Place the grid on a drop of 1 percent aqueous uranyl acetate for 5 minutes (time this closely). Remove and place sequentially on three drops of water for rinsing. Drain carefully and dry in a Petri dish on filter paper. The dish serves as a convenient temporary carrier while scanning grids.

(7) Microscopy

Because the various procedures for microscopy are dictated by the design of the instrument, refer to the directions of the manufacturer or other sources.

In the later study the embedded materials were sectioned, using diamond knives, on an Ivan Sorvall MT-2B ultramicrotome and mounted on 400 mesh grids. Material was then stained for five minutes in

both 1% uranyl acetate and lead citrate solutions. Grids were examined on a Hitachi HS-8 electron microscope and images recorded at magnifications ranging from 2,000 to 50,000.

## RESULTS

The cytological evaluations of this study are reviewed as follows:  
Plate 1474. Magnification 56,310.

In this view at least three and perhaps four structures (termed "rods") are seen. All are located within membrane bound areas that are either small vacuoles or enlargements of some other membrane system such as the endoplasmic reticulum. One rod is found within a vacuole along with a collection of tiny microvesicles. This could be a paramural body because of its configuration. Another rod appears coincident with the membranous wall of it's vacuole. The rods are similar in diameter but not in length. The plane of section could account for these length discrepancies. Other features of the surrounding cytoplasm include mitochondria, one of which lacks any semblance of cristae, and an unidentified body which contains densely staining material.

Plate 1508. Magnification 29,570.

In this view, four rods may be observed within a single vacuole. Resolution does not allow the observation of their hollow tube configuration. Cell cytoplasm is clumped and mitochondria are densely staining. Large starch grains are also observed with the two plastids present.

Plate 1490. Magnification 56,310.

In this view two rods are seen within the small vacuole accompanied by a collection of microvesicles. The hollow nature of the rods is apparent. Again, the diameter is constant (800  $\mu$ ) but the length of the rods varied. The nucleus, seen adjacent to the vacuole, appears normal as do the other cytoplasmic features of this cell. The unit membrane configuration around the vacuole is plainly visible and its thickness is identical to that of the tonoplast.

Plate 1555. Magnification 34,800.

This micrograph of a lunar treated pine tissue culture cell shows five rods within a small vacuole along with several microvesicles. The remaining osmiophilic material seems to be forming into a sixth and possibly seventh rod. The orientation of this material indicates that it is forming on a central axis within the vacuolar sap and independent of the vacuolar membrane. Another small vacuole with similar dense granular material is seen in close proximity. Large spherosomes and densely staining mitochondria are also seen in this cell. Starch grains are seen in one of the two plastids, grana arrays are sparse, and the cytoplasm appears dense and somewhat disorganized in some areas. A fine order deposition is also observed throughout the cell. This material is considered a normal cellular reaction to tannin materials manufactured within the cell.

Plate 1543. Magnification 34,800.

Two rods of uniform length are observed within this cell. This is in contrast to those inside of vacuoles which were seen in other

plates. The cytoplasm surrounding these rods is consistent with that observed in pine tissue culture cells devoid of the rod structures.

Plate 1485. Magnification 47,500.

Two rods of the type observed in the other micrographs are seen in this view. These rods are in close proximity to paramural bodies one of which is connected to the cell membrane. The cytoplasmic consistency and plastid are normal for this tissue.

Plate 1484. Magnification 56,310.

In this view a rod shaped structure is within the cytoplasm and unbounded by any vacuolar membrane. However, one terminus of the rod is apparently connected to a small vesicle. This may be an intermediate stage in the formation of the rod structures. While the cytoplasm of the "host cell" is quite normal the adjacent cell is highly disorganized in one area. This heavy clumping is probably due to tannin deposits occurring in the adjacent cell. If so, then this disorganization must be considered normal.

Plate 1522. Magnification 56,310.

In this view of a pine cell, several crystals are seen within the central vacuole. At least four and perhaps five of these arrays are similar to the rod structures observed in the other plates. The other features of the micrograph are normal. The osmiophilia of the material and the section thickness are such that rod shapes are not discernable within the crystal masses.

## DISCUSSION

The pine tissue cultures were observed to undergo a necrotic reaction two weeks after their treatment with lunar material, during the Apollo 11 quarantine tests. The rod like structures were observed only in tissue treated with lunar material in the Apollo 12 quarantine tests.

Although the micrographs in this report are ordered to suggest that rod synthesis occurs within small vacuoles the reverse maybe true. The rod production could take place in the cytoplasm and the structures then engulfed within the vacuoles. This sequence would allow rod degradation by means of the poly-phenolic compounds produced by the pine tissue culture cells and stored within their vacuoles as ergastic materials.

This report does not mean to imply that a virus infection was generated by or from the lunar material in the pine tissue culture cells. However, no other cellular inclusion, yet reported in the literature, approximates those structures. Their origin, function, synthesis, and life history remain unresolved.

NOTE: This is the only report by Texas A & M University that will include prints of micrographs. Due to the unusual nature of the findings we feel it is in the interest of all concerned to document the observations completely. It is of some importance to review the history of this sample. It was collected by Dr. C. H. Walkinshaw, U. S. Forest Service NASA/MSC and fixed by him within the constraints

of the quarantine facility. The tissue was removed from the Class III cabinetry completely fixed in the manner required by the Bio-protocol. The material was then given to a NASA contractor, Paul G. Mahlberg, Ph. D. - University of Indiana, Bloomington, Indiana for cytological evaluation. Contract documentation and reports did not include any reference to the structures described in this report. Upon termination of the contract, the embedded materials were returned to NASA/MSC where they were re-sectioned and examined by this contractor for use in comparative cytological evaluations of bio-effects resulting from treatment of pine callus with Apollo 14, 15, and 16 lunar fines.

Copies of these micrographs have been sent to Dr. Katherine Esau, Riverside, California, and to Dr. Russell Steere, Beltsville, Maryland for their evaluations. No reference to lunar treatment was made in the correspondence accompanying the micrographs. Their evaluations will be submitted to NASA/MSC as an addition to this document.

Report on Pine T. C. Examined For  
Bacterial Contamination

1. That part described as "Green", small areas of culture having slight green cast and found on peripheral areas of whole culture. This particular sample was excised from the top of the culture.

Most (80-90%) of cells were "alive" using criteria that included: normal nuclei and organelles (esp. mitochondria) without any evidence of membrane rupture and as structurally organized as healthy differentiated plant cells. These cells had large vacuoles and little ergastic content. Some areas did contain the osmiophilic material being synthesized at the vacuolar membrane boundary. Mitochondria appeared very normal. Chloroplasts were well developed with grana found (abundant) in every plastid. Starch deposition was also commonly observed. Cytoplasm consistency was about average. Ribosomal activity was considered moderate. Cell walls and plasma-membranes, all intact "bacteria" found in the intercellular spaces at one edge of the sample, an illustration depicts what is meant by edge of the culture.

The number of bacteria was not extensive but were in sufficient numbers to say that they were well established within the tissue. Bacterial untrastructure was identical to that previously described in infected pine seedlings. Intercellular areas were clear of any debris other than the bacterial contaminants occasionally found.

2. That tissue described as "black" or "brown". Collected from culture areas assumed to be older tissue that was deep within the culture and very close to the media surface. NO cells were alive. Complete tissue disorganization and degradation. Resinous and lipid remains were most pronounced in the "ghost" cells examined. Plastid and organelles debris were easily distinguishable within the dense cytoplasm remains. Intercellular spaces filled with debris including bodies that appeared to be coalesced tannin droplets. Very few bacteria were noted in this tissue.

3. Tissue that was termed "tan" was found beside green areas of culture and from visual observation appeared to be the best cells of the culture. These cells were found in this particular culture in all levels of the geometry. Ergastic deposits were much more frequent in these areas than were observed in the green area. Evidence of osmiophilic material could be observed in the cell walls and intercellular spaces. This area of the culture contained bacteria but not in the abundance observed in the green area.

Some correlation exists between either the nutritional state of the culture areas or the presence of tannin compounds (ergastic deposits) in regard to the number of bacteria found in the intercellular spaces.

Bacterial colonies did not form on the agar substrate of the tissue culture. Thus, bacterial contamination can remain inapparent unless tissue is studied microscopically.

Generalized Methods and Materials For all Missions  
(14, 15 and 16)

Secondary pinus sp. cultures were supplied to NASA for quarantine studies by the Biology department of the University of Houston. These cultures, pinus palustris and pinus lambertiana, were originally obtained from the University of Georgia and were generated from embryonic tissue grown from disinfected seeds. All ensuing generations of the cultures were maintained on a semi-solid media formulated from the recipe of Brown and Lawrence. Both pinus palustris and pinus lambertiana were used in the Apollo 11 quarantine studies while only the pinus palustris was used in the Apollo 12 tests.

All experimental tissues were recultured to new media several weeks before proposed challenge with lunar materials. Culture containers were maintained under gnotobiotic conditions before and during the quarantine experiments. Cultures were randomly placed in a Lab Line Biotrionetic incubator at a temperature of  $23^{\circ} \text{C} \pm 0.5^{\circ}$  with light spectrum and intensity unspecified. An air turnover of 2 times per hour was maintained throughout the tests with intake and exhaust parts covered with biological filters.

Challenge was accomplished by the addition of 0.22 gram aliquots of Lunar, Sterile Lunar (dry heat sterilization), and Earth (synthetic lunar) materials. During Apollo 11 no Earth sample was used. Apollo 15 and 16 missions included a similar quantity of Iowa soil as one test treatment. Untreated cultures served as controls in all experiments.

During Apollo 11, Pinus palustris and Pinus lambertiana were studied in addition to the other species (Glycine, Helionthus, Nicotiana, Zea and Oriza). Only the pine studies were reviewed. Ultrastructural characterization for the later missions included: Pine and tobacco during Apollo 14, soybean during Apollo 15, and sunflower during Apollo 16. Species selection is as yet unselected for the Apollo 17 study. In all cases, the material studied cytologically consisted only of plant tissue cultures and representative samples (2 each treatment) were collected at weekly intervals until sample supply was exhausted (5 week duration).

#### General Tissue Prep for All Samples

##### Methods and Materials:

Tissue preparation: Tissues prepared for electron microscopic examination were carefully excised to minimize any physical damage and were quickly immersed in a 6% glutaraldehyde solution (a 0.1 molar diphosphate buffer at a pH of 7). Fixation was for 12 to 16 hours at 0-4° C and was followed by three washes in the diphosphate buffer for periods of 30 minutes each. Post fixation was accomplished using a 2% osmium tetroxide solution (0.1 molar buffer at a pH of 7) for 30 minutes. The surplus osmium solution was removed by means of three distilled water washes of ten minutes each.

Intermediate staining involved tissue exposure to a 1% uranyl acetate solution in water for 12 to 16 hours. This was followed by two more washes in water to remove any remaining stain. Dehydration was carried out in a graded series of ethyl alcohol at concentrations ranging

from 30 to 100% with a immersion time of 15 minutes in each dilution. Two impregnation steps using anhydrous propylene oxide removed all traces of the ethyl alcohol. These two steps were of 15 minute periods.

Dilutions of the propylene oxide-resin mixture at a 3:1 1:1, and 1:3 ratio were employed in the infiltration steps and tissue was exposed to each dilution for 1 hour. Embedment consisted of two one hour exposures to the undiluted resin mixture using vacuum to remove all traces of the propylene oxide and insure maximum incorporation of the plastic into the cells. The resin used in these procedures was that described by Winborn in 1965 and includes DER-334, TAC, DDSA, and DMP-30. Mounting was carried out in preformed polyethylene capsules filled with the plastic. Tissues were allowed to settle to the capsule bottom before centering and subsequent polymerization at 60° C for 40 hours.

Sections were cut on an Ivan Sorvall MT-2B ultramicrotome using diamond knives. These sections (ranging from grey to pale yellow) were mounted on precleaned 300 or 400 mesh athene type grids and stained for five minutes in a 1% uranyl acetate solution and for four minutes in the lead citrate solution of Reynolds (1963). Each staining step was followed by washes in distilled and filtered water to remove any traces of the uncombined stain. Sections were then lightly coated with carbon using evapoative techniques and examined on a Hitachi HS-8 electron microscope. Images were recorded at magnifications ranging from 2,000 to 50,000..

Prior Reports on the Apollo 11 & 12  
Quarantine Studies

What Dr. Paul Mabberg (Biology Department, University of Indiana) reported on Pine Tissue Cultures tested during the first two quarantine missions.

1. Resin accumulation in lunar treated tissues.
2. Resin probably derived from vacuole membrane surface. (These not unique for pine).
3. Partial wall development - not unique.
4. Nucleus and division seems normal.
5. Dumbbell structures in all treatments and controls.
6. Nuclear and golgi membrane complexes.

Dr. Baur's Observations of The  
Pine Tissue Culture

1. Better cells - better test subjects for metabolism study.
2. Tannin is produced within central vacuole.
3. Dumbbells are worth reporting.
4. Cells lived longer with L & SL. (lunar and sterile lunar) treatments.
5. Nucleus, and mitochondria were unchanged.
6. Vascular activity was enhanced by L & SL treatment.
7. Nutrition was probably the cause of the effect.
8. Chloroplasts were much better in the lunar cultures.
9. Response was not species specific in the pine.

10. These cells would be good to use in future nutritional studies.
11. Three types of ergastic material were formed (microspherules, macrospherules, and froth deposits).

## Morphology of *Nicotiana tabacum* cells grown in contact with lunar material

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Suspension and stationary habituated tobacco tissue cultures were examined histologically and cytologically after treatment with 0.22 g of lunar material. The treated and untreated tissues differed in chloroplast structure and distribution, degree of cell association, cytoplasmic vacuolation and vesiculation, and living to nonliving ratios.

BAUR, P. S., C. H. WALKINSHAW, R. S. HALLIWELL et V. E. SCHOLES. 1973. Morphology of *Nicotiana tabacum* cells grown in contact with lunar material. *Can. J. Bot.* 51: 151-156.

Des tissus de tabac habitués à la culture en suspension stationnaire ont été examinés histologiquement et cytologiquement après traitement avec 0.22 g de matériel lunaire. Les tissus traités et non-traités différaient par la structure et la distribution des chloroplastes, le degré d'association cellulaire, la formation des vacuoles et des vésicules cytoplasmiques et l'abondance relative des cellules mortes par rapport aux cellules vivantes. [Traduit par le journal]

### Introduction

Habituated callus cultures of *Nicotiana tabacum* L. treated with lunar material collected during the Apollo 11 or 12 missions exhibited an increased green pigmentation and remained viable several months after untreated controls had died (Walkinshaw *et al.* 1970). Subsequent studies by Weete and Walkinshaw (1972) confirmed observations on increases of pigmentation in lunar-material-treated tobacco cultures. Also, a change in the relative proportions of the sterols was detected from comparative analyses of the treated and control tissues (Weete *et al.* 1972).

Because treatment of tobacco callus tissue with lunar material affected the concentrations of pigments and sterols, it seemed essential to examine these tissues for possible morphological and cytological differences. The cytological response of tobacco cells, in both suspension and callus tissue cultures, treated with lunar material is described in this report.

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### Materials and Methods

Cultures of habituated tobacco callus, maintained at the University of Houston (Chen and Venketeswaran 1968), were grown in liquid or on semisolid medium. The liquid cultures were initiated by the inoculation of 1-cm<sup>3</sup> (about 1 g) pieces of actively growing callus into 125-ml culture flasks containing 45 ml of medium modified from Murashige and Skoog (1962) (Table 1). Flasks containing the inoculum and those containing only culture medium were placed on a platform shaker operated at 125 cycles per minute and provided with alternating 12-h light (about 1500 ft-c) and 12-h dark periods. The temperature varied from 23 to 27°C during the experimental period.

After 14 days of growth, 0.22 g of dry-heat-sterilized (160°C for 16 h) lunar material was added to each of five suspension cultures and to five flasks containing medium. Five suspension cultures and five flasks of medium were left untreated for controls.

Ten days after treatment, the flasks were removed from the shaker and the contents prefiltered through Whatman No. 3 filter paper to separate cells, cellular debris, and lunar material from the medium. Filtrates then were passed through 0.25- $\mu$  filters and analyzed by atomic absorption spectrometry for Al, Ca, Fe, Mg, Si, and Ti. Total cellular mass (wet weight) was determined by gravimetric means without correction for any weight contributed by lunar material adhering to, or embedded within, the tissue.

Samples (1 mm<sup>3</sup>) were excised from the periphery of the suspension tissues for electron microscopy. All remaining material was fixed for light microscopy. Tissue samples were fixed in 6% glutaraldehyde (pH 7.0, 0.1 M diphosphate buffer, 0-4°C) for 24 h (Sabatini *et al.* 1963). Tissues were postfixed in 2% osmium tetroxide for 30 min (Pease 1964), dehydrated in ethyl alcohol series, and em-

bedded in the epoxy resin mixture of Winborn (1965). Thin sections were cut on an Ivan Sorval MT-2B ultramicrotome using a diamond knife, stained with uranyl acetate (Huxley and Zubay 1961) and lead citrate (Reynolds 1963), and examined with a Hitachi HS-8 electron microscope.

Samples for light microscopy were cleared in xylol, dehydrated in ethyl alcohol, and embedded in paraffin. Sections 8  $\mu$  thick were stained according to the Papanicolaou Technique I (Reagan 1963).

Callus cultures that were newly subcultured on White's 10X medium (White 1963) were each treated with 0.22 g of lunar material. The conditions under which these cultures were maintained were identical with those described by Weete and Walkinshaw (1972). Small portions were excised from the margins of each of six treated and six control cultures at 3-week intervals for 12 weeks. These samples were prepared for examination in the same manner as were those collected from the suspension cultures.

The lunar material used in this study was recovered from algal cultures treated during the Apollo 12 quarantine and was prepared in the manner described by Weete and Walkinshaw (1972).

### Results and Discussion

At the time of treatment, 20–30% of each suspension culture consisted of free cells. This production of free cells, partly from new cell

TABLE 1  
Modified Murashige and Skoog medium used  
in this experiment

Component	Concn.	Cation	Concn. contributed by inorganic com- ponents, ppm
NH <sub>4</sub> NO <sub>3</sub>	0.66 g/l	Ca	63
CaCl <sub>2</sub>	0.176 g/l	Fe	7
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.148 g/l	Mg	14
KNO <sub>3</sub>	0.76 g/l	Si	0
KH <sub>2</sub> PO <sub>4</sub>	0.068 g/l	Al	0
H <sub>3</sub> BO <sub>3</sub>	0.248 g/l	Ti	0
MnSO <sub>4</sub> ·4H <sub>2</sub> O	4.46 mg/l		
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	3.44 mg/l		
KI	0.332 mg/l		
NaMoO <sub>4</sub> ·2H <sub>2</sub> O	0.01 mg/l		
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.001 mg/l		
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.001 mg/l		
Ferric tartrate	40 mg/l		
Glycine	3.0 mg/l		
Thiamine-HCl	0.1 mg/l		
Ca pantothenate	2.5 mg/l		
$\alpha$ -Naphthalene- acetic	0.5 mg/l		
2,4-D	1.0 mg/l		
Sucrose	20 g/l		
Coconut milk	150 ml/l		
Edamin	1 g/l		

NOTE: pH adjusted to 6.3 with dilute HCl.

formation and partly from disintegration of the initiate, continued in the control cultures until 50–60% of the total culture mass consisted of free cells when the experiment was terminated. No free cells were observed in the lunar-material-treated suspension cultures at the termination of the experiment. This absence of free cells in treated cultures may have resulted from the abrasive nature of the particulate lunar material (42  $\mu$  or less in diameter with 35–40% of the particles 1  $\mu$  or less), changes in the culture nutrition, or increases in the binding forces of adjacent cells.

Control cultures changed color from their initial light green to tan at the time of treatment. At termination, controls were still tan, but treated cultures had become dark green. This change agreed with the findings of Weete and Walkinshaw (1972) for tobacco and Silverman *et al.* (1971) for the green alga *Chlamydomonas reinhardtii*.

The texture of the suspension culture initiate was firm. However, at the time of treatment with the lunar material, it had become a friable mass. Control cultures continued to crumble until the termination of the experiment with the cell mass in the culture comprising only 40–50% of the total (2.212 g). The lunar-material-treated cellular mass (1.510 g) was firm and solid at collection and resembled a typical callus grown on semisolid medium.

At the termination of the experiment, 50–60% of the cells in the control suspension cultures were determined to be nonviable (lacking any evidence of cytoplasm) by histological examination. In treated cultures, only 20–30% of the cells were dead. Viable cells in untreated cultures examined by light microscopy contained large (80–90% of cell volume) central vacuoles surrounded by a thin layer of parietal cytoplasm. The nuclei, always surrounded by plastids, were pressed to the cell wall (Fig. 1A). These cells were similar to those described by Ball and Joshi (1965) in their study of tobacco callus cells grown in liquid culture.

Also, vacuoles were observed in the lunar-material-treated cells, but their volume seldom exceeded more than 50% of the cell. Although control cultures were larger than treated cultures at experiment termination, the weight estimation of viable cells per culture was greater in the treated tissues ( $2.212 \times 0.55 = 0.995$  g per con-

trol culture as opposed to  $1.510 \times 0.75 = 1.133$  g per treated culture). When vacuolar content is considered, the response of the treated tissues is more evident. Cytoplasmic content of the control cultures was estimated at 0.15 g per culture ( $0.99$  g cells  $\times$  0.15), whereas, treated cultures averaged 0.57 g per culture ( $1.13$  g cells  $\times$  0.50). Thus, the cytoplasmic content of the treated cultures was four times that observed in the controls.

Numerous transvacuolar strands were observed radiating from the centrally located, well-defined nucleus of the treated cell (Fig. 1B). No difference in cell size, cell wall thickness, or staining was noted between treated and control suspension cultures. Cell division was not as evident in the controls as in the treated cultures.

Lunar particles were observed in nearly all crypt areas (Fig. 1B) but were not found within any cell. These particles were often observed adjacent to, or sandwiched between, viable cells. Such an incorporation of the lunar materials into the treated cultures resulted from the almost complete suspension of the lunar particles during culture agitation. Incorporation of the lunar material in the crypt area also must be considered as a factor in cation uptake. Walkinshaw *et al.* (1970) postulated the extracellular dissolution of the lunar materials by secreted acids. If this is the mechanism, the crypt areas would afford isolation from the turbulence of the medium that would remove most of the soluble salts formed during hydrolysis.

Analyses of the medium filtrates failed to reveal any contribution of soluble cations made by the lunar materials after exposure to the cultures or to the medium (Table 2). The tobacco tissue cultures did not dissolve sufficient amounts of lunar material to affect the concentration of cations in the cell-free fluids. Other studies (Duff

*et al.* 1963; Henderson and Duff 1963; Webley *et al.* 1963; Silverman and Munoz 1970) have shown the solubilization of phosphates, silicates, aluminum, iron, and magnesium from rock materials by fungi and bacteria. Silverman *et al.* (1971) concluded that the pigmentation changes brought about in the bacteria *Serratia marcescens* and *Pseudomonas aeruginosa* grown in contact with Apollo 11 lunar material may have resulted from a leaching of iron from the lunar fines. Although the lunar material in this experiment was recovered from previous tests on algae, it still caused a response in the tobacco cultures.

Cells from the control suspension cultures, when examined in the electron microscope, were found to contain large vacuoles with numerous plastids in the folds of the convoluted nuclei (Fig. 2A). No ergastic depositions were noted within the vacuoles. Well-defined mitochondria, Golgi bodies, and endoplasmic reticulum were observed in the cytoplasm surrounding the nucleus and adjacent to the cell wall. Plastids of control cultures were small and densely stained. These organelles frequently contained numerous opaque globules. Prolamellar bodies were infrequent and starch granules were absent. Except for plastid morphology, the ultrastructure of these cells closely resembled descriptions of tobacco tissue culture cells made by Nakata and Hildebrandt (1967).

Ultrastructurally, the lunar-material-treated suspension cells showed a higher degree of vesicular activity than untreated ones. Many of the vesicles appeared to originate from Golgi bodies and were apparently involved in the forming of phragmoplast (Fig. 2B). This observation agrees with the intact tissue studies of Frey-Wyssling *et al.* (1964). Pinocytotic-like vesicles, apparently formed by infolding of the plasmalemma, fre-

TABLE 2  
Concentration of soluble cations found in the liquid medium of *Nicotiana tabacum* shake cultures treated with lunar material

Treatment	Concn., ppm						pH of medium
	Ca	Fe	Si	Mg	Al	Ti	
Medium + cells + lunar material (0.22 g each)	87.1 $\pm$ 3.85	2.2 $\pm$ 0.29	22.5 $\pm$ 1.23	60.5 $\pm$ 1.35	0.06	2.5	6.0
Medium + cells	89.1 $\pm$ 8.08	1.3 $\pm$ 0.7	21.4 $\pm$ 2.95	64.3 $\pm$ 14.1	0.06	2.7	6.3
Medium + lunar material (0.22 g each)	88.6 $\pm$ 5.42	0.055	18.7 $\pm$ 1.35	40.8 $\pm$ 1.6	0.06	2.5	6.4
Medium	88.3 $\pm$ 7.12	0.05	18.3 $\pm$ 3.2	42.3 $\pm$ 4.51	0.25	2.5	6.6

NOTE: Elements are reported as oxides. Values are the means of the five cultures in each group.

quently were observed in the lunar-material-treated cells. These were noticeable especially in the vicinity of lunar particles (Fig. 3A). Mitochondria, Golgi bodies, and endoplasmic reticulum observed in the treated cells were similar in structure and distribution to those found in the untreated cells.

Most of the plastids in the treated tissues were large, densely stained bodies containing grana lamellae and osmiophilic droplets (Fig. 3B). Some were similar to those noted in the control tissues (Fig. 2A). Although chloroplasts of treated cultures were more or less elongated or ovoid, some resembled the cup-shaped forms observed in *in vitro* cultures of artichoke reported by Tullett *et al.* (1969). These unusual forms were noted also in the controls. Plastids of treated cells were similar in appearance to those observed in cells of *in vitro* cultures of *Daucus carota* (Israel and Steward 1967) and *Oxalis dispar* (Sunderland and Wells 1968) grown under increased lighting. In the current experiment with tobacco, treated cultures may not have received as much illumination as the controls because of the shading by the lunar material, yet their plastids were more developed.

In the callus culture experiment, control cells examined 3 weeks after subculture resembled the controls in suspension culture (Fig. 4A). However, chloroplasts contained sizeable quantities of starch and small opaque vesicles distributed throughout the stroma (Fig. 4A). Prolamellar bodies were not observed. All other cytological features of these cells were similar to those reported in tobacco tissue culture cells by Nakata and Hildebrandt (1967).

Cells collected 3 weeks after application of lunar material to callus show grana lamellae, osmiophilic droplets, and starch deposits in many of the chloroplasts (Fig. 4B). Crystalline bodies similar to those described by Cronshaw (1964) were present in the cytoplasm of both control and lunar-material-treated cultures (Fig. 4C). Cytoplasmic density appeared to be greater in treated tissues as compared to controls. The percentages of dead cells (80–90%) and vacuolar to cytoplasmic volume (70–80%) were the same in both groups. Variability of cellular ages found within each culture was taken into consideration when ultrastructural observations were made. Only those cells considered mature were compared from culture to culture and between treatments.

Six weeks after treatment, controls appeared the same as the 3-week treated cells. Except for chloroplast development, controls in the callus experiment continued to resemble the treated cells 3 weeks younger in age.

Six weeks after the application of the lunar material, the cells from treated cultures contained highly developed plastids with large starch granules, four to six lamellae, and many osmiophilic droplets (Fig. 5A). These cells were similar in structure to those found in tobacco tissue cultures past the log phase of growth (Laetsch and Stetler 1965). Slight reductions in the percentage of dead cells (70–80%) and vacuolar to cytoplasmic volume (60–70%) were observed in this tissue.

Aside from continued improvement in chloroplast structure and increases in the percentage of live cells, the only feature unique to the 9-week-old treated culture was the unusual array of mitochondria found in one cell (Fig. 5B). This network of one or more organelles contained electron-dense granules embedded in the ground substance. We have never observed this conglomerate in any other plant tissue or tissue cultures. However, it is suggestive of the calcium deposits in mitochondria of animal cells observed by Martin and Matthews (1970).

Twelve weeks after application of lunar material, the treated cells closely resembled intact differentiated tobacco tissues. These cells exhibited well-developed chloroplasts, large convoluted nuclei, distinct vacuoles lacking ergastic material, and cytoplasm rich in organelles (Fig. 6A). Vesicular activity was minimal compared with that observed in the suspension culture. The percentage of dead cells in the treated callus cultures (60–70%) apparently had stabilized because this was the estimated value determined for the 9-week cultures.

Chloroplast development in 12-week-old control cultures was not as complete as that found in the treated cells. However, large plastids that contained opaque vesicles and starch granules were more common than those containing grana lamellae (Fig. 6B).

The callus culture findings agree with the observations of Weete and Walkinshaw (1972). Pigmentation increases noted in tobacco callus cultures can be correlated with the degree of chloroplast development, increase in living to dead cell ratios, and by differences in vacuolar

volume. All three factors could contribute to the increased pigment content per gram of tissue.

It can be concluded from these observations that lunar material is in no way detrimental to tobacco tissue culture cells. However, the lunar material induced a number of biological responses in these tobacco cells. Of particular importance was the acceleration of plastid development and a retardation of vacuole enlargement. These findings imply that lunar material affects the inorganic composition of the treated cells. Extensive investigations now are being conducted at the NASA Manned Spacecraft Center Lunar Receiving Laboratory to define the nature of these elemental changes.

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#### EXPLANATION OF FIGURES

List of abbreviations: C, cytoplasm; CA, crypt area; Cr, crystal; CW, cell wall; ER, endoplasmic reticulum; G, granum; Gb, Golgi body; Lp, lunar particle; M, mitochondrion; MG, mitochondrial granule; N, nucleus; Od, osmiophilic droplet; Ov, opaque vesicle; P, plastid; S, starch; Ts, transvacuolar strand; V, vacuole; and Ve, vesicle.

FIG. 1A.  $\times 465$ . Light micrograph of cellular aggregate from control suspension culture stained with Papanicolaou stain. Large vacuoles, small amounts of cytoplasm, and densely staining nuclei characterize the cells found in this tissue. FIG. 1B.  $\times 465$ . Light micrograph of cells from lunar-material-treated suspension culture stained with Papanicolaou stain. Distinct transvacuolar strands, centrally located nuclei, and central vacuoles typify the cells in this tissue.

FIG. 2A.  $\times 18\ 150$ . Electron micrograph of control suspension culture at termination of the experiment. Densely staining plastids lacking any evidence of starch were routinely observed within the involutions of the nucleus. Central vacuoles, lacking evidence of ergastic deposition, were common in the control tissues in the suspension cultures. FIG. 2B.  $\times 28\ 840$ . Electron micrograph of suspension culture 10 days after treatment with lunar material at termination of experiment. Numerous vesicles that seemed to originate from the Golgi bodies were apparently involved in wall synthesis of this cell found in the tissue mass from a lunar-treated suspension culture.

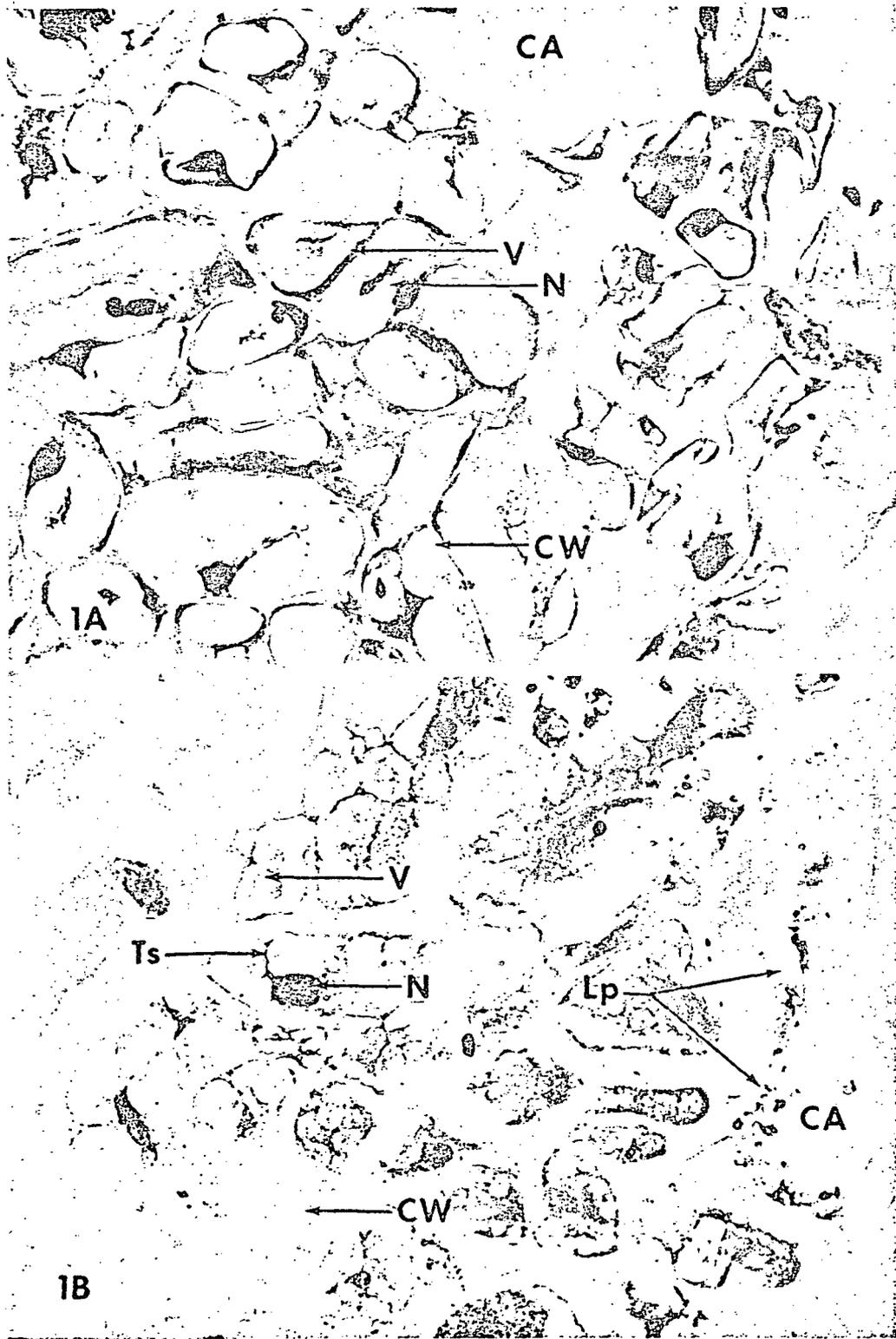
FIG. 3A.  $\times 21\ 200$ . Electron micrograph of lunar-material-treated suspension culture at termination of experiment. Pinocytotic-like vesicles were observed in close proximity to lunar particles. FIG. 3B.  $\times 23\ 600$ . Electron micrograph of lunar-material-treated suspension culture at termination of experiment. Well-developed chloroplasts containing grana, osmiophilic droplets, and opaque vesicles were observed routinely in the treated suspension cultures.

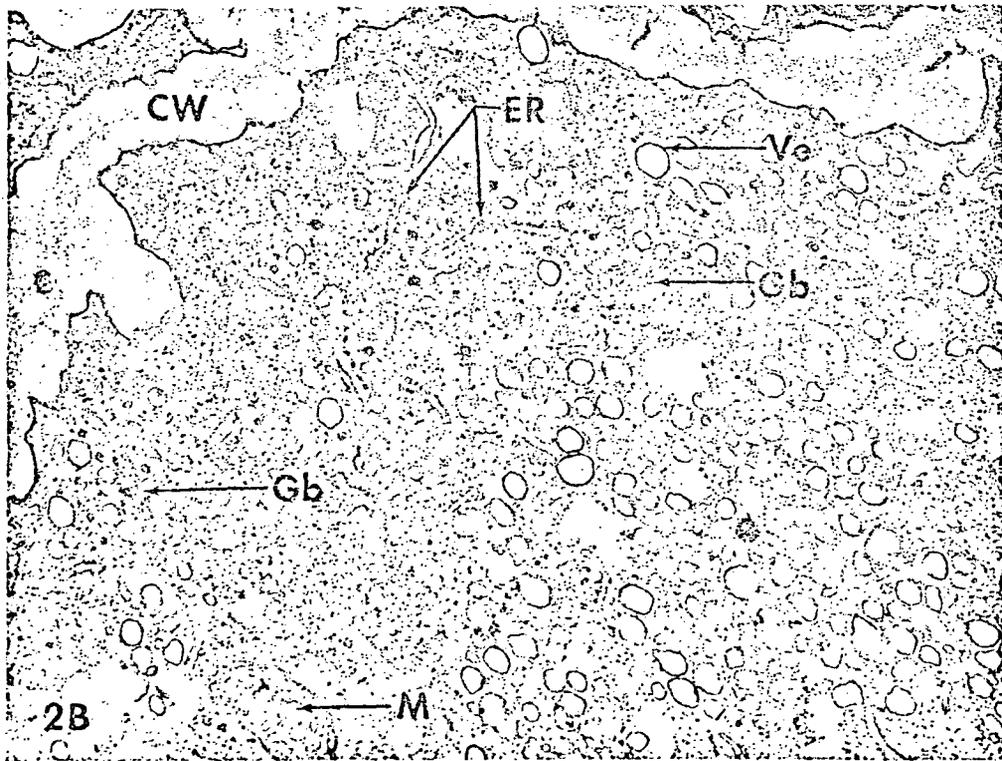
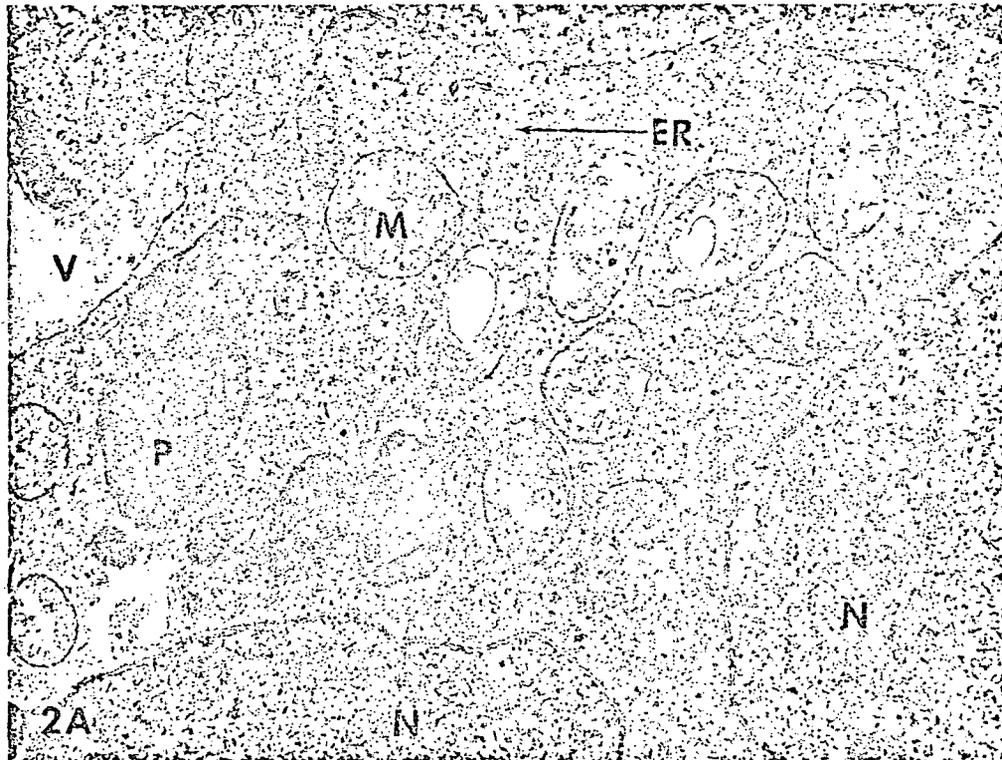
FIG. 4A.  $\times 11\ 250$ . Electron micrograph of 3-week tobacco callus control culture. Large plastids containing starch and opaque vesicles, and lacking evidence of prolamellar bodies were common in the 3-week-old control tissue from the callus experiment. FIG. 4B.  $\times 20\ 250$ . Electron micrograph of tobacco callus culture 3 weeks after application of lunar material. Well-developed chloroplasts, containing grana and starch with occasional osmiophilic droplets, were observed routinely in the callus tissue examined 3 weeks after treatment with lunar material. FIG. 4C.  $\times 38\ 950$ . Electron micrograph of 12-week control callus culture cell containing a paracrystalline body. These bodies were observed in both treated and control cells at all sampling times.

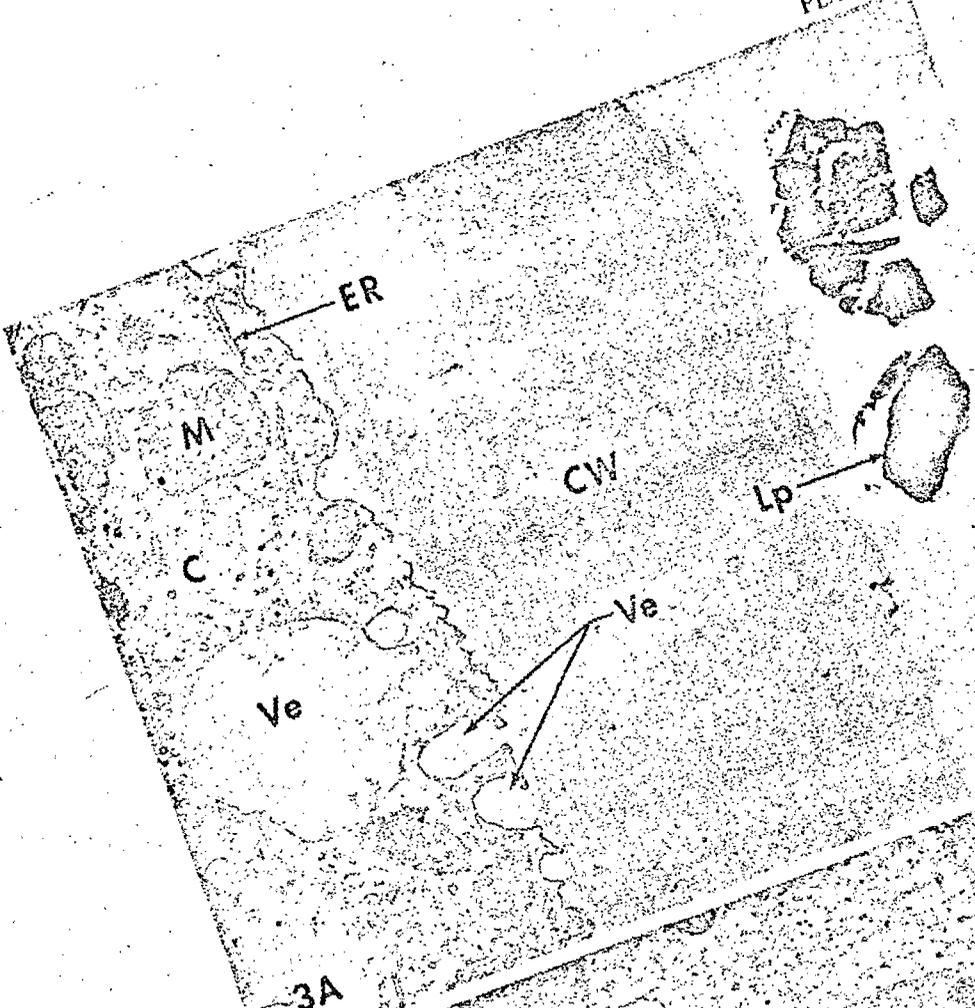
FIG. 5A.  $\times 29\ 050$ . Electron micrograph of tobacco callus culture 6 weeks after application of lunar material. Well-developed chloroplasts were observed in callus cultures grown in contact with lunar material for 6 weeks. This plastid typified those observed in other treated cells sampled at this time. FIG. 5B.  $\times 21\ 000$ . Electron micrograph of tobacco callus culture 9 weeks after application of lunar material. Conglomeration of mitochondria containing electron-dense granules in the ground substance is seen.

FIG. 6A.  $\times 21\ 250$ . Electron micrograph of tobacco callus culture 12 weeks after application of lunar material. Chloroplasts and mitochondria in the cytoplasm were in close proximity to the nucleus of lunar-material-treated callus culture cells. The central vacuoles lacked evidence of ergastic deposition. FIG. 6B.  $\times 21\ 650$ . Electron micrograph of 12-week control callus culture cell. The plastids observed in control culture cell samples 12 weeks after initiation of the experiment exhibited starch granules, osmiophilic droplets, opaque vesicles, and densely staining ground substance characteristic of this tissue. The organelles in this view were in proximity to the highly convoluted nucleus.

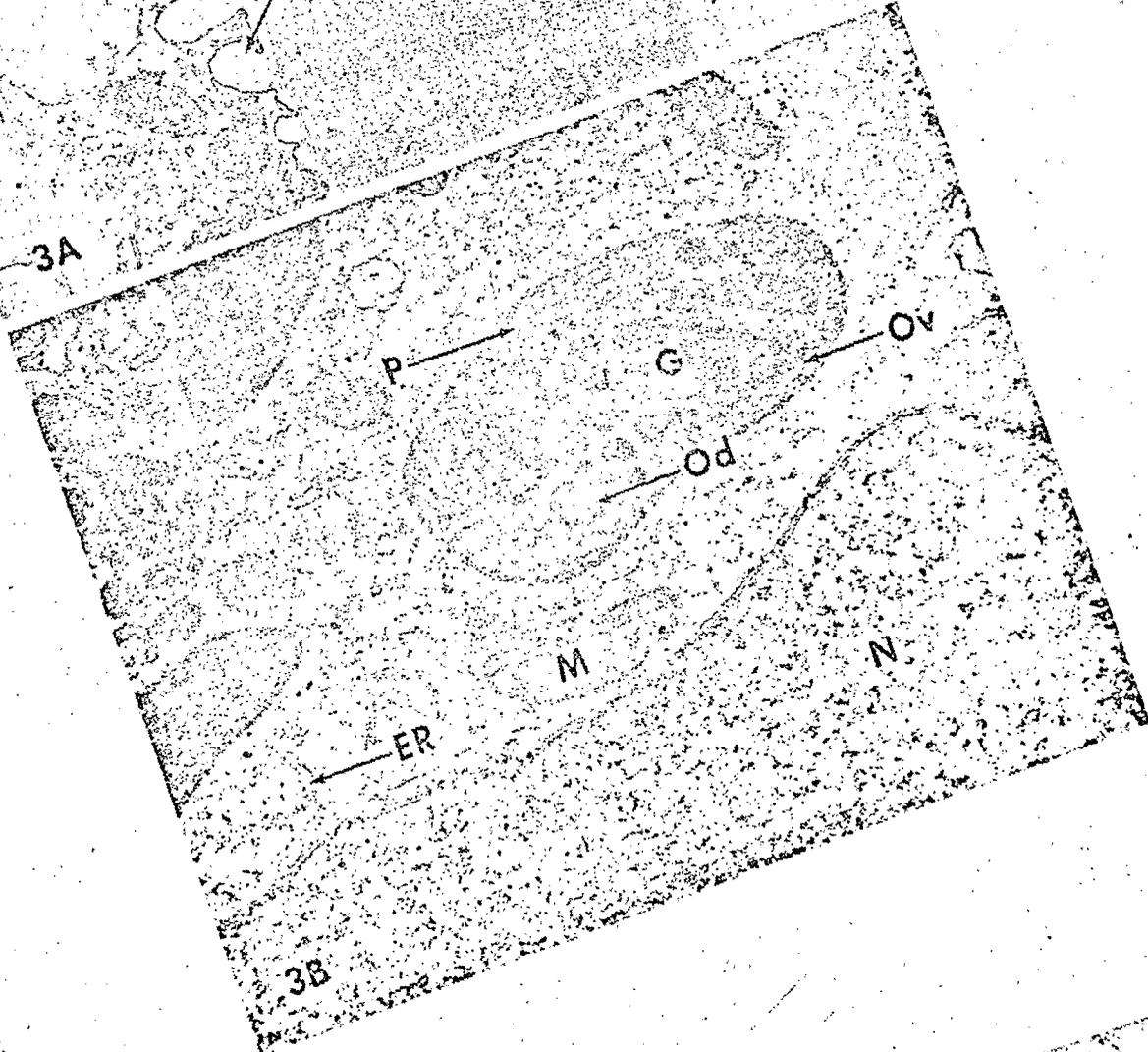
NOTE: Figs. 1-6 follow.



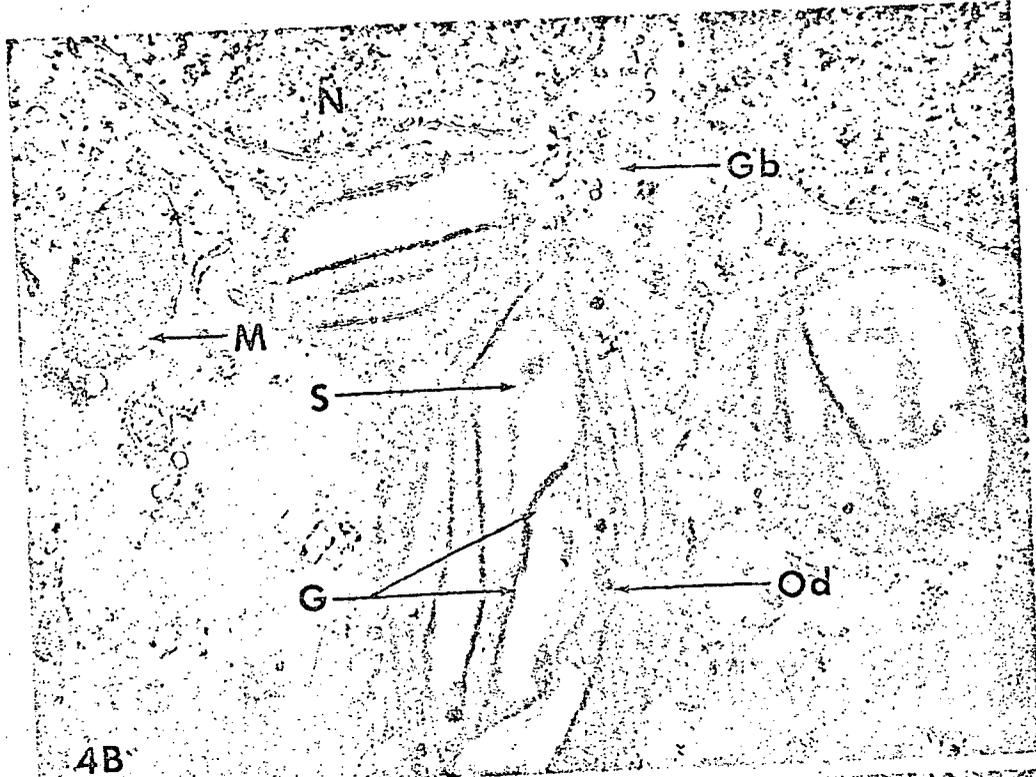




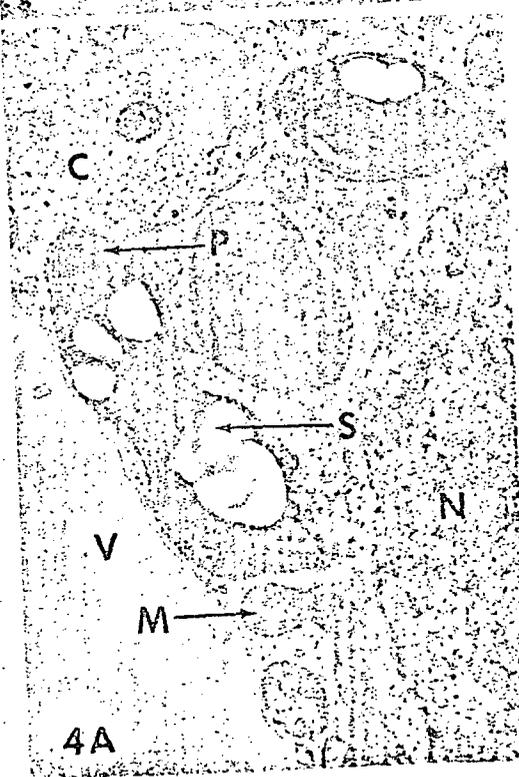
3A



3B



4B



4A



4C

