# NORTHERN ILLINOIS UNIVERSITY

**Scanning and Transmission Electron Microscopy of Various Plant and Animal Tissue**

**A Thesis Submitted to the**

**University Honors Program**

**In Partial Fulfillment of the**

**Requirements of the Baccalaureate Degree**

**With University Honors**

**Department Of Biology**

**By Robert Stockley**

**DeKalb, Illinois**

**Graduation Date May 13, 2000**

Student name: Robert Stockley

Approved by: Sonja Hanzely, Professor of BIOS

Department of: Biological Sciences

Date: March 20, 2000

HONORS THESIS ABSTRACT
THESIS SUBMISSION FORM

AUTHOR: Robert Stockley

THESIS TITLE: Scanning and Transmission Electron Microscopy of Various Plant and Animal Tissue

ADVISOR: Dr. Lazlo Hanzely ADIVISOR'S DEPT: Biology

DISCIPLINE: Biology YEAR: 2000

PAGE LENGTH: 102 BIBLIOGRAPHY: Included ILLUSTRATED: YES

PUBLISHED: NO LIST PUBLICATION: na

COPIES AVAILABLE: Hard copy

ABSRACT: The focus of the work is to learn basic and advanced techniques in the preparation of plant and animal tissue for both the TEM and SEM. Techniques used would include fixation, critical point drying, heavy metal coating, and photography of the SEM samples as well as negative staining and shadowing for the TEM samples (e.g. collagen, bacteria). Electron microscopy work leads to exciting discovery about plant and animal tissues which would otherwise be beyond our sight. The minute nature of the specimens studied in this work required the powerful application of TEM and SEM microscopes. Electron microscopy is an exciting and rewarding field of research.

## ABBREVIATIONS

FiP - filiform papillae

FuP - fungiform papillae

I - intrinsic muscles

M - Extrinsic muscles

FA - fascicles or bundles

En - endomysium

EP - Epithelial surface

HC - hyaline cartilage

C - chondrocytes

Ci - ciliated cells

G - goblet cells

CF - collagen fibers

f - fimbriae

fi - filament

F - flagella

A - anther

P - pollen

O - ovary

Sy - style

Sg - stigma

GT - glandular tricome

S - stomata

T - trichomes

M - mesophyll

PP - palisade parenchyma

Z - pedipalps

* - chelicerae

# CONTENTS

## 1.0 Scanning Electron Microscope Design

The primary function of the scanning electron microscope is the analysis of the surface topography of biological specimens. By combining a high resolution with a great depth of focus, a three-dimensional image can be observed. The SEM consists of five systems:

1). The Illuminating/Imaging System,

2). The Information System,

3). The Detection System,

4). The Vacuum System.

Each of these systems will be discussed separately.

### 1.1 The Illuminating/Imaging System

The illuminating/imaging system and its two integral parts, the electron gun and the condenser lens assembly, produce the electron beam and focus it onto the sample. The electron gun consists of three components. The first is the V-shaped tungsten filament. By applying current through the filament, an intense beam of electrons is produced and attracted down the microscope column. The second part of the electron gun is the shield enclosing the filament, with a 1-3 mm aperture that must be centered over the filament tip. The third component, positioned beneath the filament and shield, is the anode. When the electrons are emitted

from the filament, they are accelerated by the positive potential field produced by the shield and anode. After leaving the gun, the electrons pass down the "field-free" region of the column at constant velocity (Bozzolla, 1992).

The second integral part of the illuminating/imaging system is the condenser lens assembly, or the demagnifying lens assembly. It consists of a condenser lens, a final lens, and scanning coils. It serves the dual function of demagnifying the electron beam, and magnifying the image. Both lenses, acting together, demagnify the beam from 25,000 to 50,000 Å to about 100 Å. In addition, by changing the focal length, the condenser lens can control the brightness of the image that is seen on the CRT (discussed later). The final lens is also equipped with a movable aperture which intercepts excess electrons and prevents background scattering (Bozzolla, 1992). The scanning coils, located between the two lenses, move the electron beam in horizontal lines over the sample, and control the scan rate. The CRT on the SEM is similar to a television set, however, the scan rate is different. While a television screen has a fast scan rate (525 horizontal lines), a SEM has a slow scan rate (up to 100,000 lines), which is essential for better resolution. Resolution is defined as the smallest distance that two points can be separated but

still appear distinct. Most scanning electron microscopes have a resolution within range of 7 to 25 nanometers.

## 1.2 The Information System

The information system consists of the sample and its interaction with the electron beam to produce a variety of information signals. However, a typical SEM can only detect secondary electron signals and some backscattered signals (Bozzolla, 1992). The sample must be coated with a thin layer of metal to enhance the surface density for better interaction with the beam. When the electron beam strikes the coated surface of the sample, secondary electrons, and some backscattered electrons are emitted and directed to the electron detectors.

## 1.3 The Detection System

The detection system is responsible for collecting and amplifying the information signals generated by the sample/beam interactions (Bozzolla, 1992). The secondary electrons are directed to and collected by an electron detector (also termed collector), then accelerated into a scintillator, where they are translated into light. The light is transmitted through a photomultiplier and video amplifier, and eventually, to the display tube.

## 1.4 The Display System

The display system of the SEM consists of two cathode-ray tubes (CRT). The images that are displayed on the CRT's, have been reproduced exactly as they were originally scanned: line by line. The scanning electron microscope used in this class had one CRT for observation and focusing and one CRT with a Polaroid camera attached. This was used to record the image.

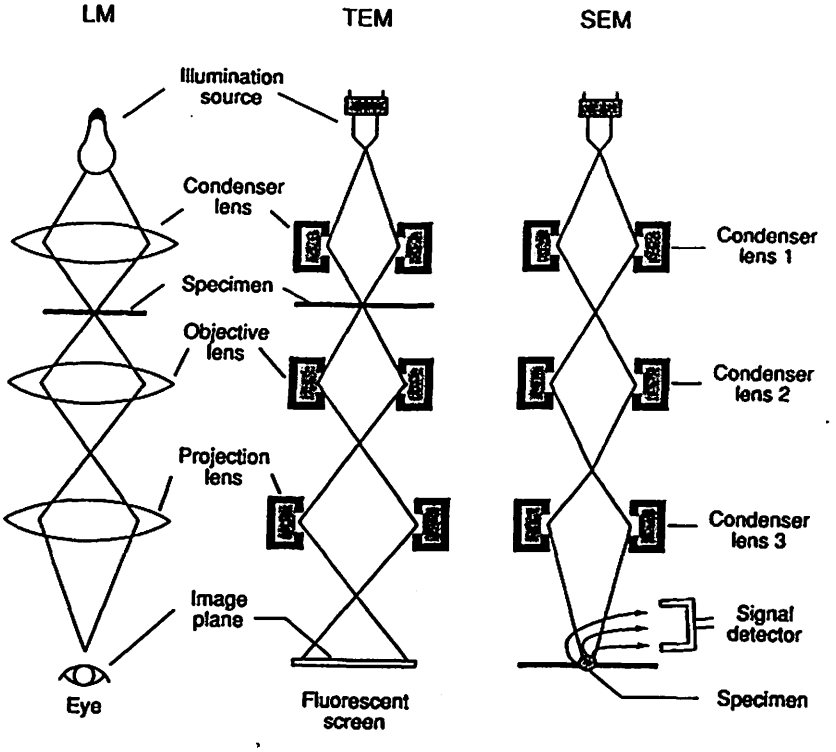
## 1.5 The Vacuum System

In any electron microscope, a vacuum is necessary to remove all gases from the microscope column. Stray gases will interact with the electrons and scatter them randomly, giving rise to glare, noise, reduced contrast, electrical discharges, beam fluctuations, or sample contamination (Bozzolla, 1992). Most microscopes must operate at a minimum pressure of $10^{-4}$ Torr. A typical vacuum system is comprised of two separate pumps. The rotary pump creates a low vacuum of $10^{-2}$ Torr, and the diffusion pump takes the system from low vacuum to high vacuum, near the range of $10^{-5}$ Torr. The rotary pump generates a lot of vibration, which is detrimental to the function of the SEM. It is usually situated in another room in order to eliminate the problem. The combined action of these two pumps keeps the column free of residual gases and their associated problems.

## 2.0 Design Comparison

NOTE:

The design and function of a scanning electron microscope differs significantly from that of a transmission electron microscope and light microscope. Many differences are shown in the following diagrams.




Light Microscope, Transmission Electron Microscope, And Scanning Electron Microscope Comparison

Source: Bozzola, et al. (1992)

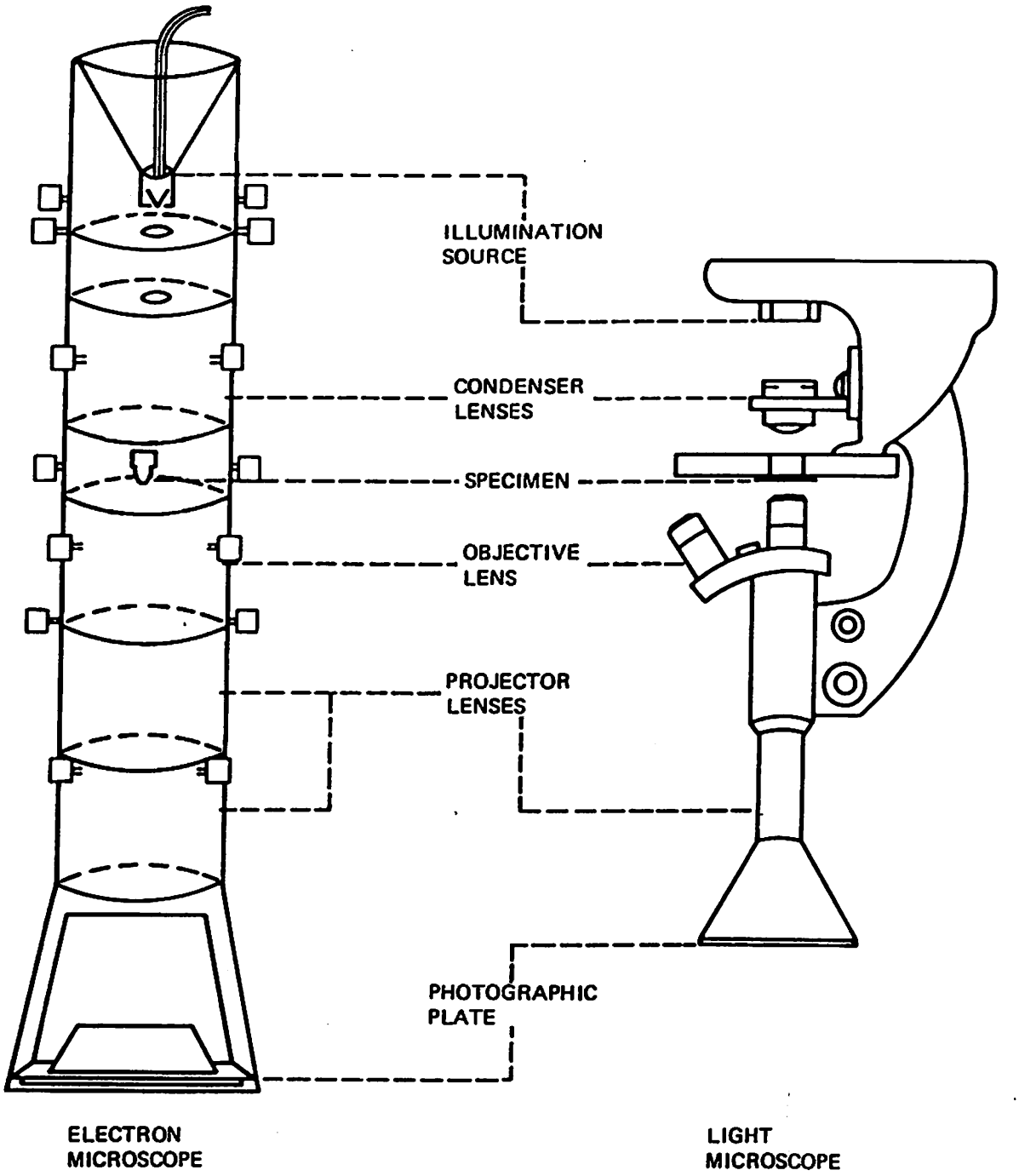
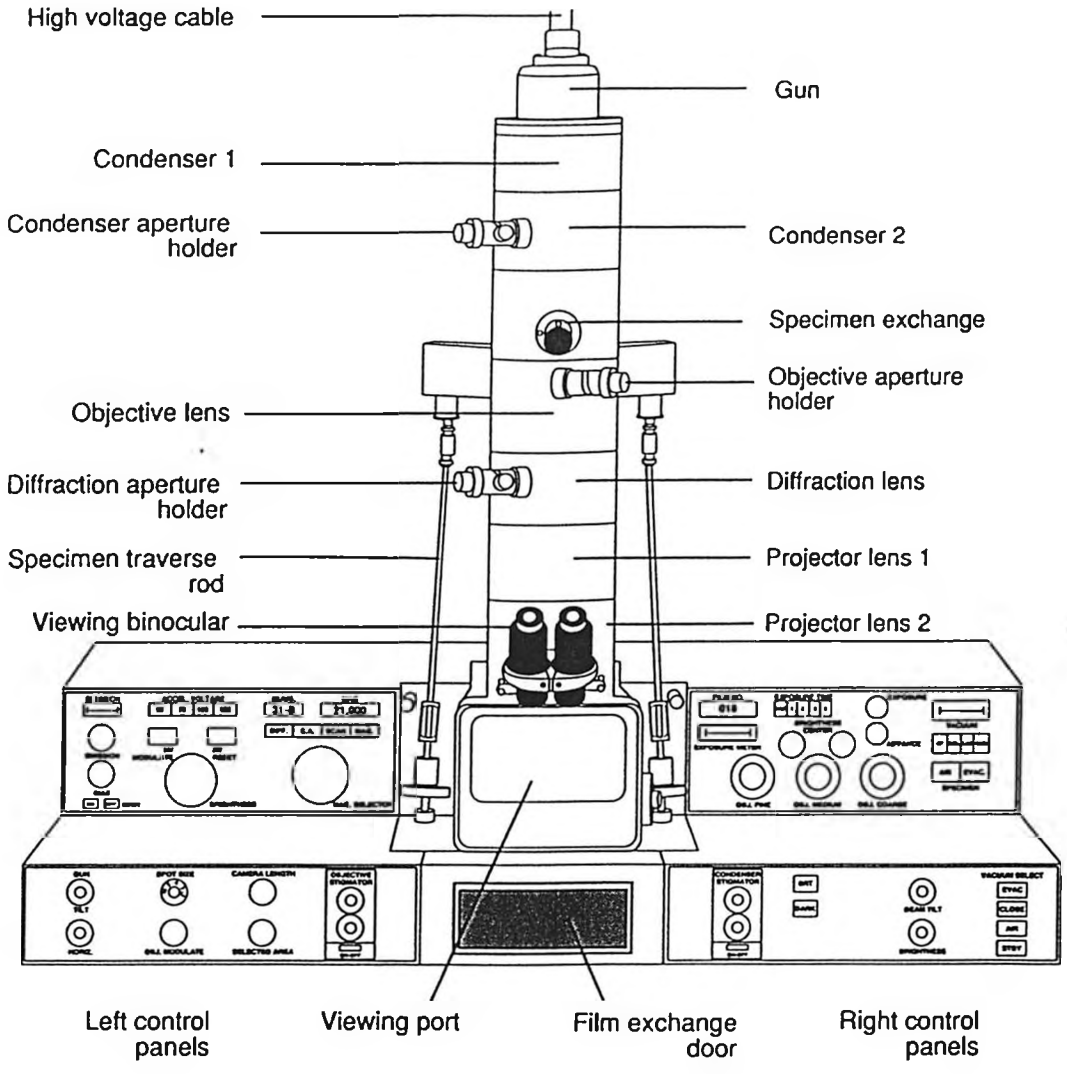



*Figure I-1.1. Comparison of the Light and Electron Microscopes*

Source: Bils, Robert F. (1974)




Source: Bozzola, et al. (1992)

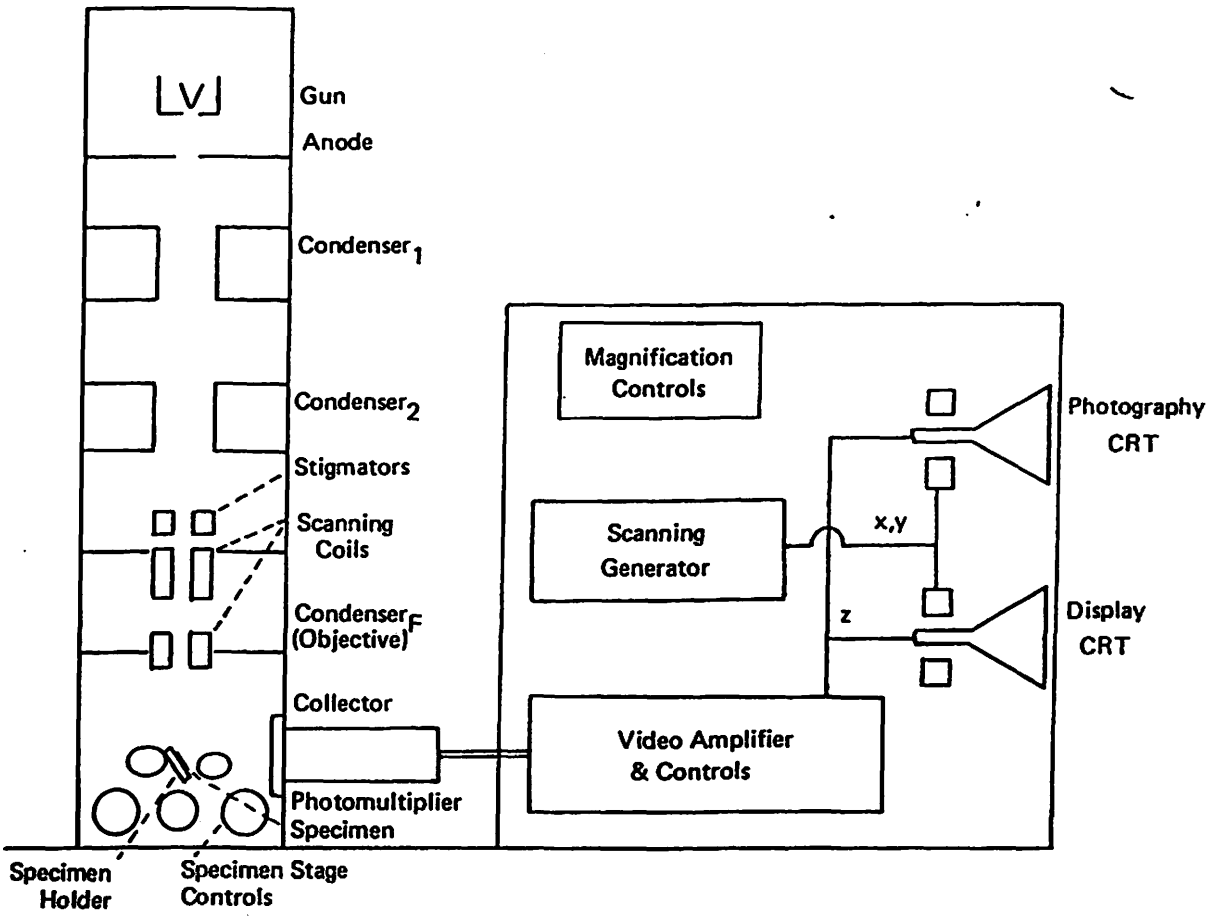



*Figure II-7.3. Scanning Electron Microscope (SEM)*

Source: Bils, Robert F. (1974)

## 3.0 Biological Specimen Preparation For The SEM

Fixation of biological samples for the scanning electron microscope involves a number of steps that would not be necessary if we were looking at something like a piece of metal. Biological samples are soft, wet, and living. We must try to kill the material as quickly as possible and yet introduce an absolute minimal amount of disruption or damage when we do this. In addition, we need to add some structural stability to the cells. Finally, all of the water must be removed from the sample.

Hundreds of different procedures exist for preparation of biological tissues for the SEM. Many are simply variations of several basic schemes. Most bulk biological samples, such as insects, leaves, pieces of tissue, can be fixed by immersion. The tissue is placed in a solution with a fixative, usually 4% glutaraldehyde. We used 4% Millonig buffered glutaraldehyde. The glutaraldehyde does an excellent job of killing the cells and adding structural stability by forming molecular cross-links. The fixative is used in a buffer solution that maintains a constant pH. A good value to use for both plant and animal tissue is pH of 7.2. Fixation in the buffered glutaraldehyde is allowed to take place for an hour or two and may be done at room temperature, or at 4°C. Some people feel that room

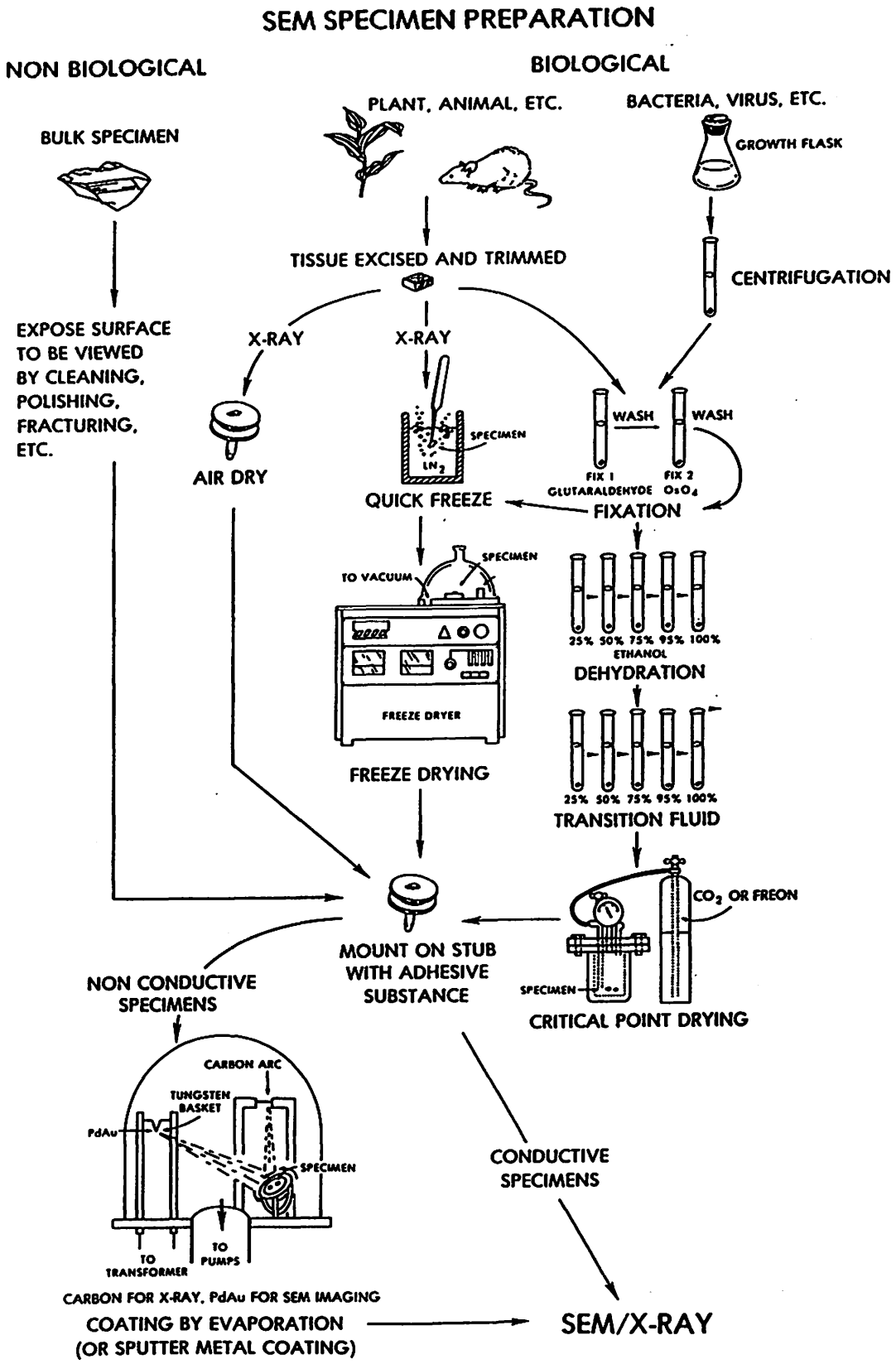



**Figure 3-1** Schematic showing sequence of events for processing biological specimens for SEM. (Courtesy of Judy Murphy.)

Source: Bozzola, et al. (1992)

temperature fixation is better and some feel that cooler fixation is better.

Size of the samples is very important. Most stubs (the metal mounts used in the SEM) are no larger than 3/8 to 1/2 inches across. Normally, samples should be considerably smaller than this. If at all possible, sample should be about 1/8 of an inch or less in at least one dimension. The difficulty with large samples is that fluids such as fixatives and dehydration solvents do not penetrate well. For instance, it is very difficult to impossible to fix many large insects whole. However, the insect can be cut into pieces and the individual parts fixed. Sometimes larger samples can be successfully used. This involves lengthening the times in all steps. At the critical point drying stage, it involves using many more rinses and flushes of a longer duration.

Usually, the samples are rinsed in the buffer alone once or twice to wash out the fixative. Next, the samples were post-fixed in 1% Millonig buffered Osmium tetroxide solution for 1 hour and again rinsed in buffer. Dehydration followed in ethyl alcohol, with acetone being the second choice. Usually fairly large gradations are sufficient such as 25%,50%,75%,95%, and 100% with about 15 minutes in each step. Some believe that for very delicate samples 10% steps

are better. We used 10% steps. There is some evidence that the transition to 100% is critical. Thus the 95% step has been included. There should be several changes in the 100% step to ensure that there has been adequate time for diffusion of the solvent into the sample and that it really is saturated in 100%. It is extremely important to ensure that the 100% solvent is pure 100% and that it has not been allowed to absorb moisture. It should be stored tightly closed. If there is doubt, a few grams of sodium sulfate added to the bottom of the bottle will absorb any moisture present.

Finally, the last step will be critical point drying. The ideal situation is to take the sample from fixation to critical point drying with no holding periods in-between. In actual practice, this may not be possible. Usually it is best to store the sample in 100%. Samples that have been stored for even several months in 100% will often show no deleterious effects. Critical point drying is now considered to be the standard method for preparation of most biological samples. It is basically a method for drying a sample whereby there are no surface tension forces. If a sample is allowed to air dry, the surface tension forces of the tissue water will cause severe shriveling and distortion. Some experimentation has been

done with drying samples saturated in alcohol (the surface tension forces of alcohol are less than those of water) or freeze-drying samples. The results are usually far inferior to critical point drying.

Critical point drying is an easy concept to understand in theory. A fixed sample is gradually taken through a solvent (usually alcohol, sometimes acetone) called the dehydration solvent. It is then placed in the chamber of a critical point dryer. The chamber is constructed of thick steel with an inlet valve, an outlet valve, a pressure gauge and a cover that can be sealed very tightly.

Attached to the inlet is a tank of carbon dioxide. The sample should be of the siphon type, i.e., an internal pipe draws from the bottom of the tank so that liquid $CO_2$ is withdrawn. The chamber is cooled before the sample is inserted to help keep the carbon dioxide in liquid state.

The cover is removed, and the sample inserted. The sample should be completely saturated with 100% alcohol before it is inserted. The cover is replaced. The $CO_2$ tank valve is opened. The chamber will now fill with liquid carbon dioxide. The pressure gauge should read about 700-900 pounds/inch$^2$. Then, purge at 0°C for 10-15 minutes. This washes out all the 100% ethanol and replaces it with $CO_2$. It

is extremely important that all alcohol is flushed out of the tissue and out of the system before proceeding.

At this point, the inlet valve and outlet valve should be tightly closed so that we have a closed system. The entire chamber is now heated. As the chamber is heated, the liquid phase will become less dense and the vapor phase will become denser in accordance with the laws of chemistry and physics. The heating is allowed to continue. The pressure in the chamber will rise during this process. Eventually, we reach a point called the critical point at around 1072 pounds/in.$^2$ and 32°C where the density of the liquid $CO_2$ equals the density of the gas. At this point, the surface tension is reduced to zero so that the delicate biological structures do not collapse. When this occurs, the sample is now dry.

Usually, the system is taken somewhat above the critical point to around 45°C and 1300 pounds/inch$^2$ and allowed to equilibrate for a few minutes. After this, the outlet valve is opened slightly and the pressure allowed to drop at a rate not to exceed 150 pounds/inch$^2$ per minute. A faster rate may cause the sample to explode or be damaged. When the pressure is zero, the cover is removed and the sample is now ready for mounting.

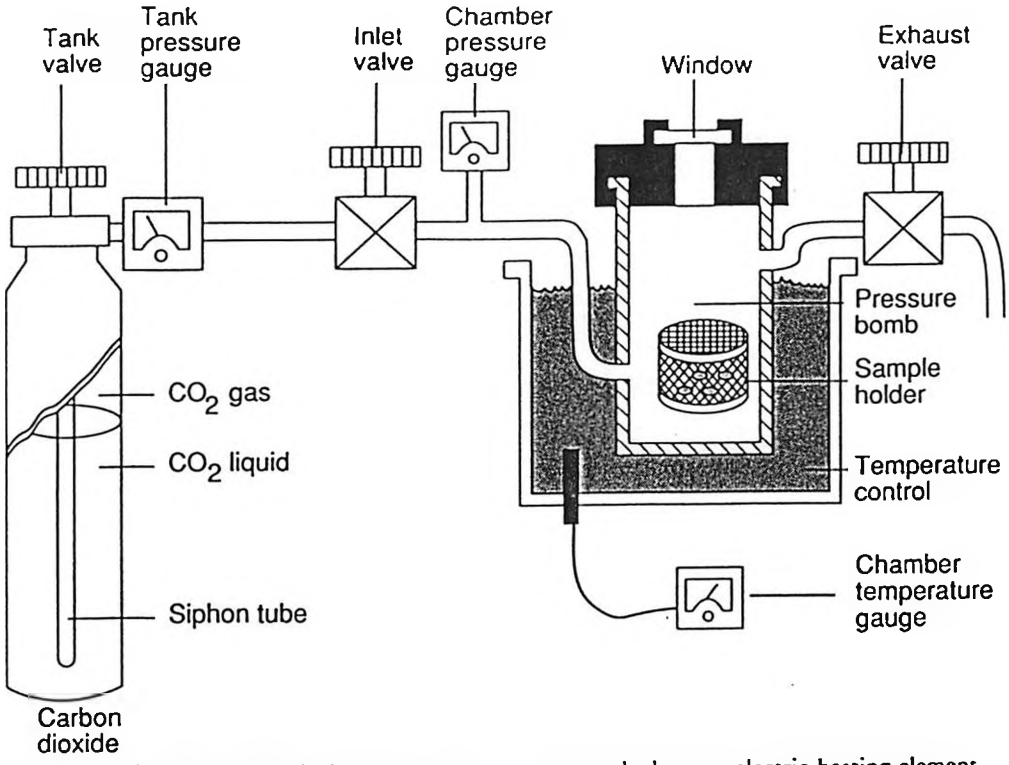



Figure 3-3 Diagram of critical point drying apparatus. The temperature of the pressure bomb may be regulated by a water bath or an electric heating element.

### Safety Precautions with Critical Point Drying

Dangerous pressures build up inside the bomb, and a few instances of the vessel cover rupturing have been reported. Modern instruments have protective discs that burst prior to the development of such pressures inside the chamber. Windows covering the chamber are of specially tested quartz or glass and should be checked for nicks or cracks before each use. It is recommended that the critical point dryer be placed inside of a fume hood or behind a shatter resistant window (plexiglass, for instance). One is cautioned not to look directly down into the pressurized chamber. Instead, a metal mirror (not glass) should be used to examine the chamber contents. The mirror should be mounted on a movable support that will fall away from the user in the event of a window failure. A well-ventilated room is necessary since the gases used in this process will displace oxygen and may asphyxiate or sicken unwary users.

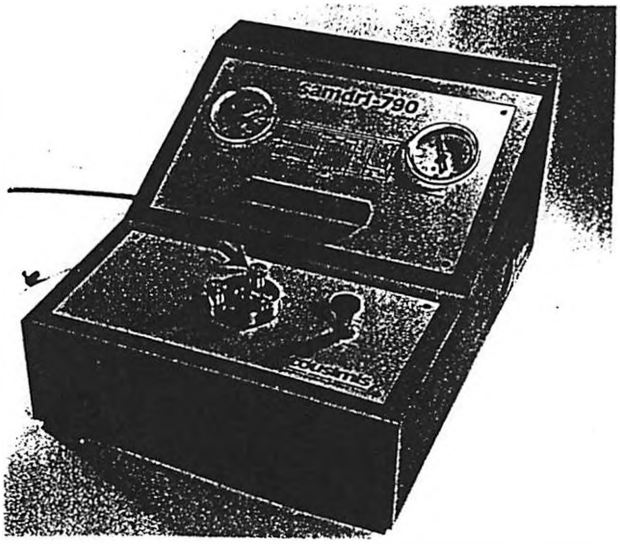



Figure 3-4 Photograph of a semiautomatic critical point drying apparatus. Specimens are placed into the recessed specimen chamber and the lid is sealed using the three thumb screws (arrow). Processing of the specimen is carried out by pressing a series of buttons (above the specimen chamber). The large dial on the left indicates the pressure inside the specimen chamber while the dial on the right indicates the temperature.

Source: Bozzola, et al. (1992)

## 4.0 Mounting Samples For The SEM

All specimens observed in a SEM require some type of mounting. Mounting should firmly fasten the sample to prevent vibration and should maintain a conductive pathway to the metal stage. Some microscopes allow very large samples (up to 3 inches or more) to be examined. These samples are often taped down or held down with metal fasteners. Small vises can be purchased which mount inside the SEM to firmly hold large samples.

Most samples observed in the SEM are mounted on small metal cylinders called stubs. The stubs may be easily inserted in the SEM and then stored in a variety of containers for viewing at a later date. Some type of cement must be used to fasten the sample to the stub. One method is to use double-stick tape. This will work with small samples. However, the tape has the tendency to contaminate the inside of the SEM because of the volatile nature of the adhesive. A better alternative is to use special adhesive tabs that deposit an adhesive film on top of the stub. This adhesive is designed for use in the SEM. The sample is then pressed onto the film.

Another method involves the use of the conductive cements such as graphite cement or silver glue. These give moderate strength and have the added advantage of being

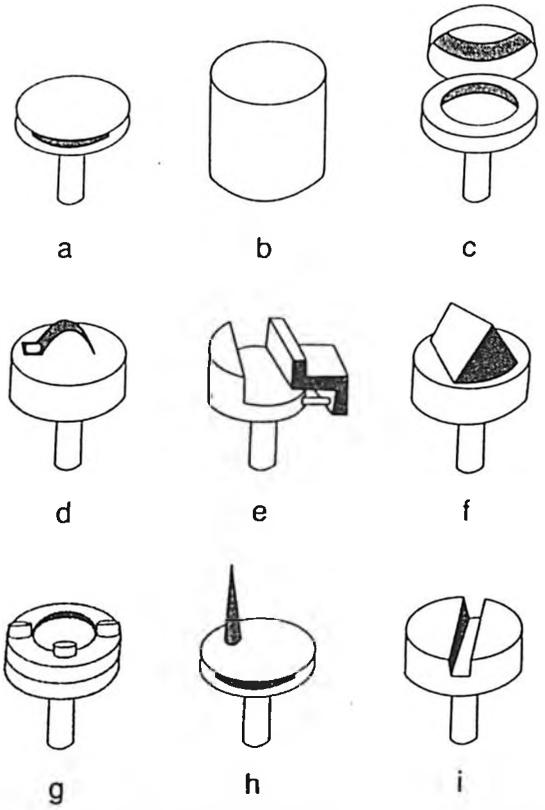



Figure 3-12 Specimen stubs used for mounting biological specimens for viewing in the SEM. The standard types are shown in a and b, while others are modifications to hold or pin down various types of specimens. (Courtesy of Judy Murphy and SEM, Inc. Redrawn with permission.)

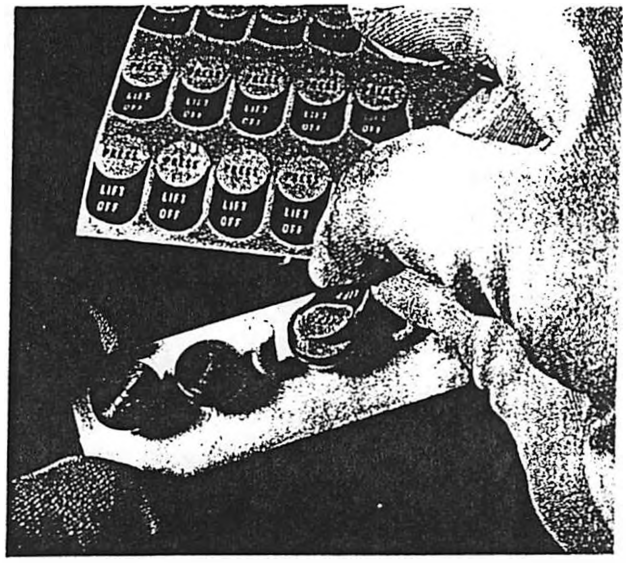


Figure 3-13 Adhesive transfer tabs are used to deposit a small amount of adhesive onto SEM stubs. The adhesive is adequate to hold most small specimens on the stub.

Source: Bozzola, et al. (1992)

conductors that can be beneficial in preventing charging which effects the outcome of photographs. Silver cement is a much better conductor; however, it costs around ten times as much as carbon cement. With the low currents involved in the sample, there is some question as to whether it really gives better results.

Because the cements are conductive, they are preferred. However, caution must be used because many biological specimens may act as a sponge and draw the wet cement up and over itself, thus destroying the surface features.

One trick-of-the-trade that may be of use with biological samples mounted using adhesive tabs or double-stick tape is to draw a fine line of graphite cement from the sample over to the edge of the metal stub prior to coating. Use a sharpened applicator stick to do this. This often will minimize charging problems because it provides a solid conductive pathway from the specimen to the metal of the stub. It is especially useful with a sample that is very rounded at the bottom.

Larger heavier samples, such as teeth, bones, metals and minerals should be mounted using glue with greater strength, such as epoxy glue. The epoxy glue with a five-minute curing time is excellent for use with SEM samples.

## 5.0 Sputter Coating Procedure

The most common method of coating specimens with a thin layer of metal is the use of plasma sputtering or sputter coating. The most commonly used system is the direct current sputtering device that closely resembles the one diagramed in Figure 3-15. Details of the principle involved and of the sputtering chamber itself are shown in Figure 3-16.

The sputtering process, after the metal stub with the attached specimen is placed in the specimen chamber; the chamber is pumped down to vacuum of 0.1 Pa using a rotary vacuum pump. The purpose of the evacuation is to remove water and oxygen molecules that might damage the surface of the specimen. This vacuuming process may take as long as 15-20 minutes.

After the desired vacuum level is achieved, an inert gas such as argon is slowly introduced into the chamber. The flow of argon is adjusted such that the vacuum is maintained at 6-7 Pa. A negatively charged, high voltage field is applied to the argon gas molecules, which ionize into $Ar^+$ and electrons. The negatively charged target, composed of a heavy metal such as gold or gold/palladium, will be struck with such force by the $Ar^+$ molecules that some of the metal ions of the target will be ejected. These

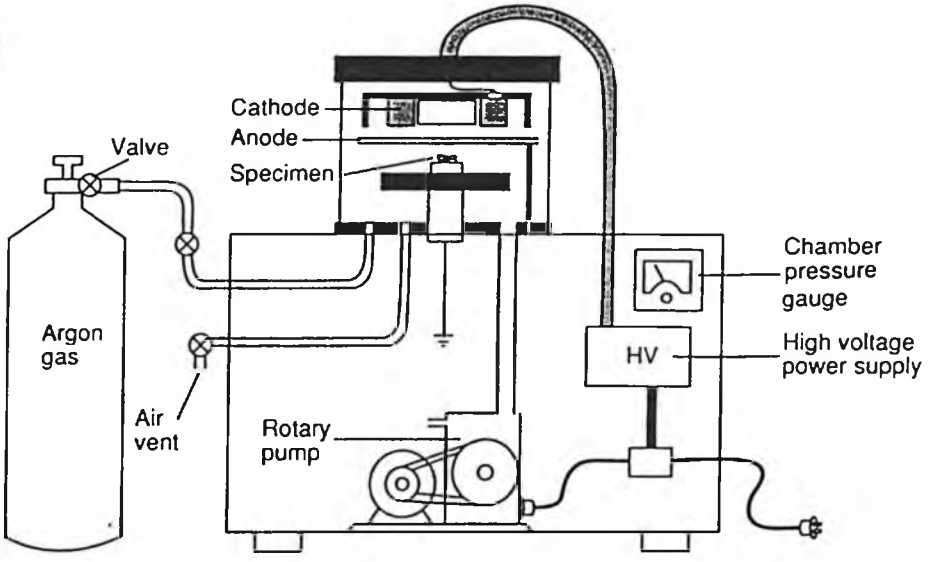



Figure 3-15 Diagram of parts of a commonly used sputter coater. A rotary pump is used to evacuate the specimen chamber to remove atmospheric gases and to permit the introduction of argon gas into the chamber. The application of a high voltage to the target causes the ionization of the argon into $Ar^+$ molecules that strike the cathode (target) and eject metal atoms that then coat the specimen.

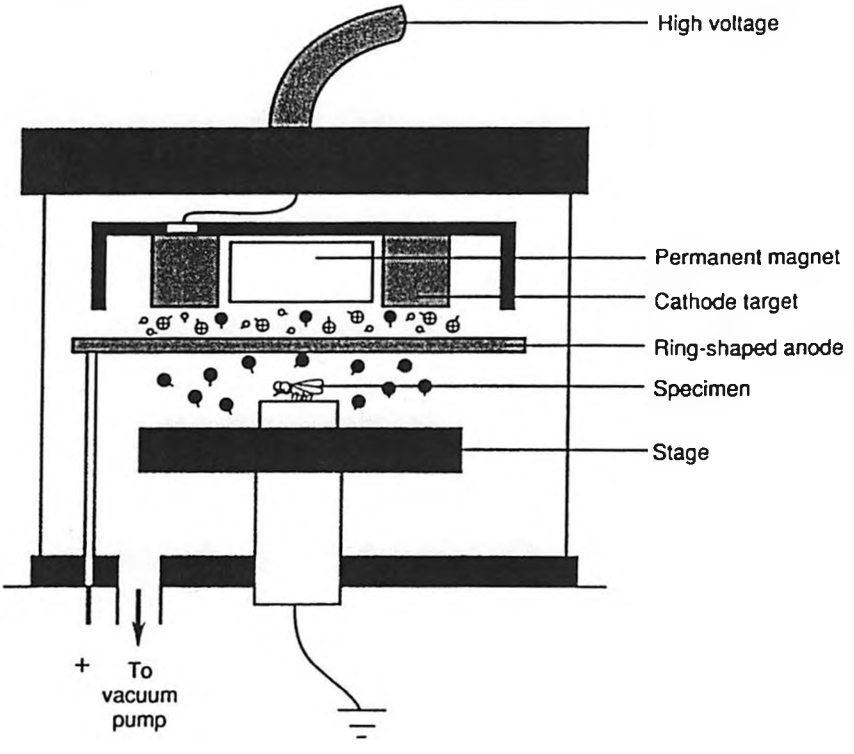



Figure 3-16 Detailed diagram of specimen chamber of a sputter coater. The metal target is struck by ionized $Ar^+$ molecules to cause the ejection of atoms of the metal target (darkened circles) that eventually coat the specimen. A permanent magnet is placed inside of the target to deflect potentially damaging electrons away from the specimen and toward the anode ring. To further protect the specimen from excessive heating, the stage may be cooled with water or by electronic means.

SPUTTER COATING

Source: Bozzola, et al. (1992)

atoms are bounced about by the various ions present in the chamber and eventually strike the specimen surface to gradually build up a metallic coating. The fact that the metal particles are knocked about in random paths is very important, since they will strike the specimen at various angles and thereby more uniformly coat the irregularly shaped specimen surfaces. The sputtering process is continued until a proper thickness of metal coating has accumulated on the specimen surface. The thickness of the required coating depends upon the topographies of the specimen. Low topographies require less coating than do high topographies.

## 6.0 Preparation of Supporting Films for EM grids

(for use in viewing shadowed and negative stained samples)

### A. Preparation of Formvar Stock Solution

1. For a (0.2-0.1%) dilution, dissolve (0.2-0.1 gm) thoroughly dried Formvar 15/95 E into 15-25 ml of dichloroethane (ethylene dichloride) in a small dry 100 ml flask.
2. Shake, in a rotary motion, until dissolved, then add dichloroethane to 100 ml mark.
3. Store in the refrigerator, making certain the flask is tightly sealed. Allow it to come to room temperature before use.

### B. Pre-clean Grids to be Coated

1. The grids should be cleaned by placing them into a tri-pour beaker and adding glacial acetic acid. Allow them to remain in the acid for 5 minutes, with gentle swirling. Rinse in distilled water (4-5 rinses) and then rinse in acetone (2 rinses).
2. Allow them to dry by spreading them out on filter paper.

### C. To Coat Grids

1. Place Formvar stock solution (0.2%) into a copelin jar.
2. Clean a pre-cleaned light microscope slide with

lens paper. A very thorough cleaning will prevent coat from attaching to slide in the next step.

3. Coat the slide with the Formvar by dipping slowly into and out of the copelin jar. Remove the slide and allow it to air-dry in a vertical position.
4. Fill a large finger bowl nearly full with distilled water. Be sure the surface is free from dust by sweeping it with lens paper.
5. Score around the edges of the glass slide with a razor.
6. Hold the slide close to the mouth and breathe on it gently. Immediately dip the slide into the water at about a 30° angle, to the surface of the water and gently lower the slide into the water. The formvar film will float off onto the surface.
7. Pick up a pre-cleaned grid and bend it slightly in the center by applying a little pressure at the center. Place the grid, dull side down, onto the floating film. Tap each grid lightly to assure good contact with the film.
8. Lower a clean glass slide almost horizontally over the film. Push down through the water surface, invert under the water, lift out and drain.

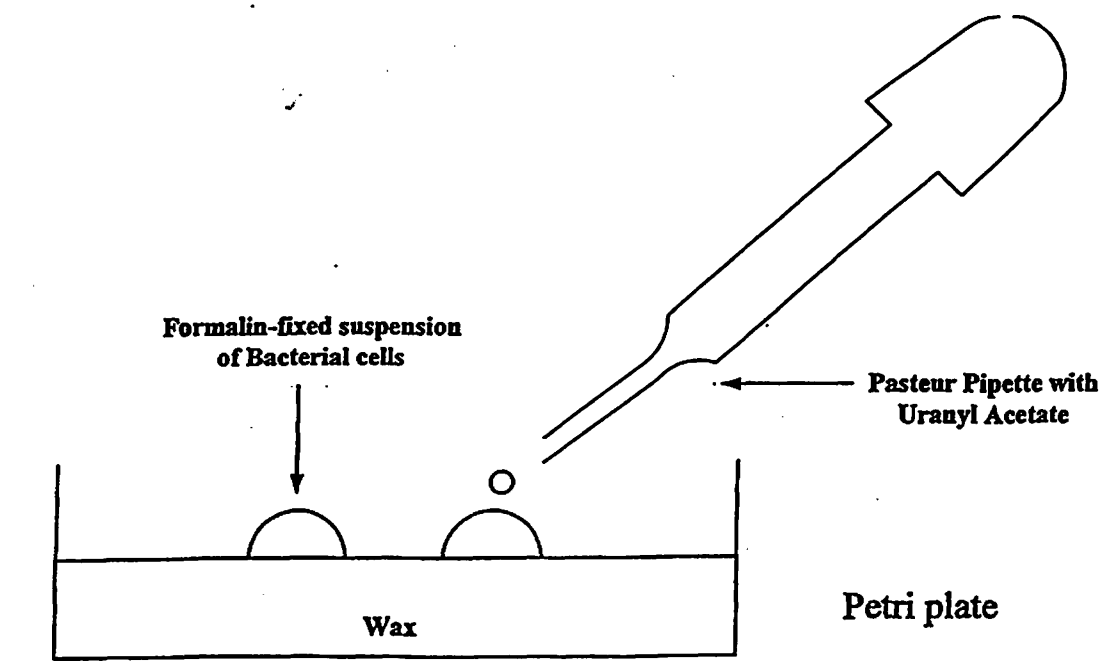
9. After the grids and film dries, the film can be strengthened and stabilized by evaporating a thin film of carbon over it in a high vacuum evaporator.

10. The grids can then be carefully taken off the slide with fine forceps and examined under phase contrast microscopy to ascertain their quality. The grids should be stored in a dessicator.

## 7.0 Preparation of Bacterial Suspension

Preparing the bacterial suspension requires five steps.

1. Pipette 10 ml of the isolated environmental sample growing in broth into a 15 ml centrifuge tube.
2. Centrifuge the bacteria until a pellet is formed. Care must be taken that undue force is not used during the centrifuge process that could cause damage or detachment of the flagella.
3. Using a Pasteur pipette, draw off the supernatant and re-suspend the cells in 5 ml of distilled water. Vortex and wait five minutes.
4. Centrifuge the bacteria again, until a pellet is formed; draw off the supernatant with a Pasteur pipette and add 2 ml of the 2% formalin. Vortex the cells and allow to fix for 5 minutes.
5. Centrifuge to form a pellet and draw off the formalin until just a small amount of liquid is left around the pellet.
6. A concentrated amount of bacteria can now be used.



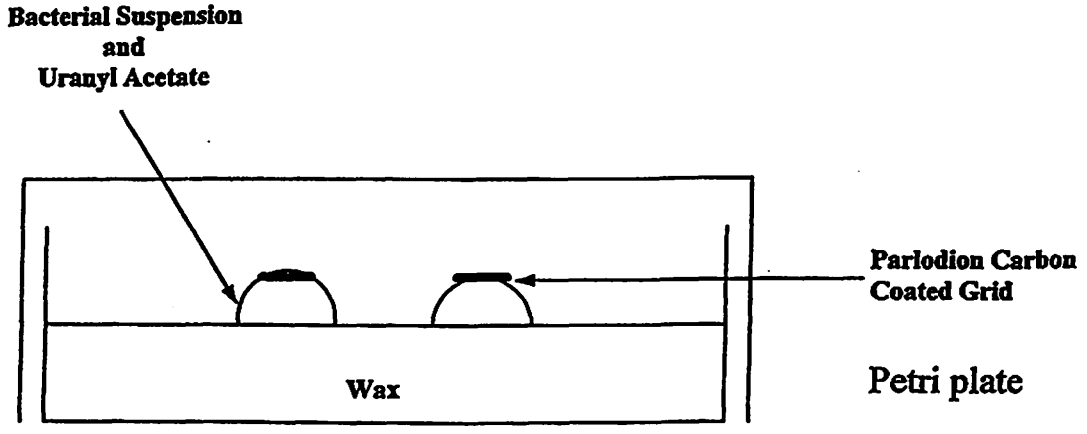




**Figure 9.5 Preparation of specimens for electron microscopy**

SOURCE: EM Lab Manual

## 8.0 Negative Staining of Bacteria

Preparing negative stained bacteria requires five steps.

1. Place two separated drops of formalin-fixed suspensions of cells on a wax-surfaced Petri dish.
2. Add a drop of 2% aqueous uranyl acetate stain to each drop of formalin-fixed suspension of cells (Figure 9.5). Do not draw up the stain from the bottom of the bottle.
3. Place a Formvar-carbon coated grid film side down on the combined drop (Figure 9.5).
4. Allow the grid to float on the drop for 10-15 minutes.
5. Remove the grid from the drop. Blot the grid carefully (on the bottom side) on clean filter paper. Store grids in Gel capsules for later viewing using the transmission electron microscope.

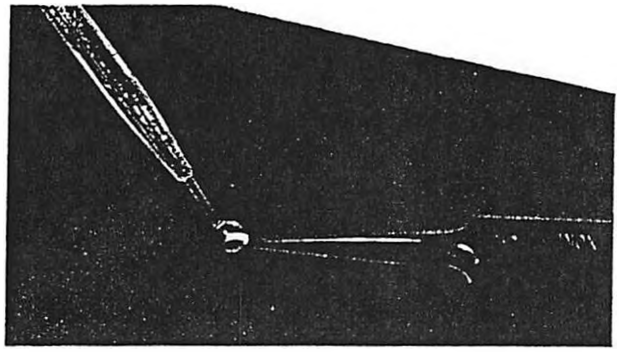


**Figure 5-14** One method of negative staining involves deposition of the specimen onto a coated grid that has been locked into a forceps (shown). After allowing the specimen to adsorb to the substrate, a drop of negative stain is applied to the grid.

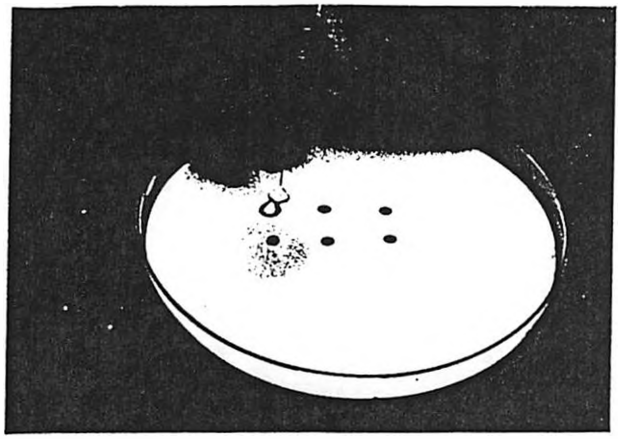


**Figure 5-15** Negative staining can also be accomplished by mixing equal volumes of specimen and negative stain and depositing the mixture on a grid using either a loop (as shown) or a pipette.

Source: Bozzola, et al. (1992)

## 9.0 Metal Shadow Casting For The TEM

Metal shadow casting is often used in conjunction with negative staining techniques in the study of small particles such as viruses, bacteria, flagella, etc. This technique is used to create differential contrast in specimens that usually lack apparent detail due to a uniformly electron transparent nature. Evaporated metal is applied at an angle and collects on features that extend above the background of the film. The "back" side or "shadow" of these features remain free from metal.

The selection of an angle for deposition and the evaporant metal are both dependent upon the particle sizes, magnification range to be employed, and required resolution. In general, large particles are shadowed at higher (30° to 50°) angles than very small particles (5° to 30° range) and metals of high melting points such as platinum are used in higher magnification/resolution situations.

## 10.0 Procedures For Metal Shadow Casting

### A. Preparation Of Specimen Grids

1. Place a drop of bacterial suspension on each Formvar-carbon coated grid that is held with forceps. The suspension is kept on the surface of the grid for 10 minutes, after which the excess

suspension is removed using filter paper. The grids will dry immediately. You may also use an aspirator to spray a virus suspension on the grids. Blot off excess after 1 min. with a piece of filter paper.

2. Place the grids (can be done as group) on a glass slide prepared by placing a thin strip of double-stick tape. When dry, place grids edgewise along tape.

**B. Evaporation Of Metal**

1. Set up the vacuum evaporator with a tungsten wire as per instructions for evaporator type.
2. Select a small piece of gold-palladium wire and measure it. Record the length in millimeters. Ordinarily, 5 to 10 mm is sufficient. Place the bent piece of metal across the V-shaped tungsten wire taking care not to contaminate the metal by excess handling.
3. Make sure that all tape from the slide/grid holder is removed except that holding the grid in place. You may also detach the grids and place them on clean slide.
4. Place the grids into the evaporator according to predetermined geometry to obtain a 30° to 45°

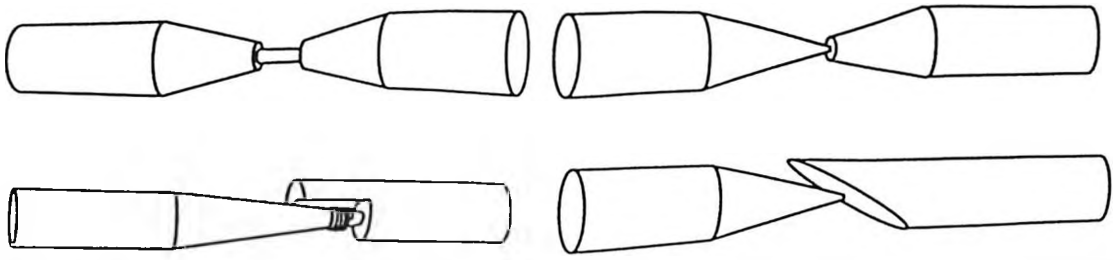


Figure 5-22 Carbon rods can be sharpened into various shapes to form the electrodes that will be used to vaporize carbon or other metals wrapped around the carbon electrodes.

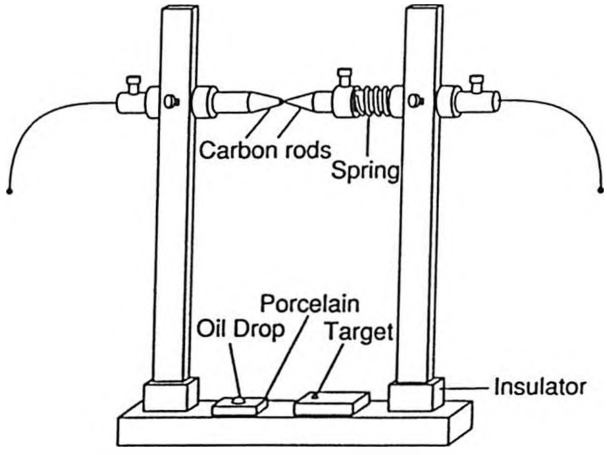



Figure 5-17 Platinum can be melted and evaporated from a heated electrode as shown.

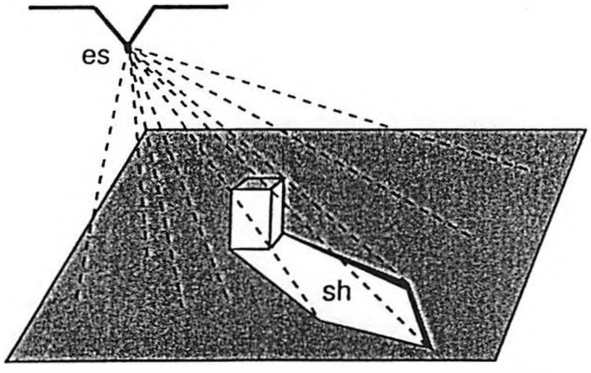



Figure 5-18 Principle of shadowing technique. Heavy metal particles travel in straight lines from the evaporating source (es) and accumulate along areas of the specimen that face the source. Other areas (sh) will receive less coating and appear lighter (less dense) in the TEM.

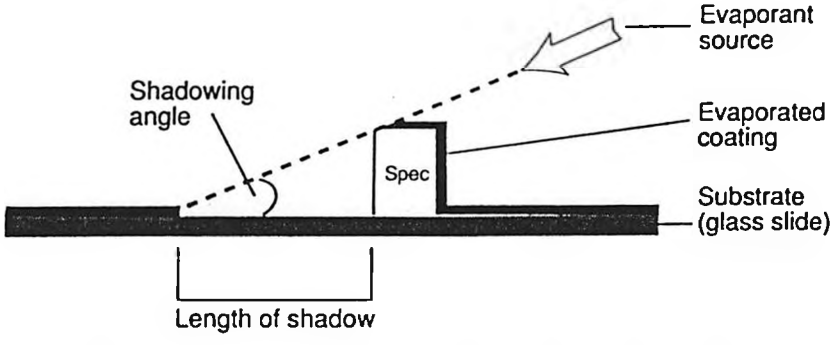



Figure 5-27 Principle of metal shadowing procedure showing deposition of metal along side facing filament source. The specimen physically prevents the vaporized metal from reaching certain areas of the substrate. These "shaded" areas will show up as electron dense, a white shadow will be generated.

Source: Bozzola, et al. (1992)

angle. To achieve this angle, we measured 2 cm up and 6 cm over from the evaporation site.

5. Pump the instrument down to vacuum according to the instructions provided. When sufficient vacuum is reached ($10^{-5}$ torr at least), evaporate the metal onto the specimen. The grids can now be examined directly in the electron microscope.

## 11.0VIEWING GRIDS WITH THE TRANSMISSION ELECTRON MICROSCOPE

The grids were placed in the specimen holder of the TEM (Hitachi HS-9) and then inserted into the TEM column after the vacuum was maintained. The high voltage was turned on, the filament was saturated, and the specimen was scanned without using apertures. An area being viewed can have the contrast increased by using apertures.

## 12.0 TEM PHOTOGRAPHY

After pictures were taken on the TEM microscope, the negatives produced were enlarged by the Durst Laboratory S-45EM enlarger. Quick prints were developed on Kodak Polymax RC paper using the Insta-print processor (model D-12). The best quick prints were chosen for the final printing. Final prints were made using Agfa Brovira fibre paper, grade 4 or 5. After the tray processing, the prints were washed in the Arkey Loadmaster print washer and dried in the Johnke print dryer. The prints were then mounted on smooth surface bristol weight paper.

:1

**FIG. 1. TONGUE Mus musculus x74**

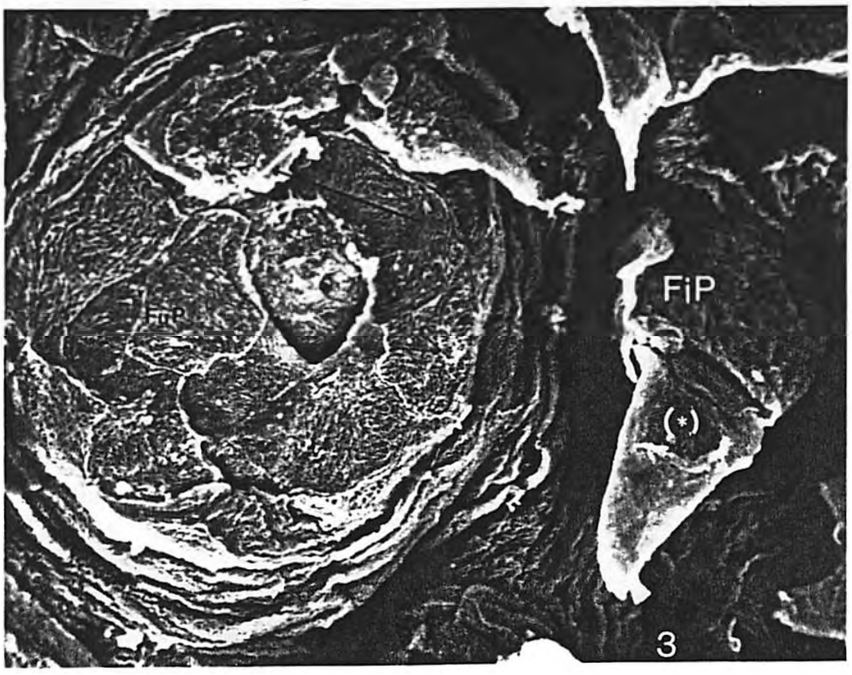
The micrograph in Fig. 1. Shows the dorsal surface of the mouse tongue. The dorsal surface is divided into symmetrical halves by a furrow called the median sulcus (**MS**). The epithelial layer of the tongue is specialized into surface projections called papillae. (Kessel and Kardon,1979). The different types of papillae can be seen clearly. The more numerous, conical projections are filiform papillae (**FiP**), and the less numerous, scattered, and flat-looking projections are fungiform papillae (**FuP**).

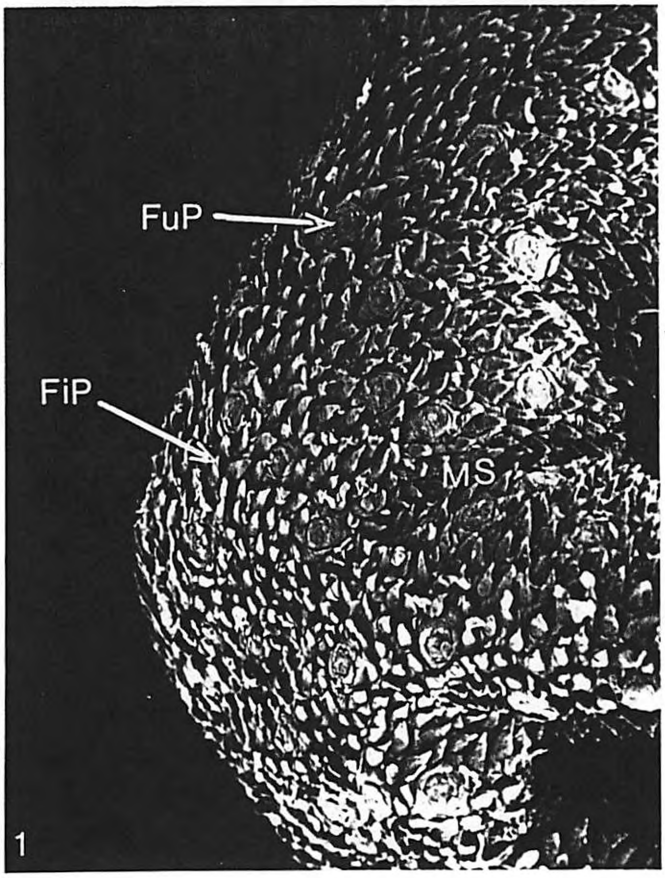
**FIG. 2. TONGUE Mus musculus x310**

This is an enlargement of three fungiform papillae (**FuP**) in a line down the center. These three are surrounded by several filiform papillae (**FiP**). This micrograph clearly shows that the fungiform papillae are larger. The flattened surfaces of the fungiform papillae are often layered with squamous cells (Kessel and Kardon,1979).

**FIG. 2. TONGUE Mus musculus x1,210**

Only one filiform papillae (**FiP**) and one fungiform papillae (**FuP**) are seen in this micrograph. The tip of the filiform papilla is covered in cornified squamous epithelium (*), which most likely helps the animal when feeding. The filiform papillae is richly supplied with sensory nerves and also serve as sensory organs for tactile sensation (Kessel and Kardon,1979). Each papilla is "shingled" with layers of flattened cells that form a stratified epithelium. Dead cells on the top layer are shed and replaced with new cells (Kessel and Kardon,1979). It appears that the fungiform papillae in this micrograph have shed its top layer of cells (arrow).


FiP
(*)
3


FuP
FiP
MS
1


FyP
EiP
2

:2

**TONGUE** **Mus musculus** **x310**

This micrograph shows a cross sectional view of the tongue. The uppermost portion of the micrograph displays the surface structures called filiform papillae (FiP). Immediately below the surface is a layer of intrinsic muscles (I) that have fibers running in various directions to modify the shape of the tongue by folding and curling it. Extrinsic muscle tissue (M) lies below the intrinsic muscle layer along with connective tissue and nerve fibers.

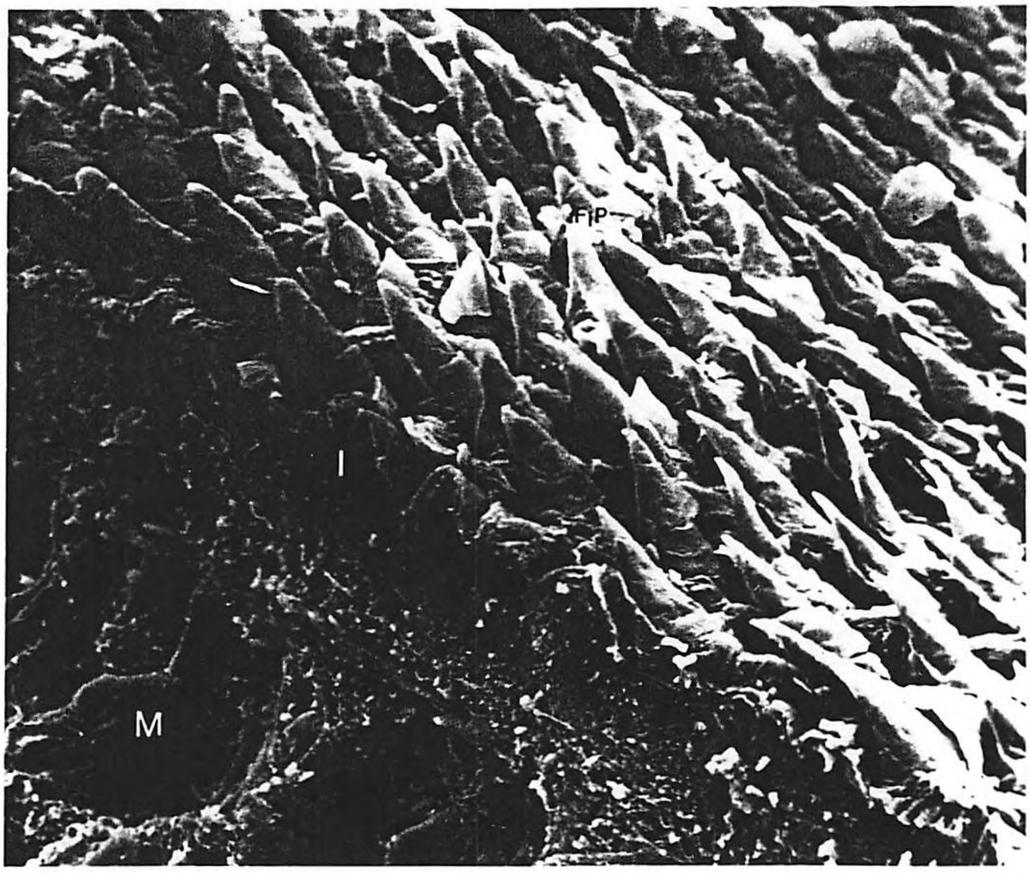
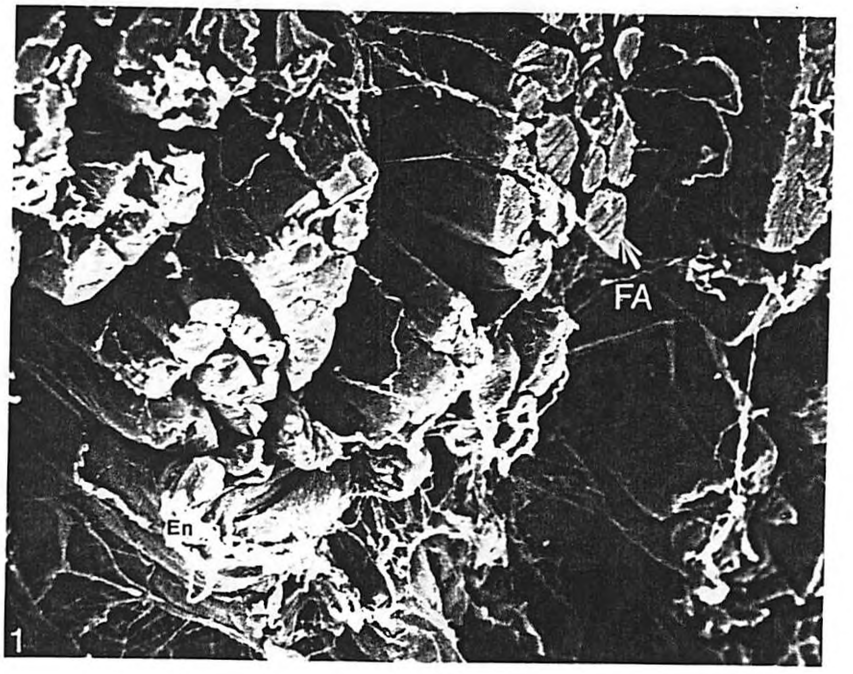

FIP
I
M

**FIG. 1. STRIATED MUSCLE TISSUE Mus musculus x210**

Fig. 1. shows striated muscle tissue that has been cut in a cross-sectional manner. This muscle is surrounded by a connective tissue sheath called epimysium; inward extensions of this sheath, called perimysium, subdivide the muscle into bundles called fascicles (FA) (Kessel and Kardon,1979).

**FIG. 2. STRIATED MUSCLE TISSUE Mus musculus x200**

Fig. 2. shows a longitudinal view of the striated muscle tissue bundles (FA). The white connective tissue, elements of endomysium (En), are present around individual muscle fibers (Kessel and Kardon,1979).


FA
En
1

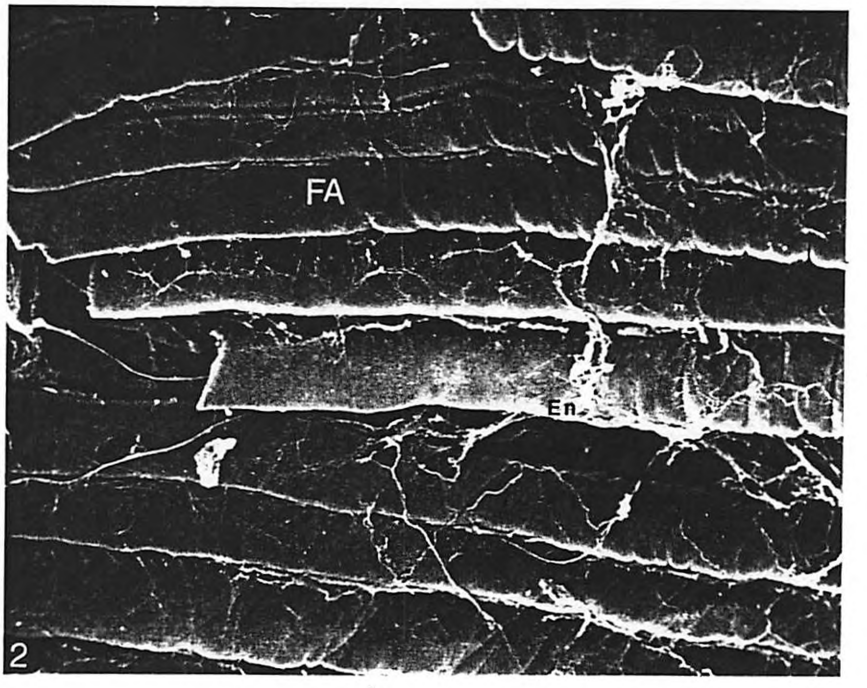

FA
En
2

**FIG. 1. TRACHEA Mus musculus x930**

This micrograph shows a longitudinally cut trachea. The bottom of the micrograph depicts the inside of the trachea or the epithelial surface (**EP**). The top shows the outside of the trachea, which is mainly connective tissue. The trachea is surrounded by rings of hyaline cartilage (**HC**). Chondrocytes (**C**) can be seen within the rings (Kessel and Kardon,1979).

**FIG. 2. TRACHEA Mus musculus x1,800**

This is an enlargement of the epithelial surface of the mouse trachea. The ciliated cells (**Ci**) are very apparent with more than 200 cilia per cell. Goblet cells (**G**) appear as flat non-ciliated regions. However, goblet cells are covered with short microvilli.

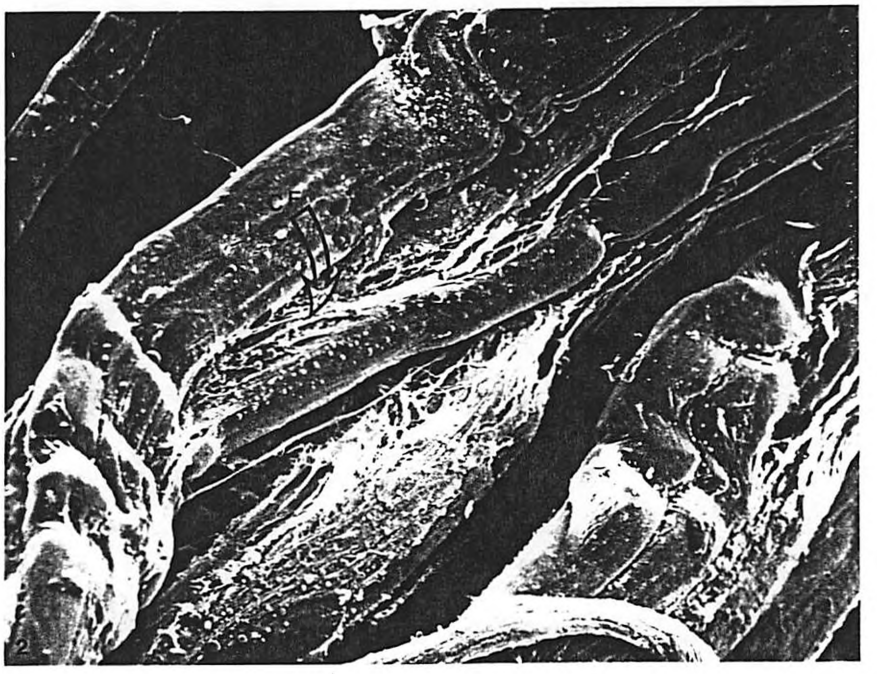

C
HC
EP

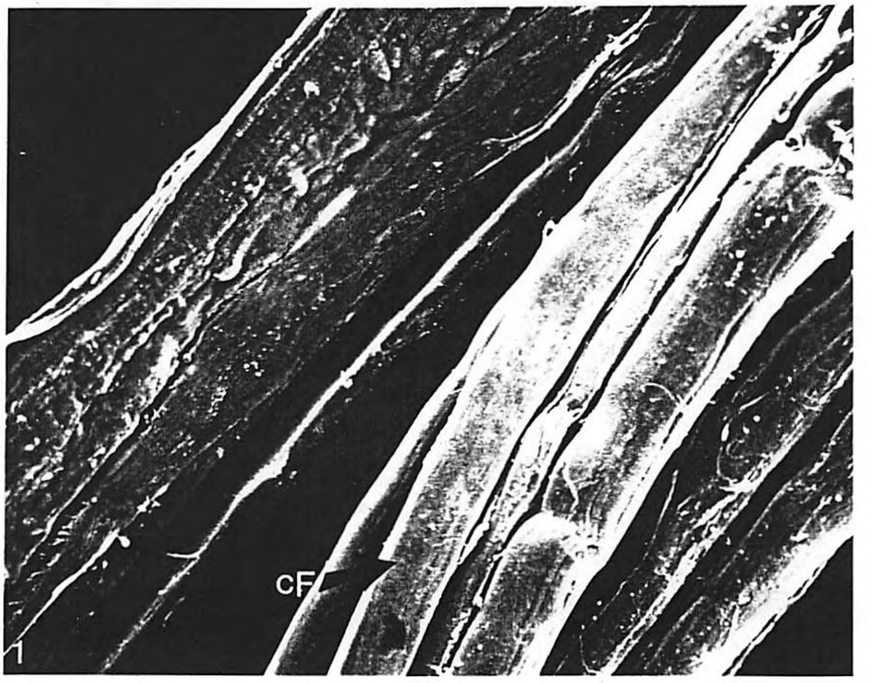

G
Ci

**FIG. 1. CONNECTIVE TISSUE** *Mus musculus* **x139**

**FIG. 2. CONNECTIVE TISSUE** *Mus musculus* **x186**

Fig. 1. and Fig. 2. Both represent bundles of collagen fibers (CF). These bundles are the foundation of the mouse tendons shown.




cF
1

:6

FIG. 1.CONNECTIVE TISSUE Mus musculus x187,500

Fig. 1. shows a negatively stained collagen fiber (CF). The banding that is visible is explained in Figure 6-4.

FIG. 2. CONNECTIVE TISSUE Mus musculus x60,000

This micrograph shows a shadowed collagen fiber (CF). The major bands are still visible. However, the banding of collagen is more clearly seen through the use of negative staining techniques, such as those used in Fig. 1.

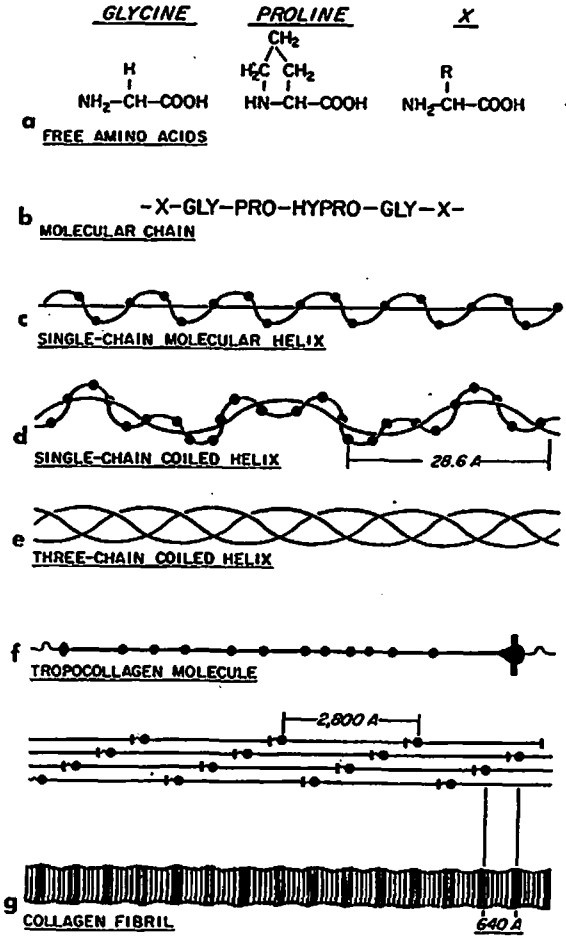
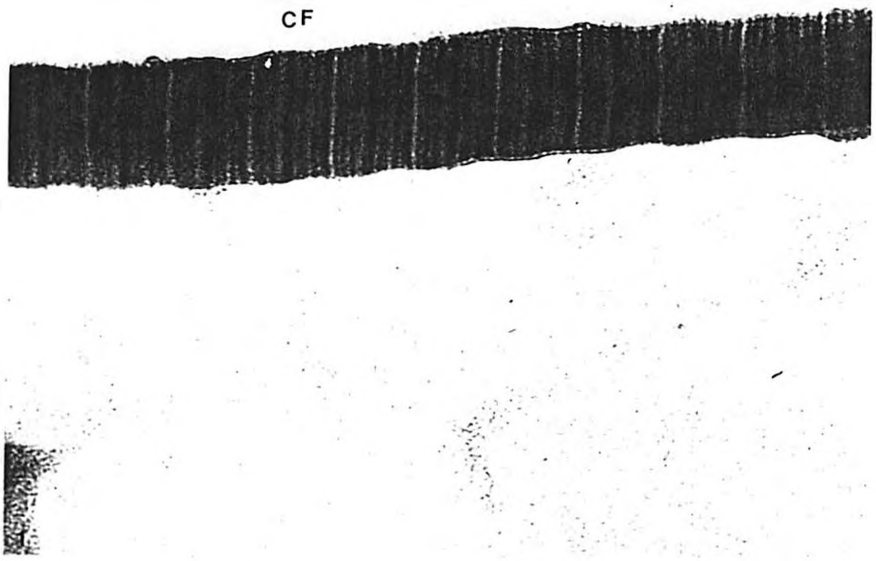
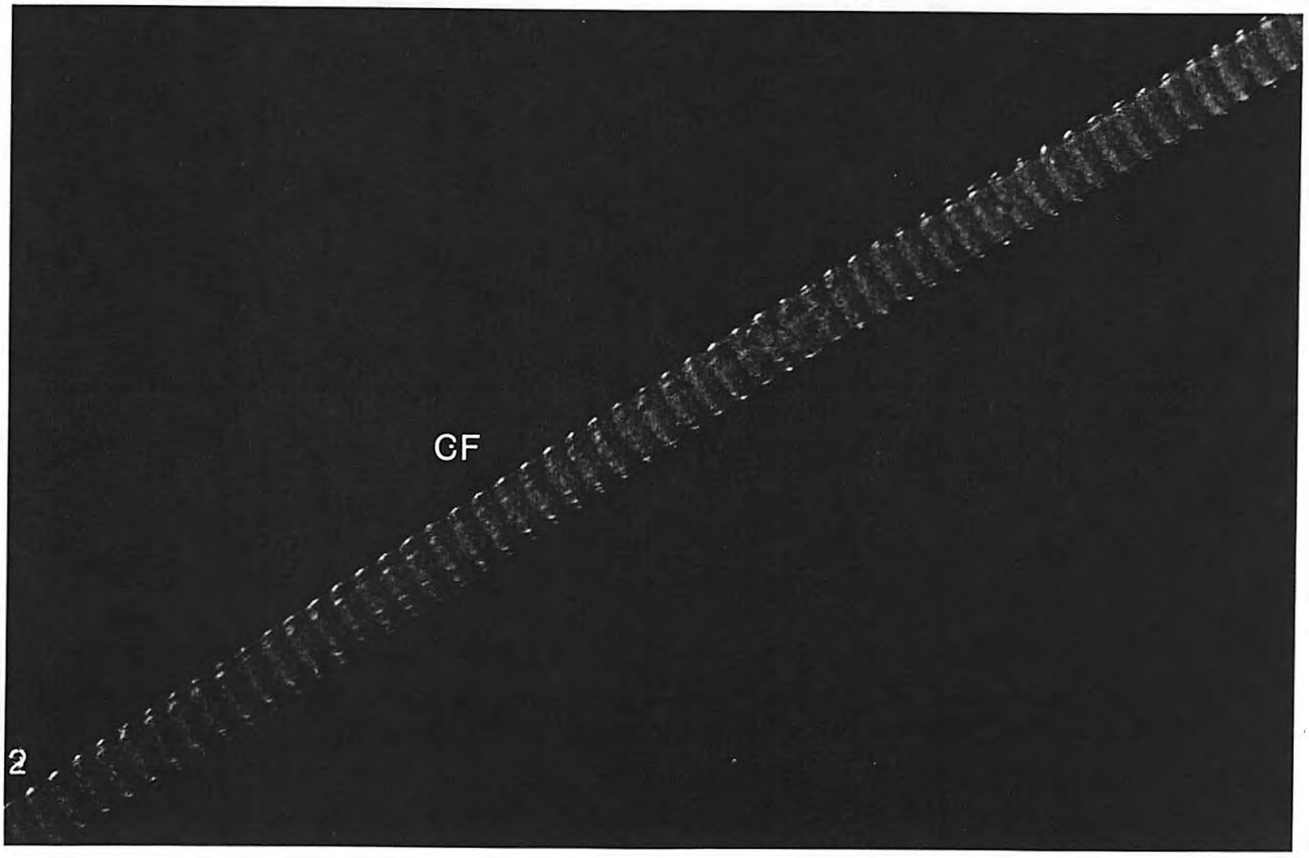



*Figure 6-4* Diagram depicting the formation of collagen, which can be visualized as taking place in seven steps. The starting materials (*a*) are amino acids, of which only two are shown; the side chain of any of the others is indicated by R in amino acid X. (*b*) The amino acids are linked together to form a molecular chain. (*c*) This then coils into a left handed helix (*d* and *e*). Three such chains then intertwine in a triple stranded helix, which constitutes the tropocollagen molecule (*f*). Many tropocollagen molecules become aligned in staggered fashion, overlapping by a quarter of their length to form a cross striated collagen fibril (*g*). (Redrawn and slighdy modified from Collagen by J. Gross. Copyright © May 1961 by Scientific American, Inc. All rights reserved.)

SOURCE: BLOOM. (1975)

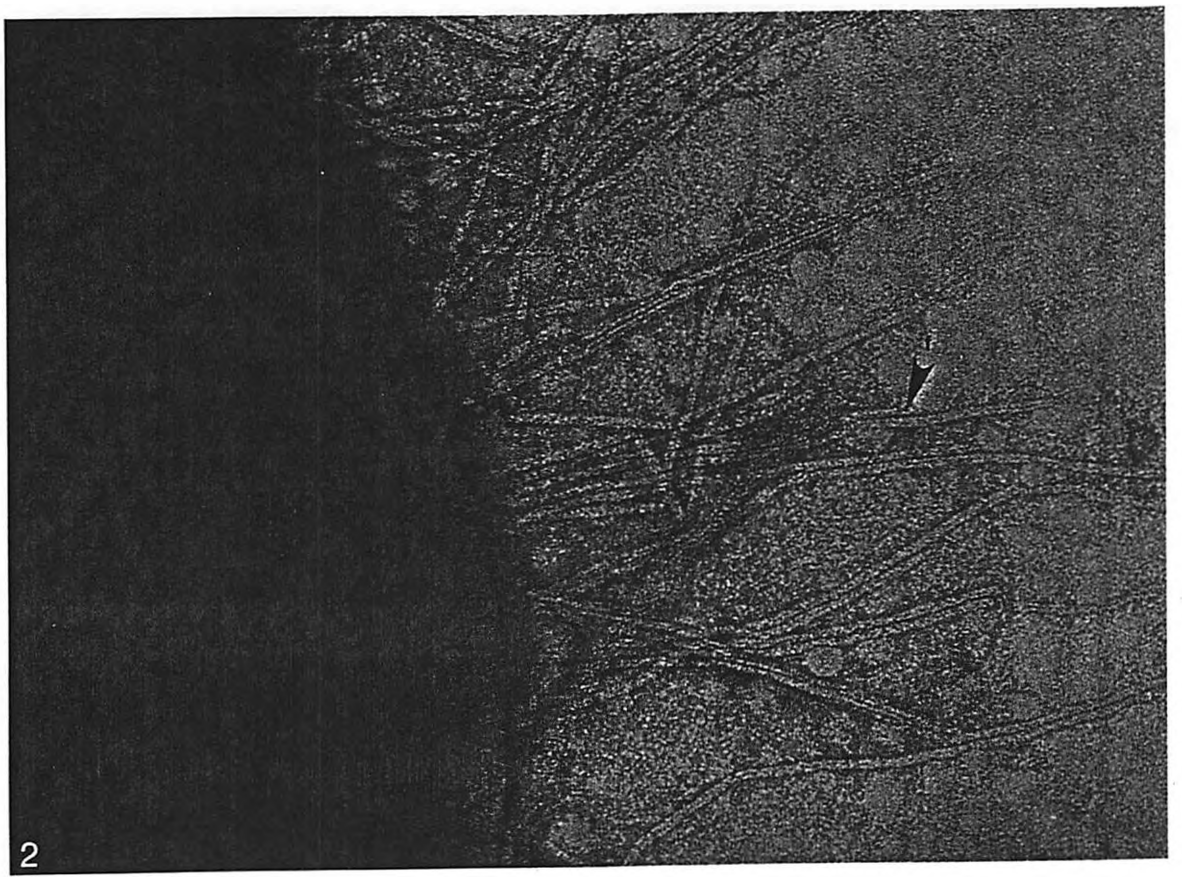

CF


CF
2

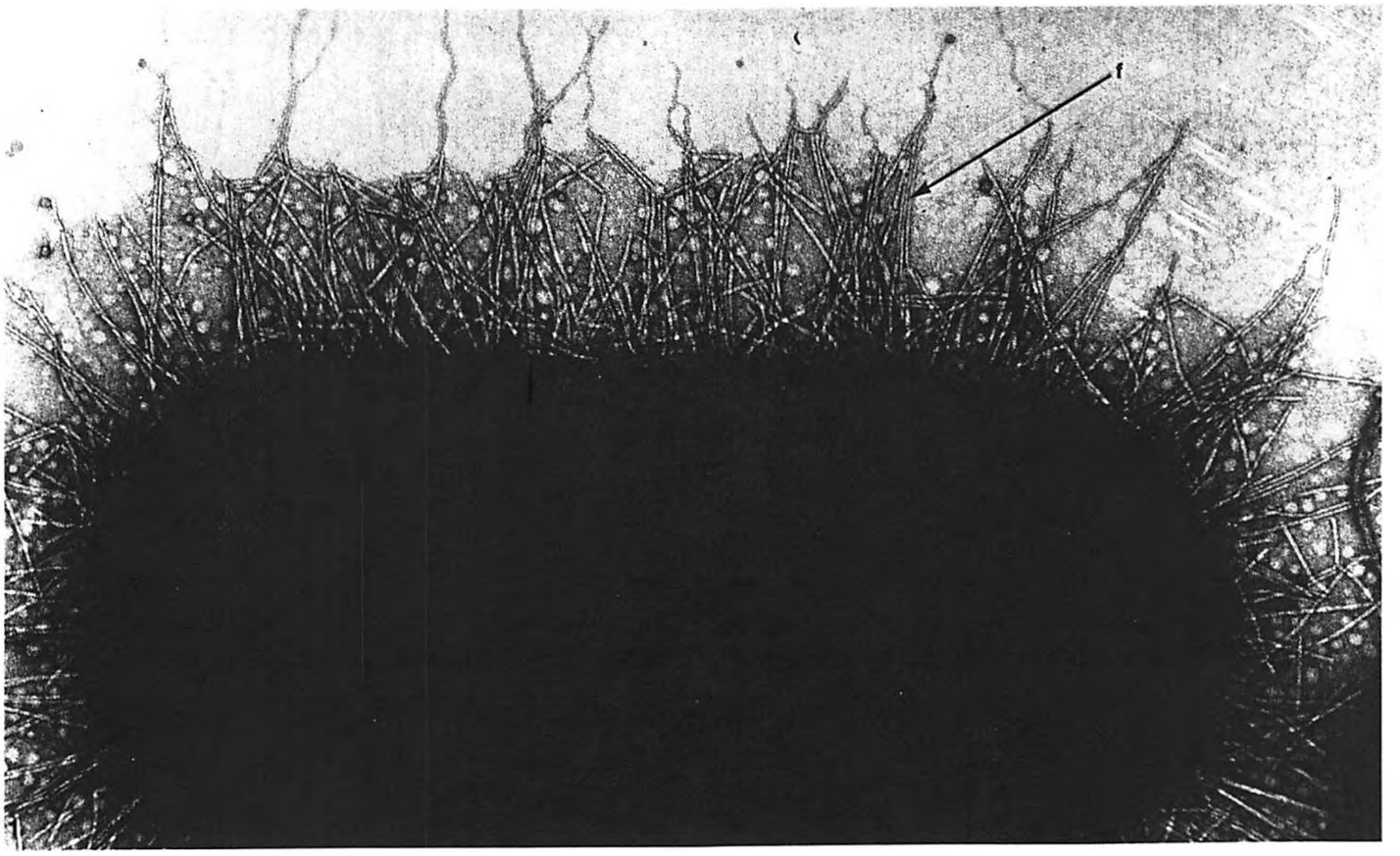
:7

**CONNECTIVE TISSUE** **Mus musculus** x60,000

This micrograph shows shadowed collagen fibers (CF) that are intertwined to form "Blair Witch" collagen.



2



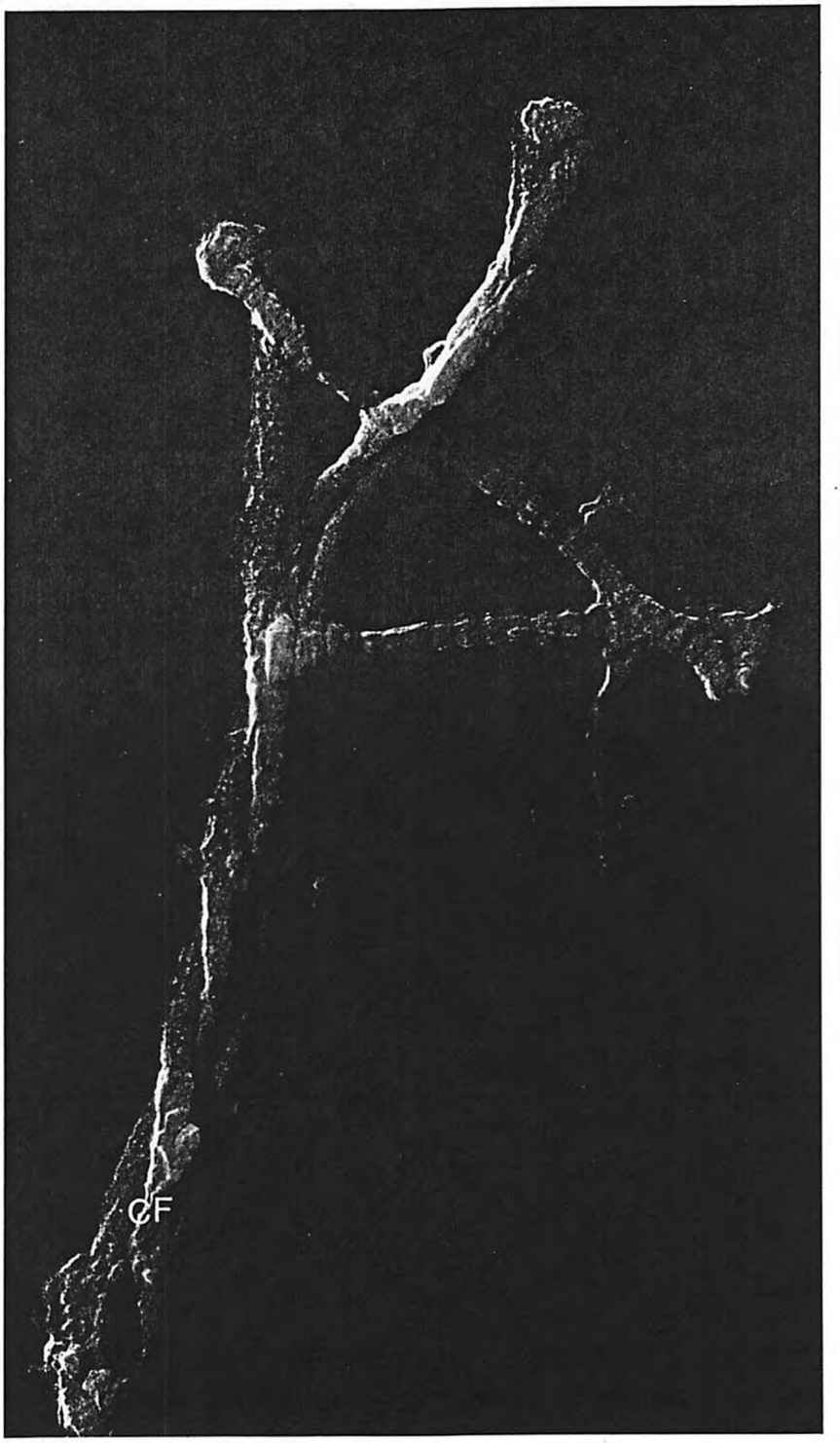

CF

**FIG. 1. Negative Stained Enterobacter aerogenes x75,000**

This micrograph shows a bacterium with its many pili or fimbriae (f) (Prescott, et al. 1999).

**FIG. 2. Negative Stained Enterobacter aerogenes x200,000**

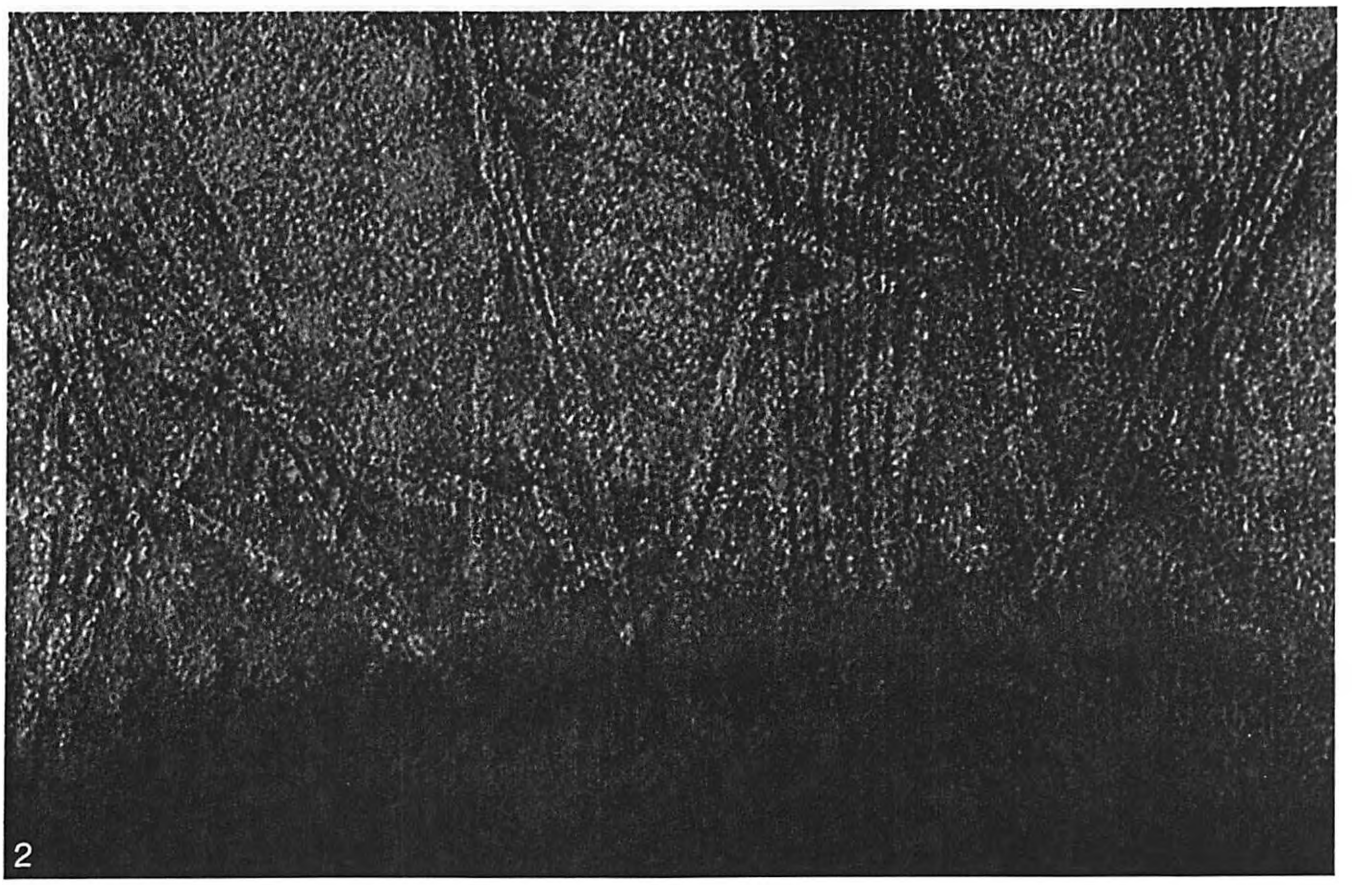
This is simply an enlargement of the fimbriae (f) in Fig. 1.

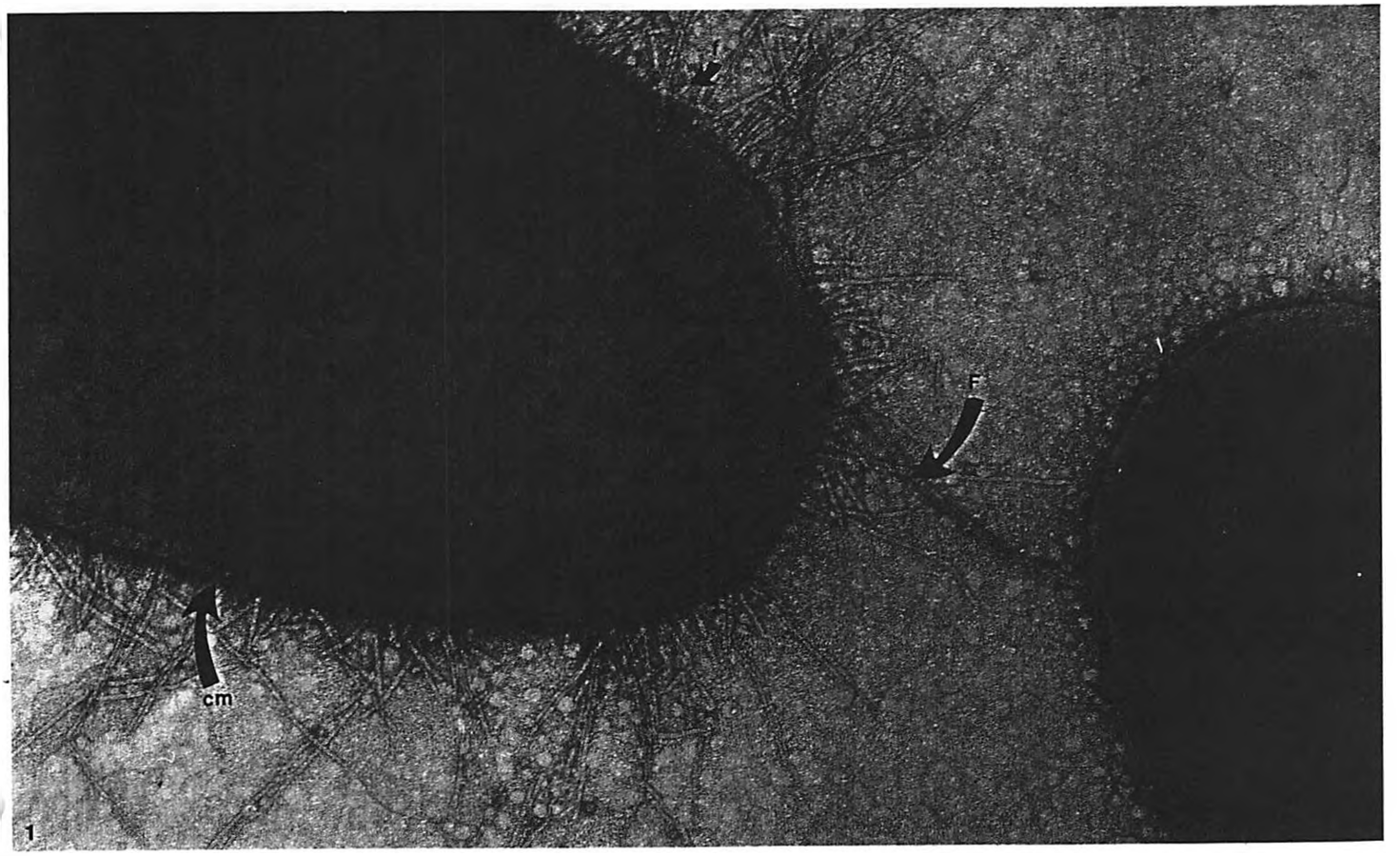
**FIG. 1. Negative Stained Enterobacter aerogenes x90,000**

The cell membrane (cm) can be seen clearly in this micrograph. This also allows for the distinction between flagella (F) and fimbriae (f) to be made. The flagellum is much larger than the fimbriae that surround it.

**FIG. 2. Negative Stained Enterobacter aerogenes x425,000**

This is an enlargement of the fimbriae (f) and flagellum (F) in Fig. 1.

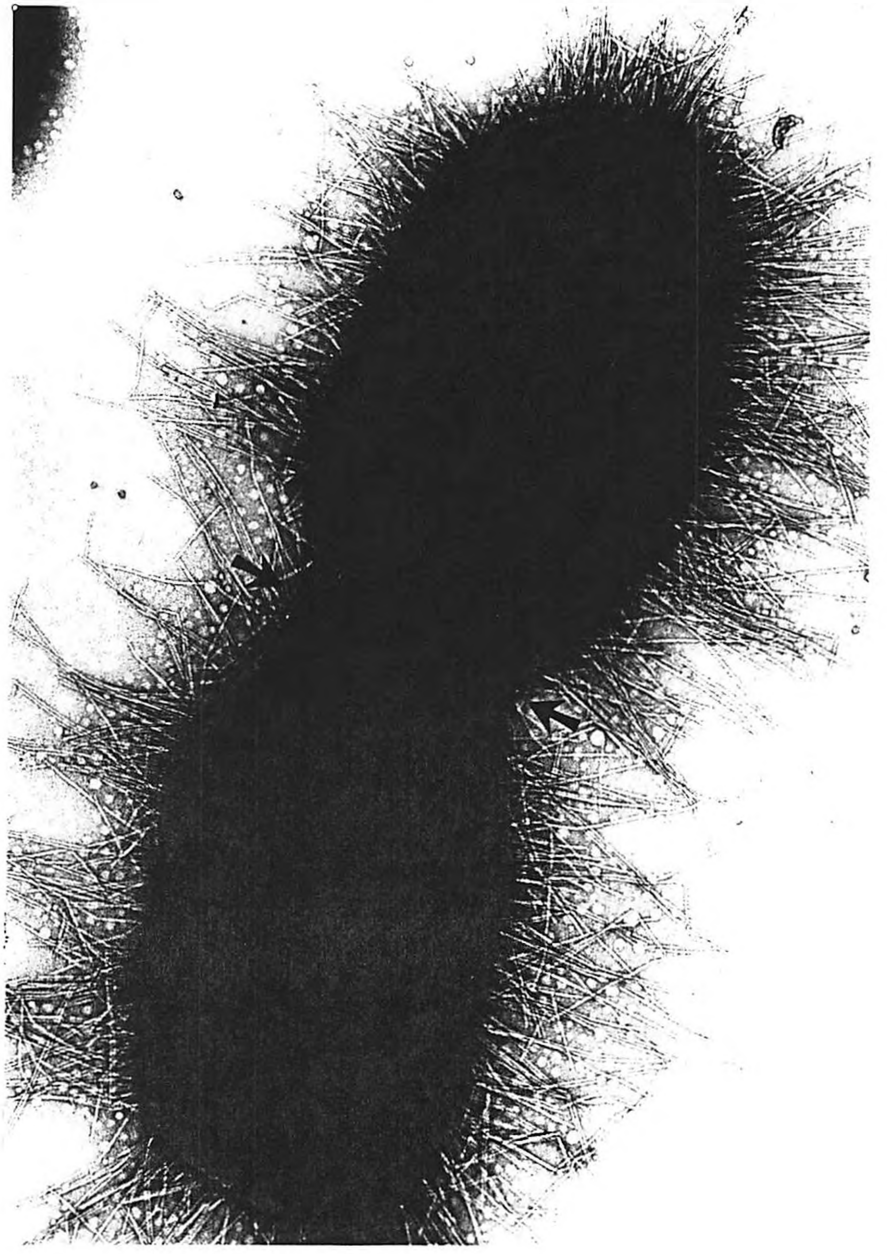

2


F
cm
1

:10

**Negative Stained <u>Enterobacter aerogenes</u> x55,000**

This micrograph shows many fimbriae (f). It is also apparent that the bacterium is dividing by fission (between the arrows).



:11

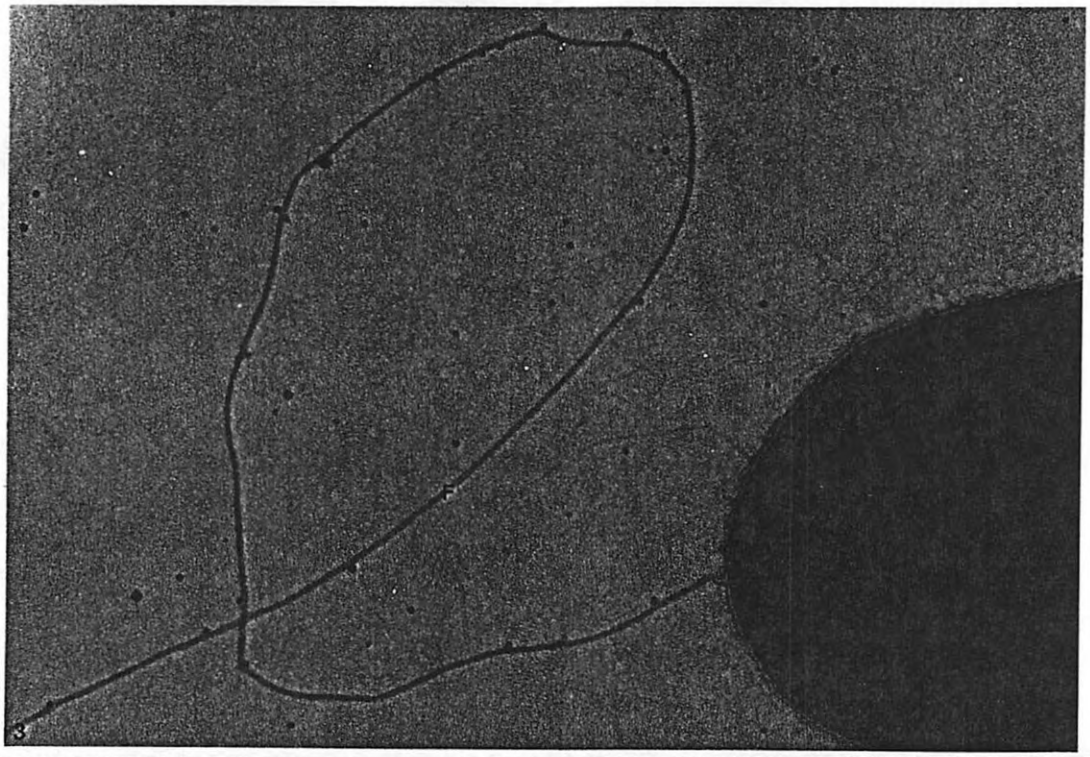
**Negative Stained Enterobacter aerogenes**

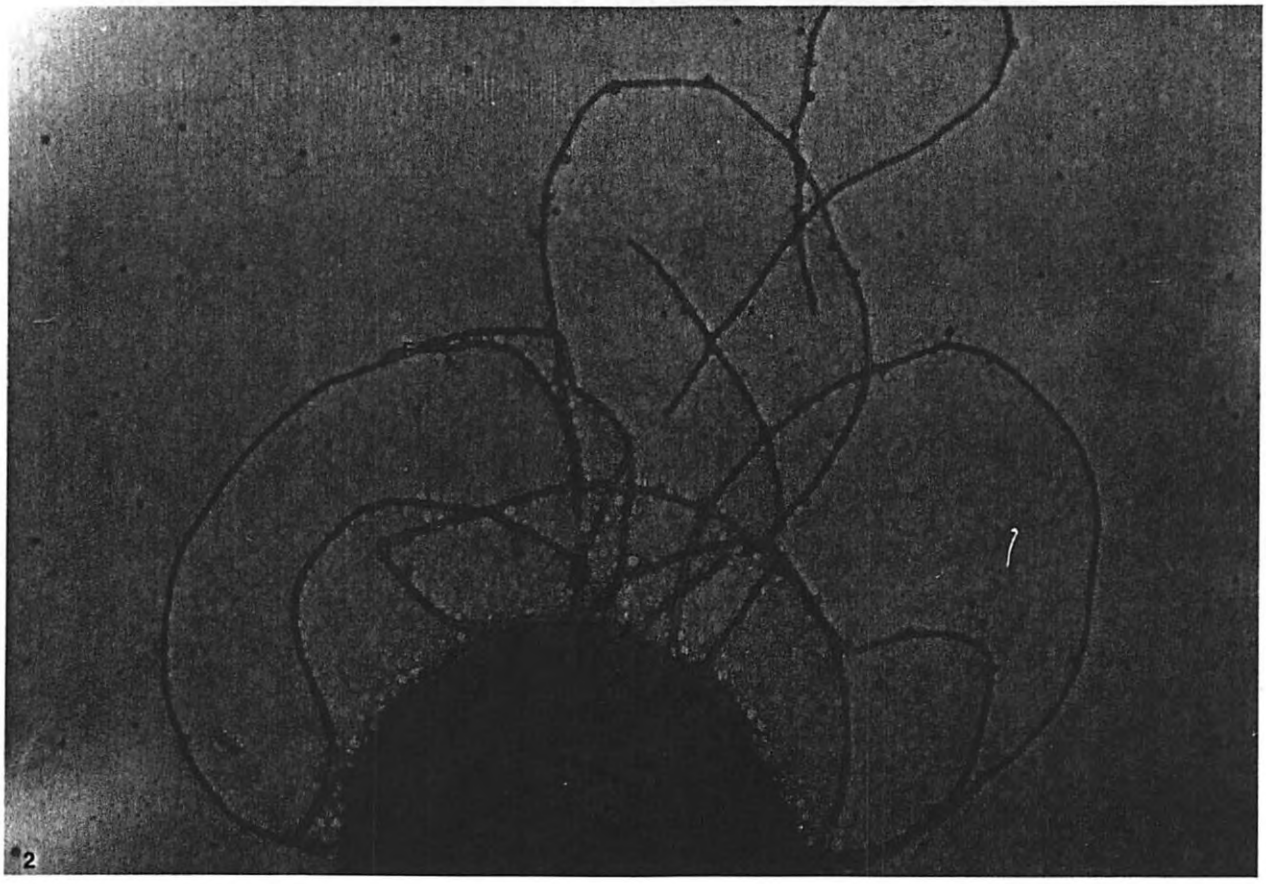
**FIG. 1. X27,500**

**FIG. 2. X55,000**

**FIG. 3. X55,000**

These micrographs all demonstrate that a bacterium can appear without any fimbriae. The flagella (**F**) are all labeled.


3

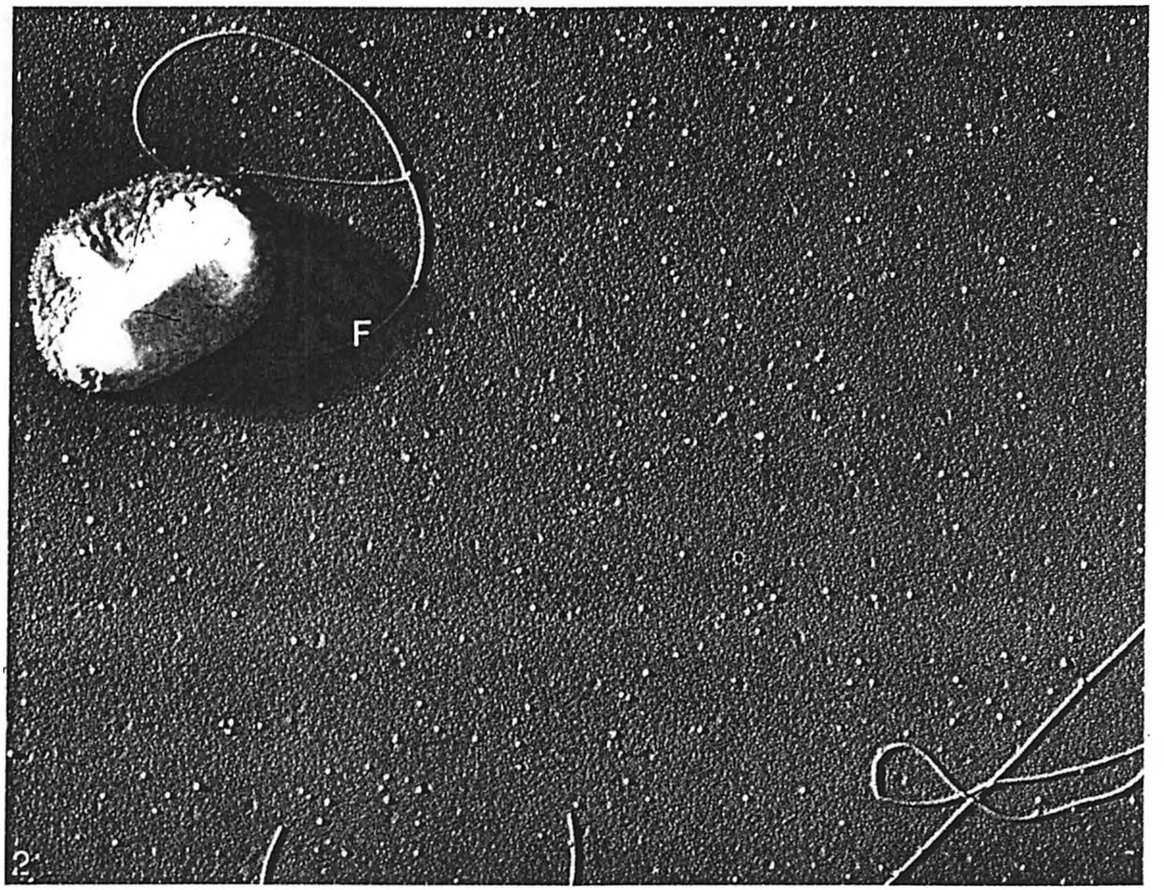

2

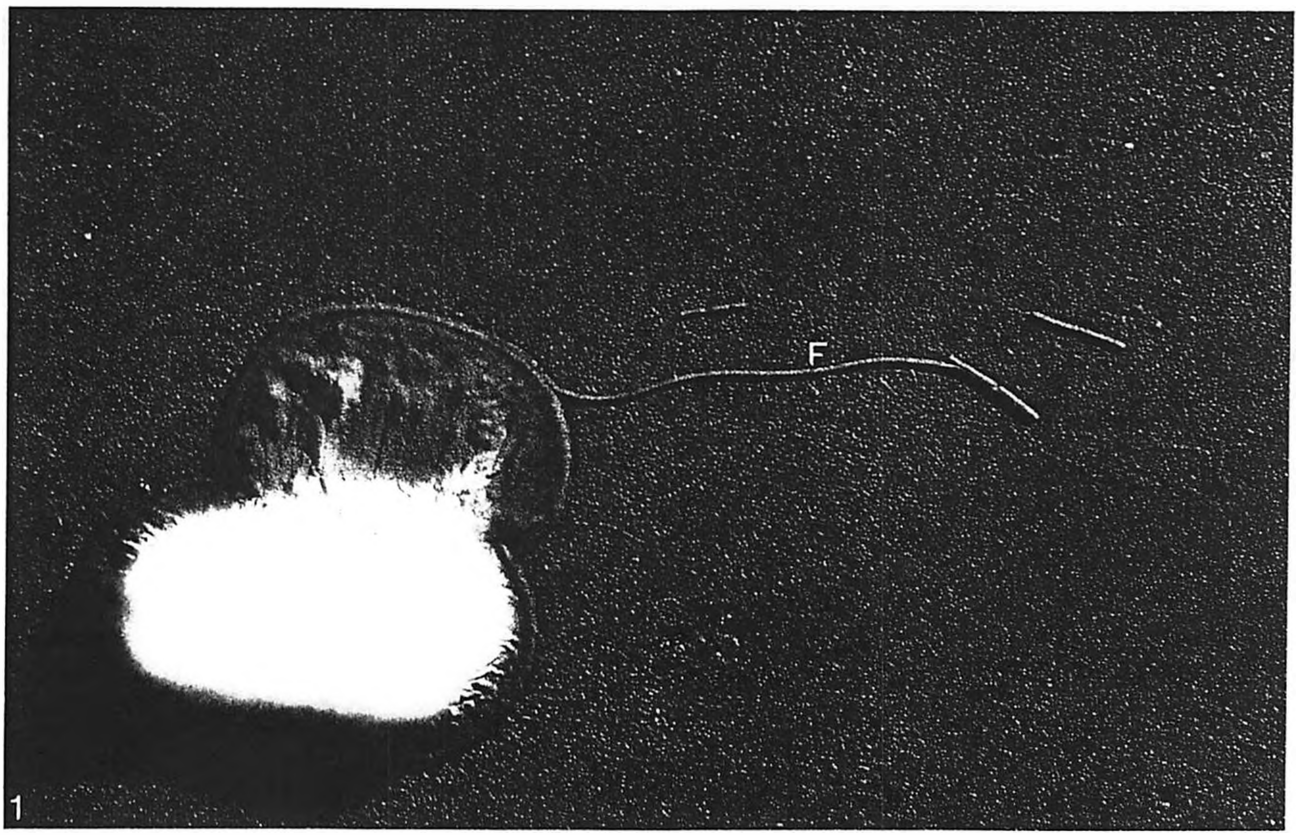
Negative Stained & Shadowed Enterobacter aerogenes

FIG. 1. X37,500

FIG. 2. X20,000

The shadowing gives these micrographs a three dimensional appearance. The flagella (F) appear more rounded using this technique.


F
2


F
1

:13

**Negative Stained & Shadowed <u>Enterobacter aerogenes</u> x20,000**

The shadowing allows the three dimensional nature of fimbriae (f) to be more thoroughly examined.

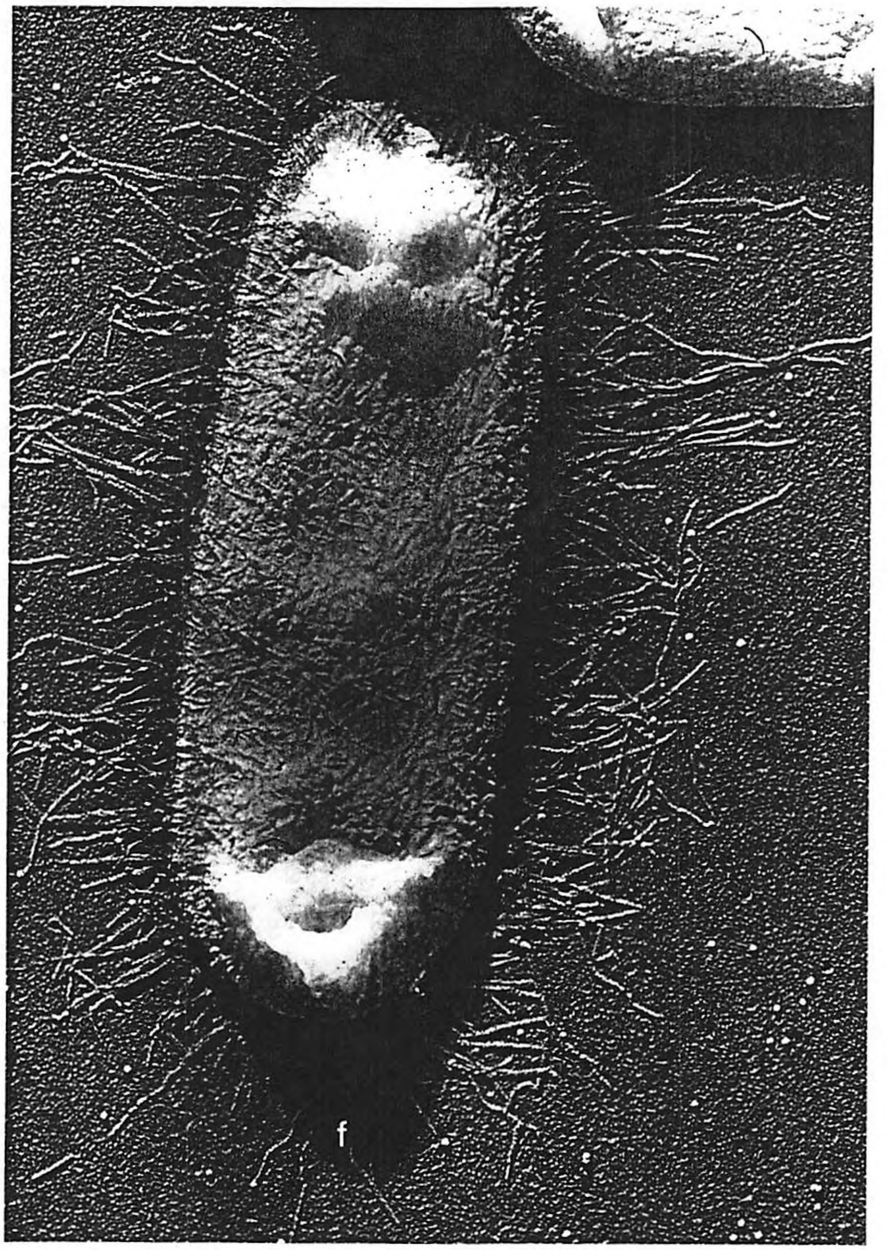
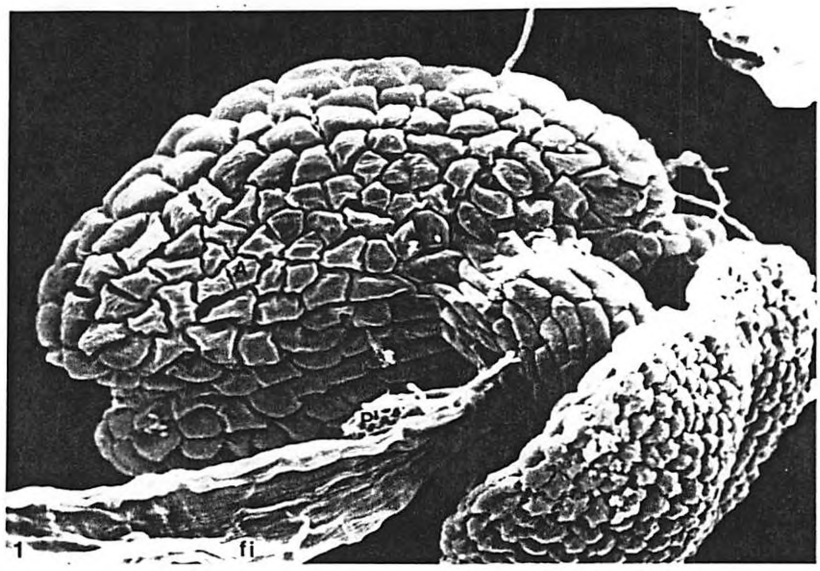

f

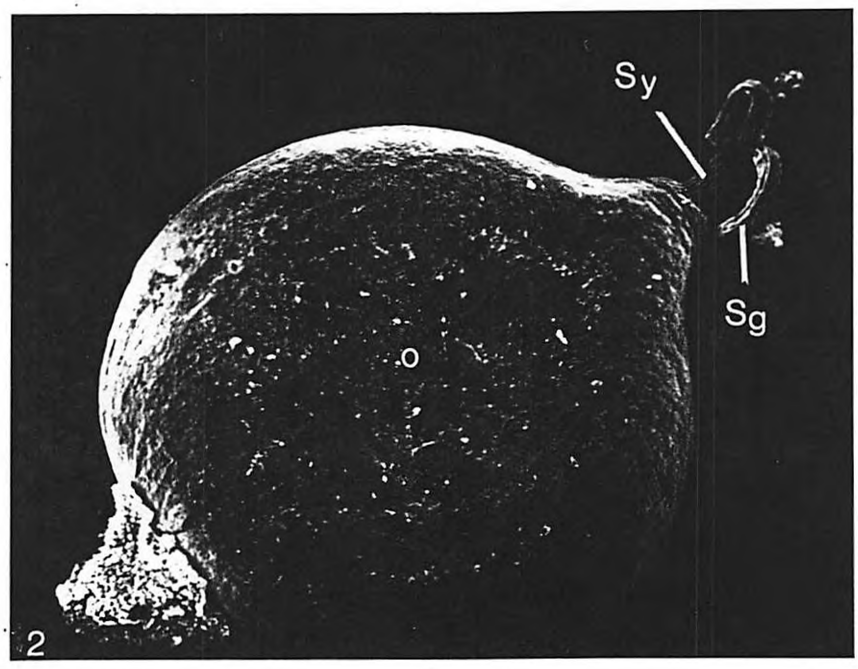
FIG. 1. KNOTWEED X330

This is the stamen of the flower parts. It is made up of the filament (fi) and the anther (A). The pollen grains reside on the anther. One pollen grain (P) can be seen.

FIG. 2. KNOTWEED X37

This micrograph shows the pistil of the flower parts. The ovary (O), style (Sy), and stigma (Sg) can be seen.


fi
1


Sy
Sg
o
2

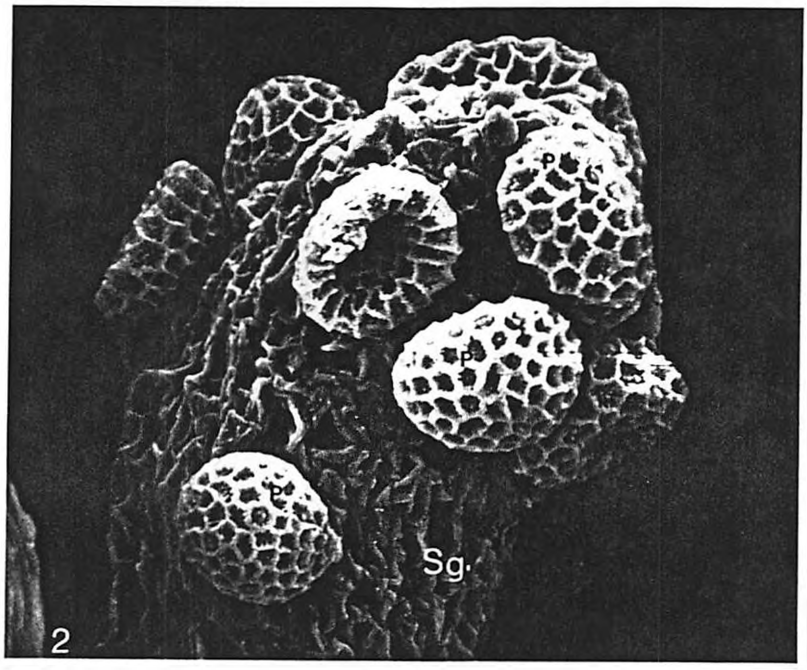
:15

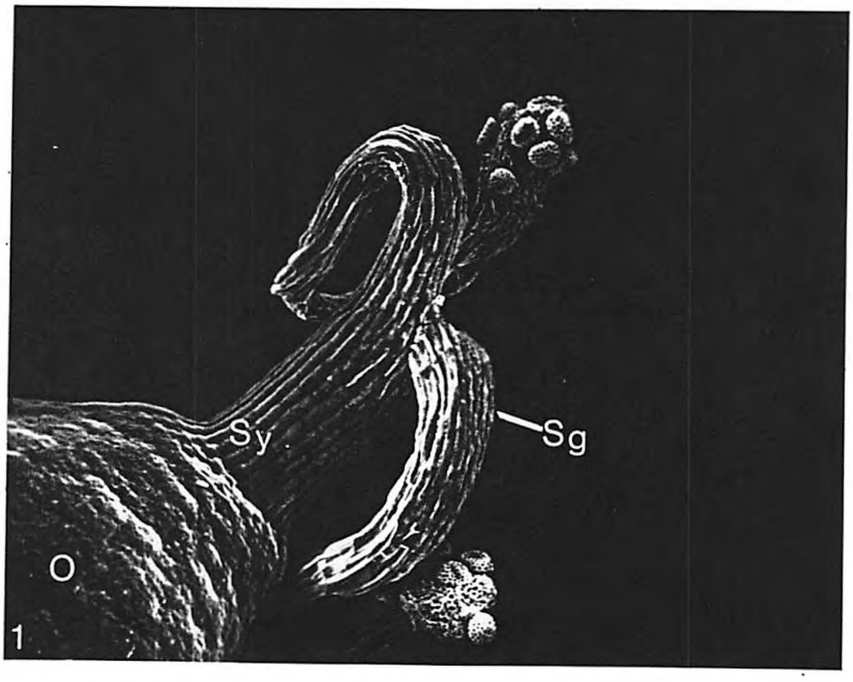
FIG. 1. KNOTWEED X127

This is an enlargement of the ovary (O), style (Sy), and stigma (Sg).

FIG. 2. KNOTWEED X750

This enlargement of the stigma (Sg) shows how pollen grains (P) attach to germinate the pistil.

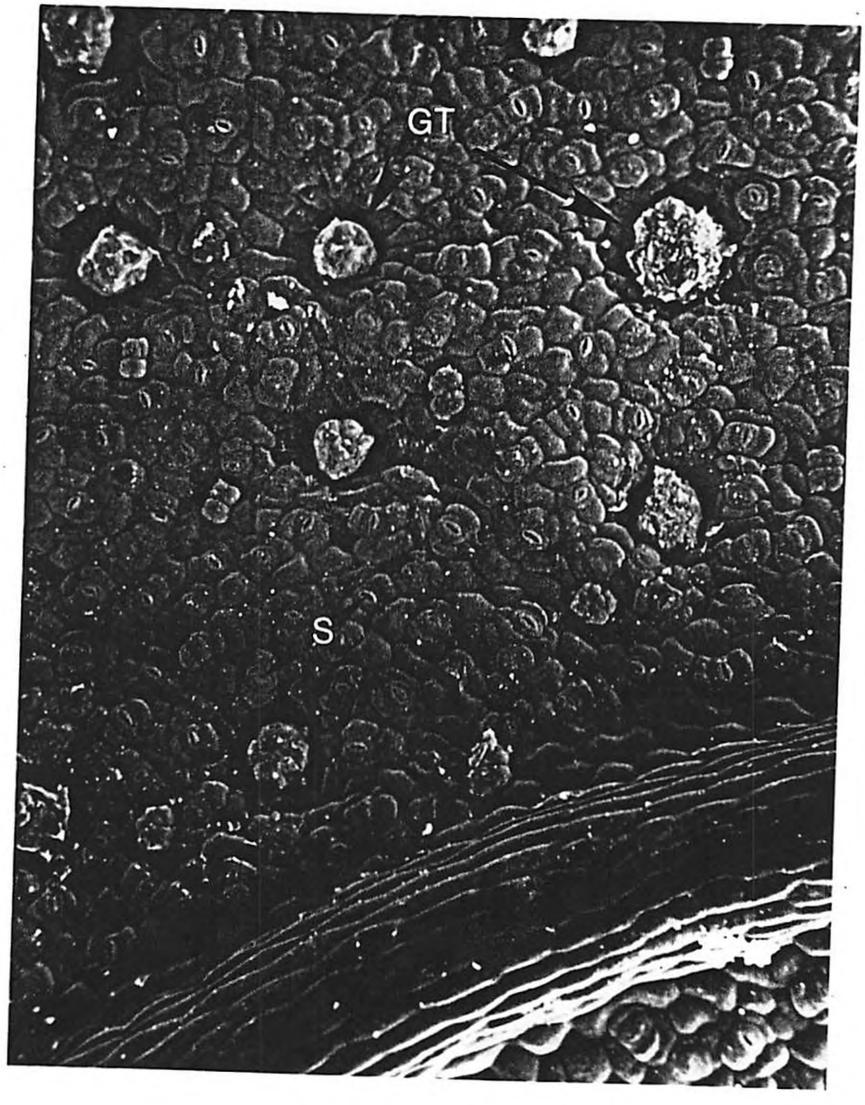

Sg.
2


Sy
Sg
O
1

:16

**KNOTWEED X238**

This micrograph shows the ventral surface of the leaf. There are many multicellular glandular trichomes (**GT**) and stoma visible.


GT
S

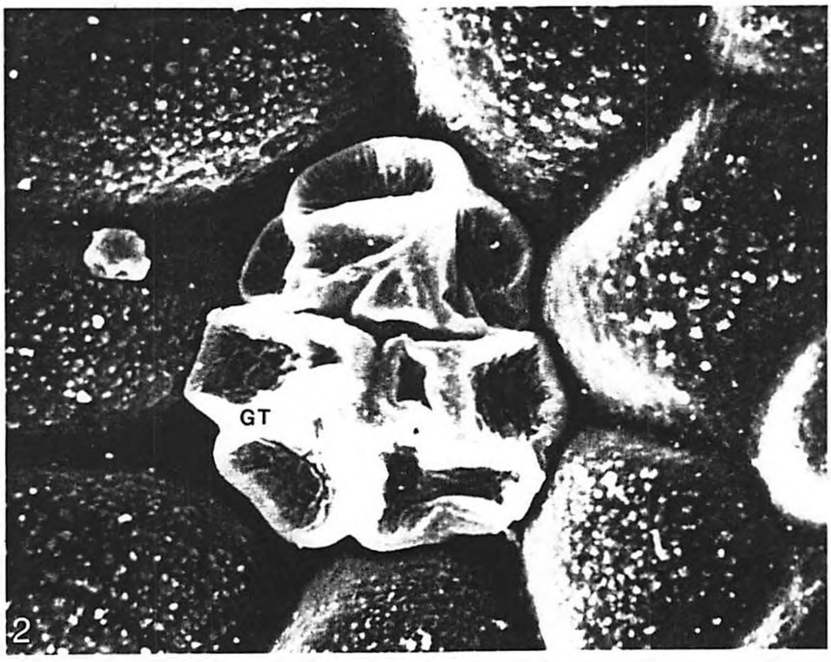
:17

FIG. 1. KNOTWEED X1,380

FIG. 2. KNOTWEED X1,840

These both show multicellular glandular trichomes (GT). Fig. 1. also shows a higher magnification of stomata (S).

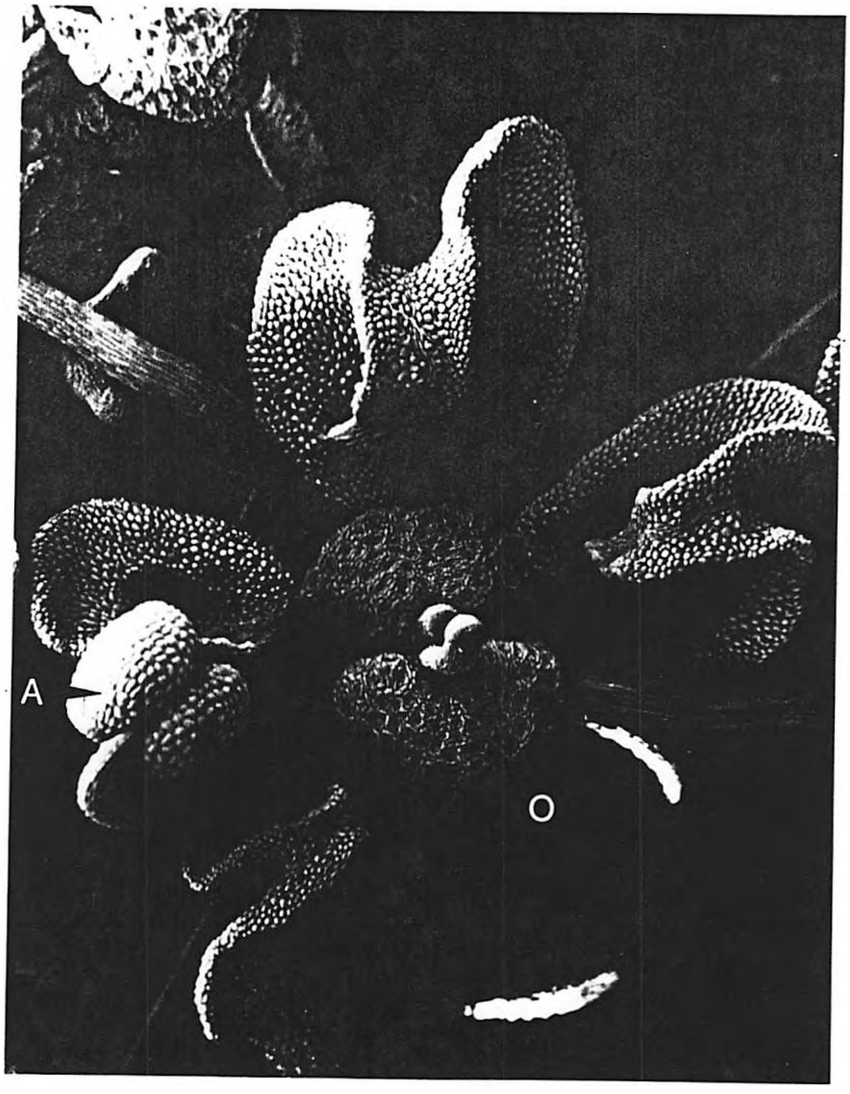

S
GT
1.


GT
2

:18

**QUEEN ANNE'S LACE x56**

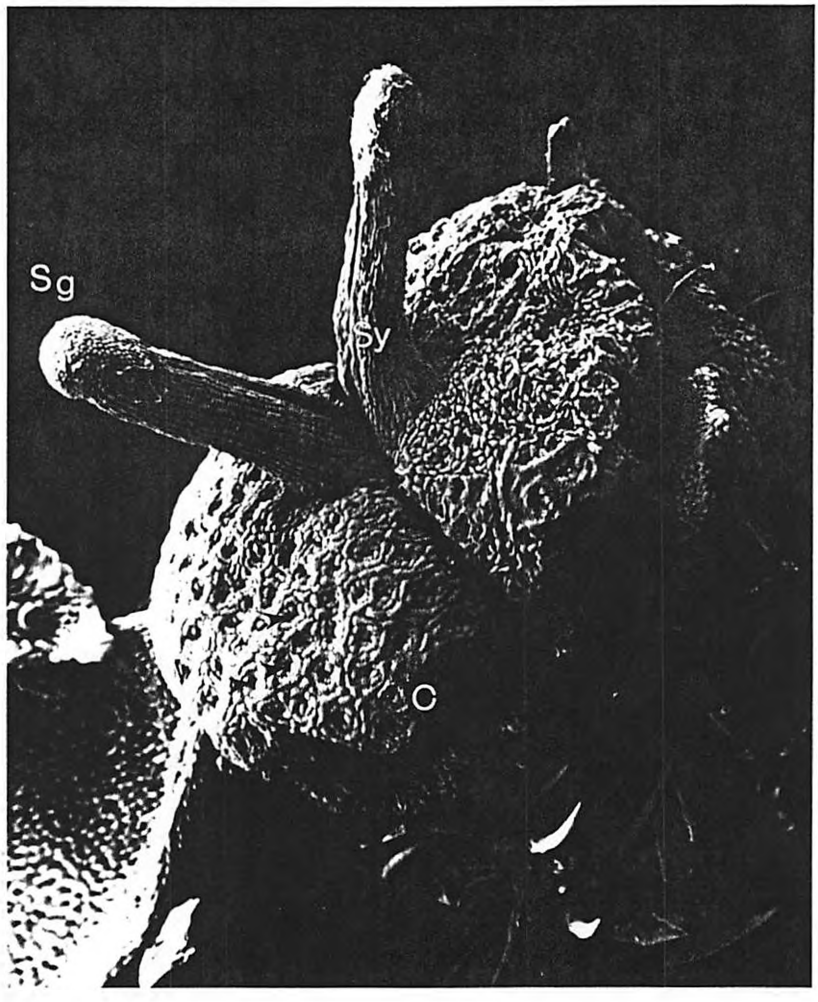
This micrograph shows the flower parts. The anther (A) and ovary (O) are seen clearly.


A
O

:19

**QUEEN ANNE'S LACE x137**

In this enlargement, the style (**Sy**), Stigma (**Sg**), and ovary (**O**) are clear. The ovary seems to have tiny openings in its surface (arrows).


Sg
Sy
C

:20

**QUEEN ANNE'S LACE x1,300**

This micrograph shows the tiny openings in the surface of the ovary in more detail (arrows).

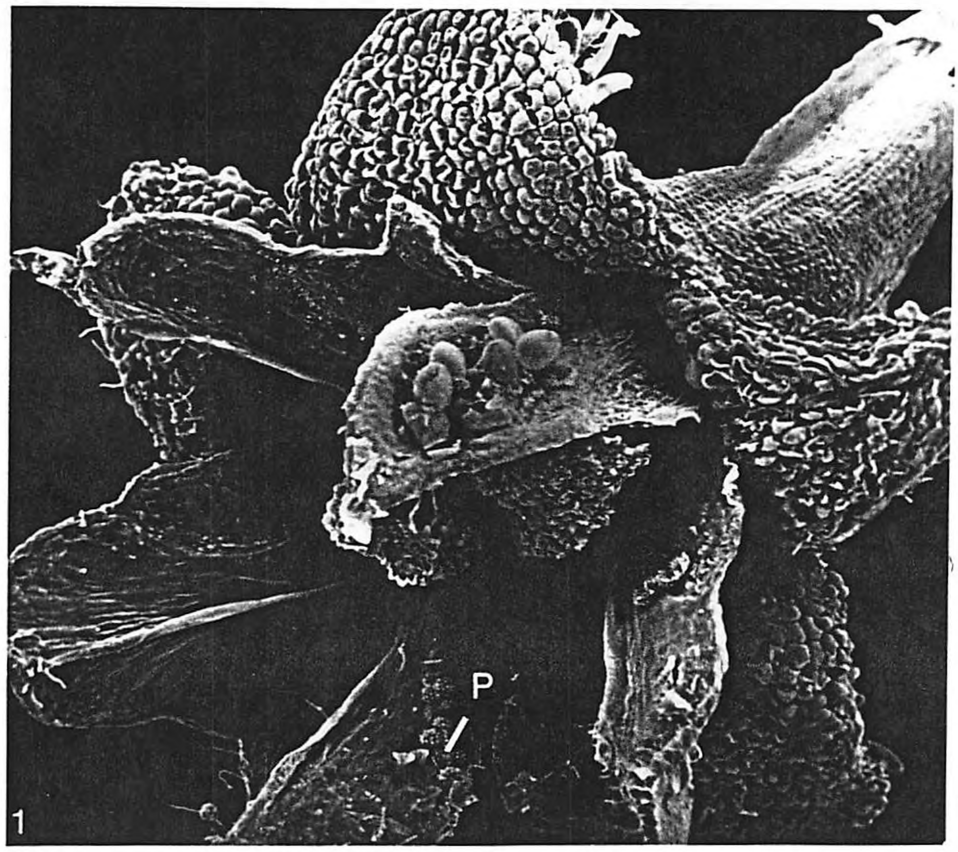
:21

**FIG. 1. BLACK-EYED SUSAN x116**

This micrograph shows the flower parts. Some pollen (P) is seen near the bottom.

**FIG. 2. BLACK-EYED SUSAN x1,405**

This micrograph shows the pollen (P) of black-eyed Susan at a higher magnification.


P
1

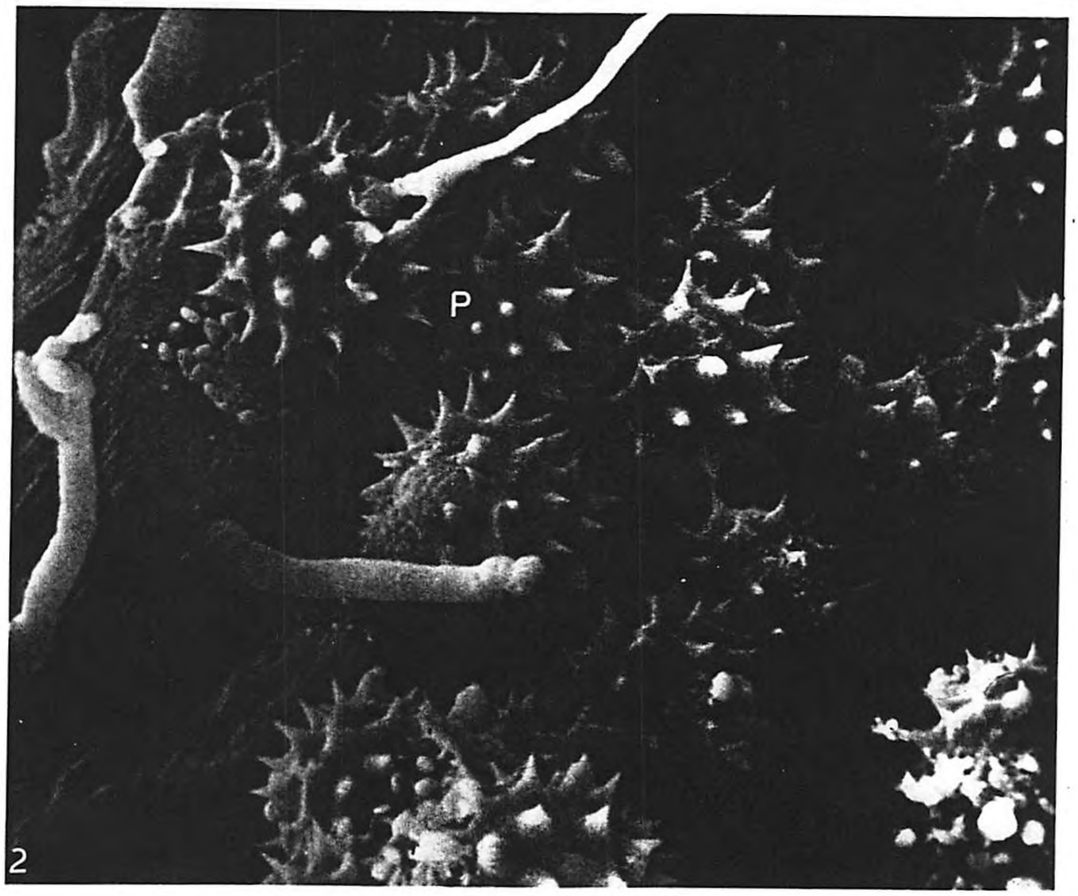
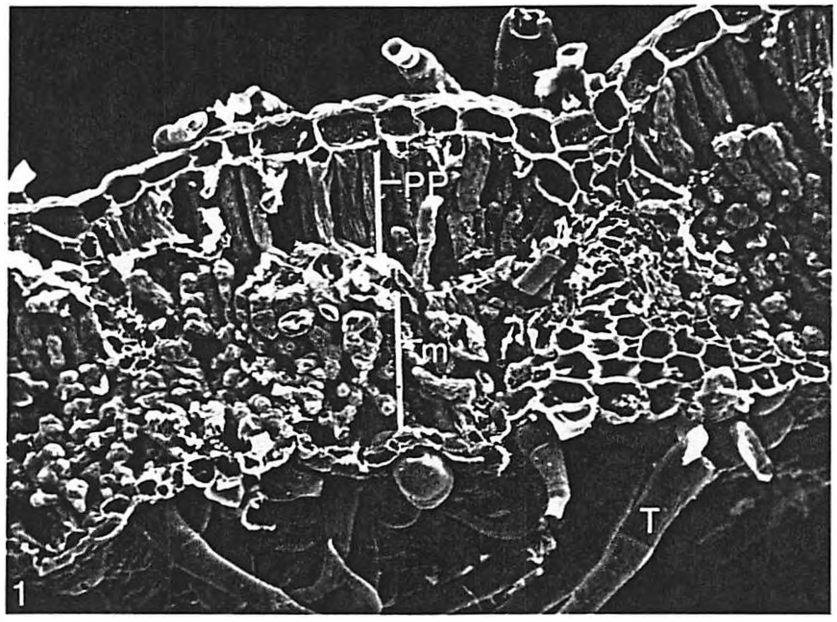

P
2

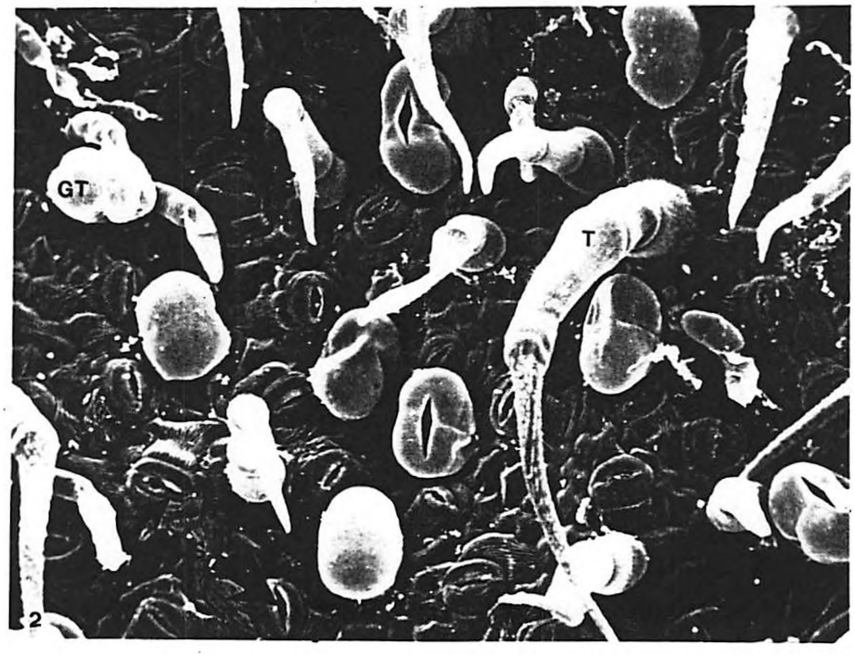
**FIG. 1. BLACK-EYED SUSAN x194**

Along the dorsal surface of the leaf there is a layer of cells called the palisade parenchyma (**PP**) that allows a large surface area for the dissolution of carbon dioxide (Mauseth, 1998). The tissue which extends to the ventral surface of the leaf is termed mesophyll (**m**). Tricomes can be seen protruding from the ventral surface.

**FIG. 2. BLACK-EYED SUSAN x270**

This shows an enlargement of the ventral surface area. The ventral surface is covered in trichomes (**T**), Glandular tricomes (**GT**), and stomata (**S**).


PP
m
T
1


GT
T
2

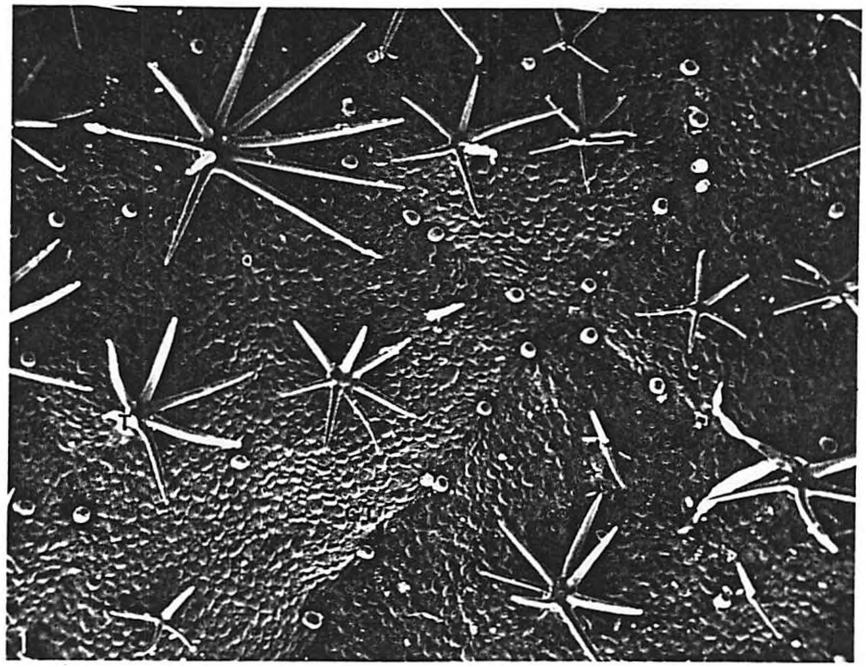
:23

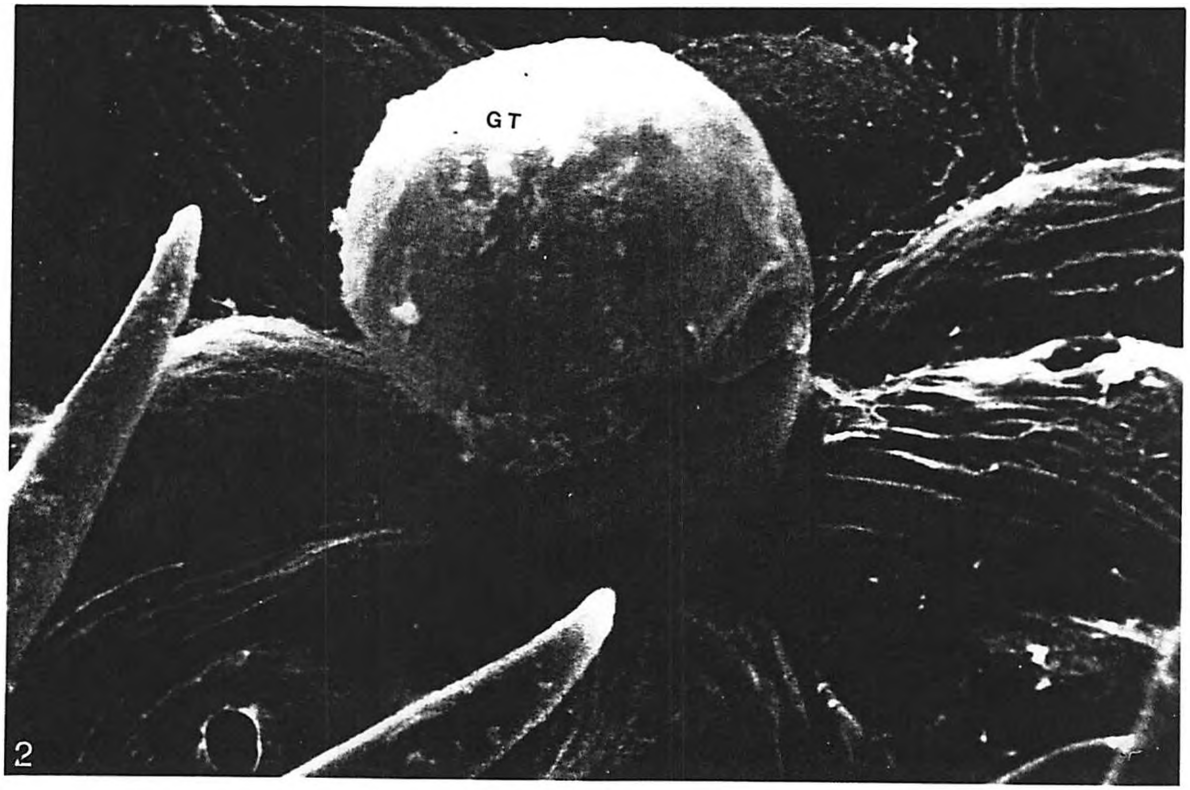
**FIG. 1. HOLLY HOCK x72**

This is a holly hock leaf that has trichomes (**T**) and glandular trichomes (**GT**) projecting from its surface.

**FIG. 2. HOLLY HOCK x2,190**

This is an enlargement of a glandular trichome (**GT**).

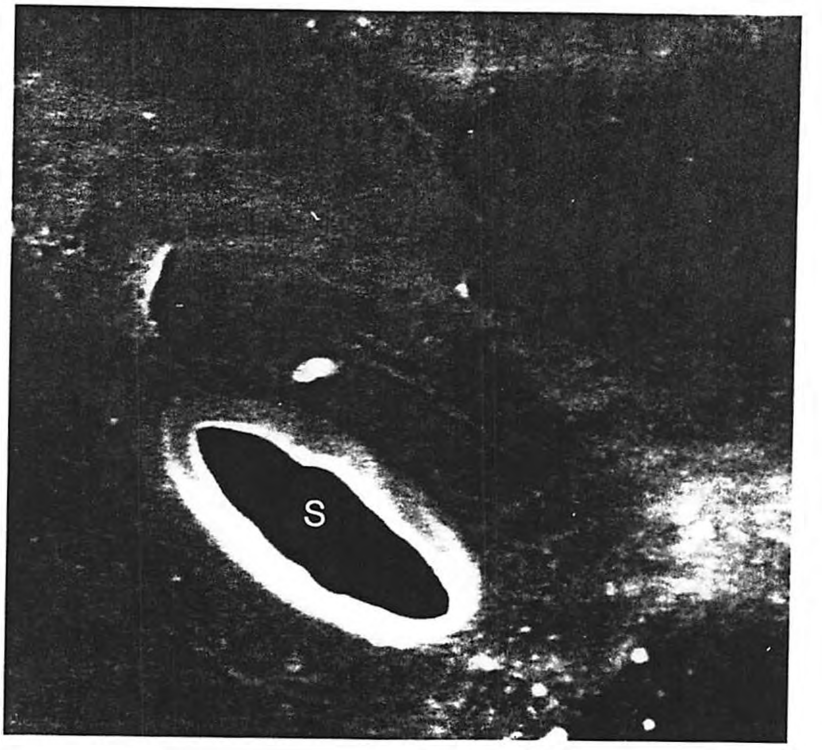



GT

:24

HOLLY HOCK x4,300

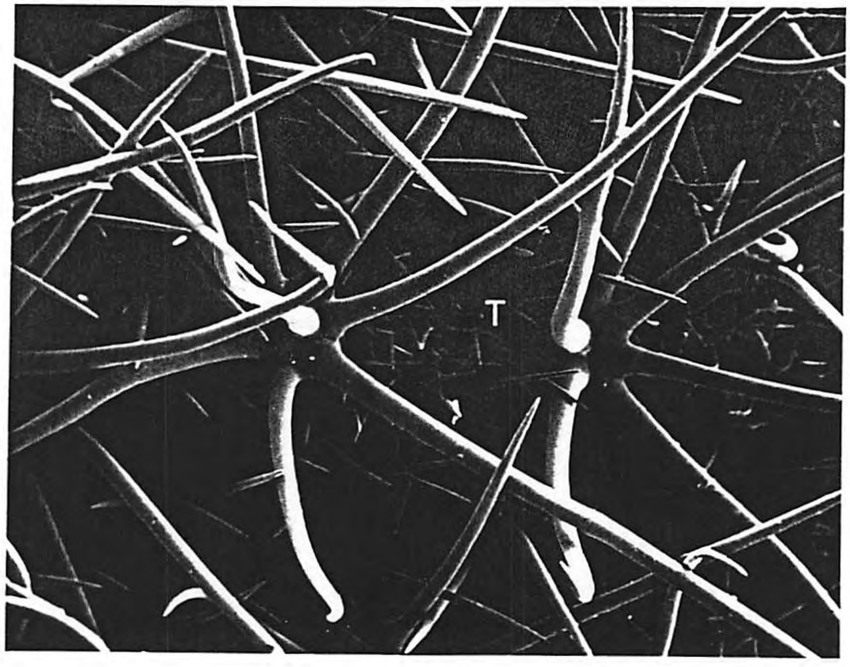
This is an enlargement of a stoma (S) on the surface of the holly hock leaf.


S

:25

**HOLLY HOCK x76**

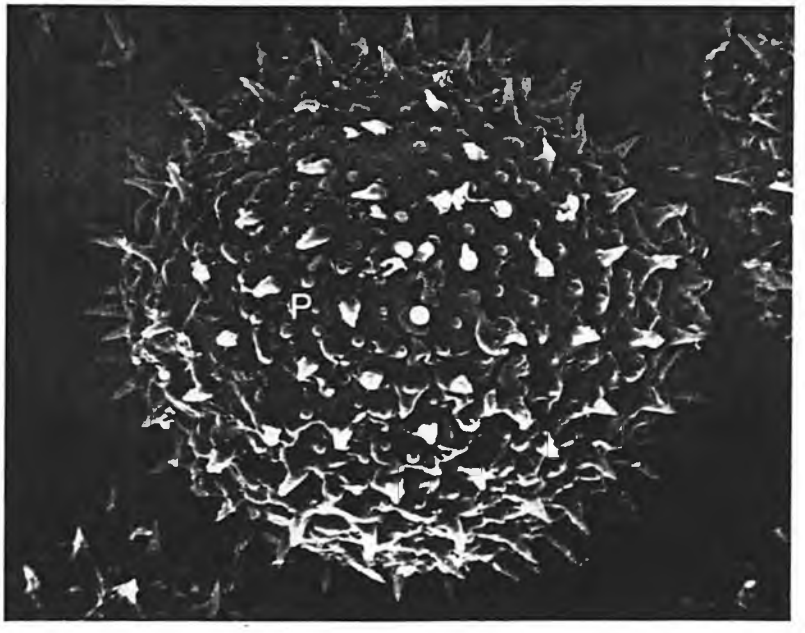
This shows many trichomes (T) protruding from the bud of holly hock.


T

:26

**HOLLY HOCK x560**

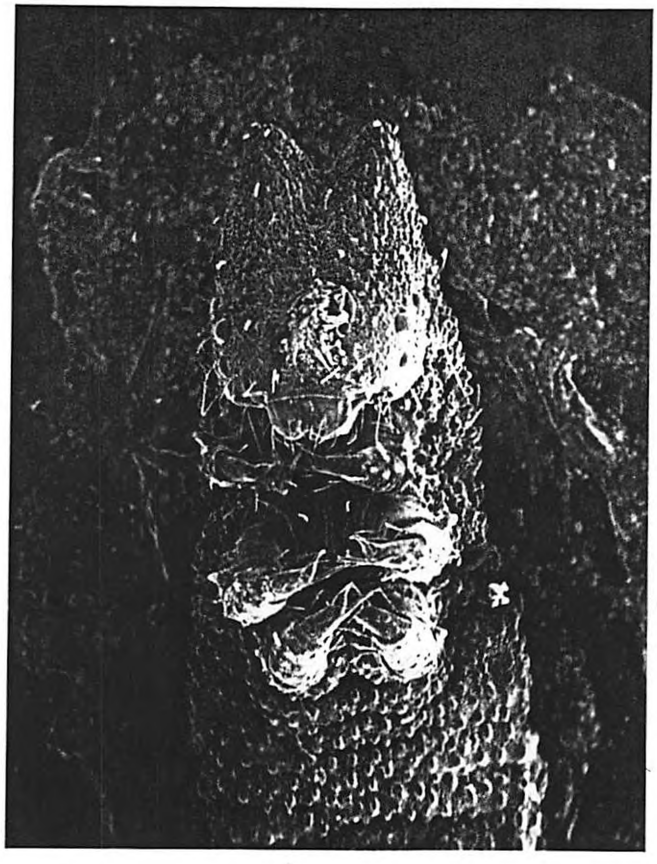
This is an enlargement of holly hock pollen (P).


P

:27

INCHWORM x32

This micrograph shows the head and front legs of an inchworm. These legs are capable of manipulating food (Borror,1976).



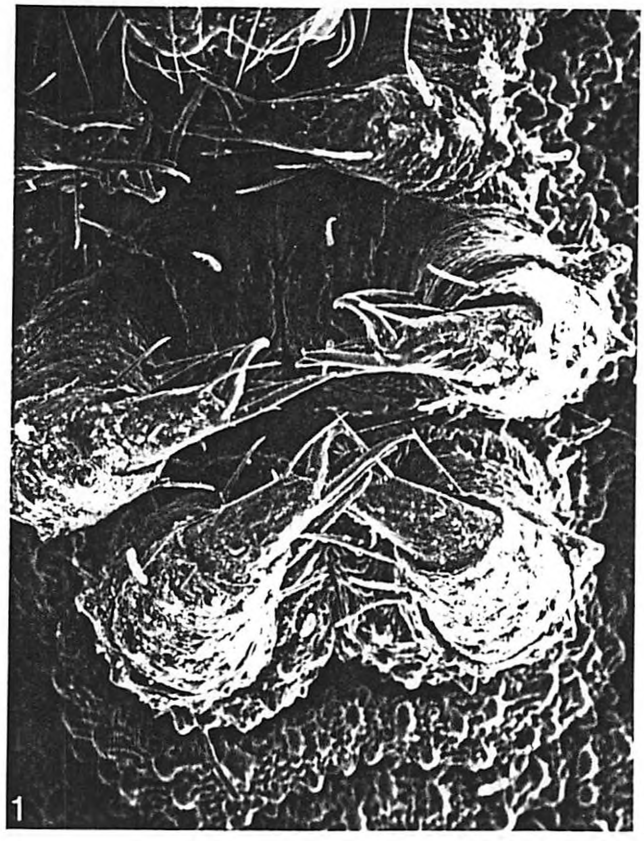
:28

**FIG. 1. INCHWORM x89**

This is an enlargement of the front legs. Little grasping claws can be seen which might be used to grasp food as well as surfaces the inchworm traverses.

**FIG. 1. INCHWORM x69**

This is an enlargement of the rear "legs" that only grasp the surface the inchworm traverses these are not capable of manipulating items.


1

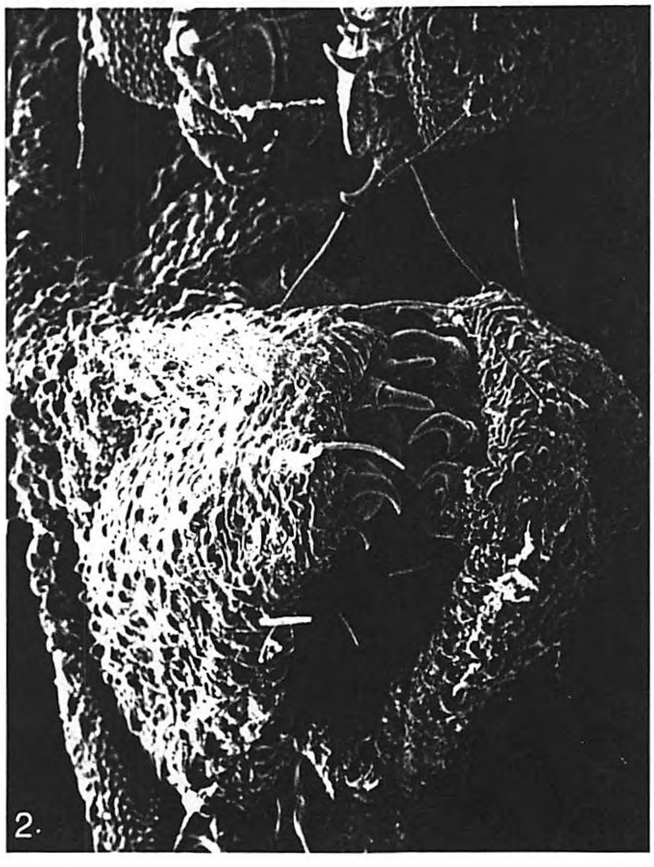
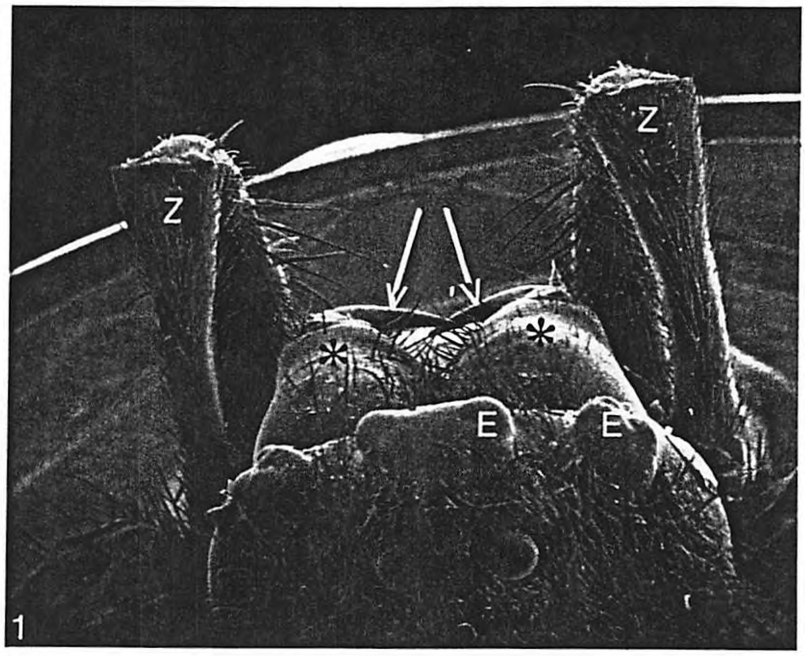

2.

**FIG. 1. GARDEN SPIDER (Argiòpe aurántia) x19**

This micrograph shows the pedipalps (Z) and the chelicerae (*) as well as the fangs (arrows) and many eyes (E).

**FIG. 2. GARDEN SPIDER (Argiòpe aurántia) x19**

This is simply a different view of the features noted in Fig. 1.


Z
Z
E
E
1

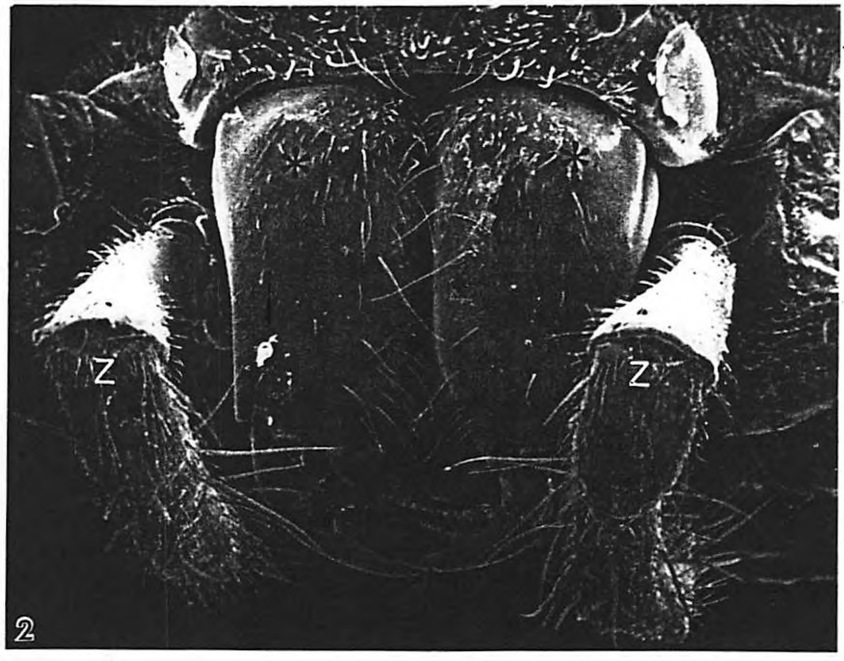

Z
Z
2

FIG. 1. GARDEN SPIDER (Argiòpe aurántia) x1,460

This micrograph shows that many spinnerets (N) are used to form the silky strands that make up a spider's webbing.

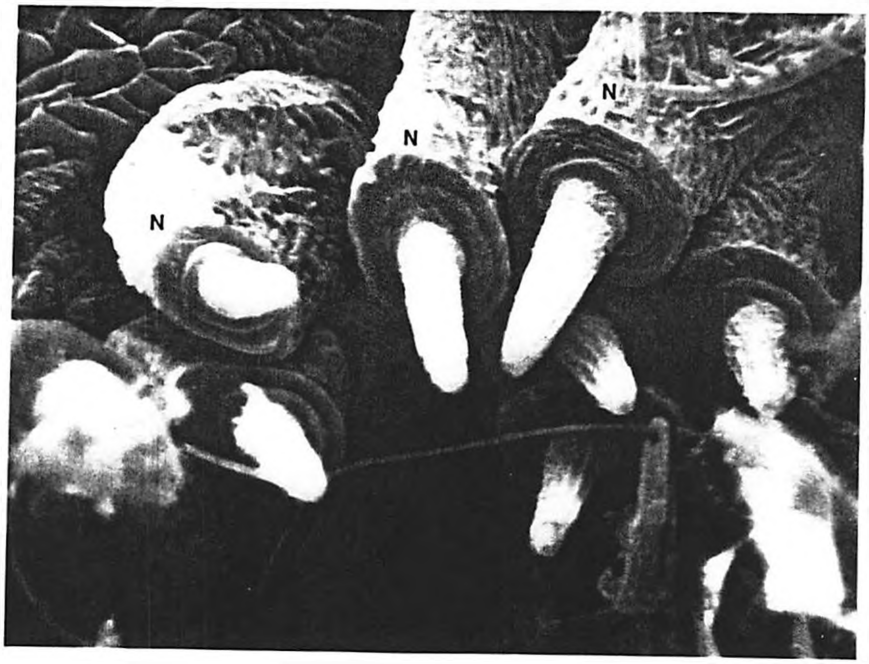
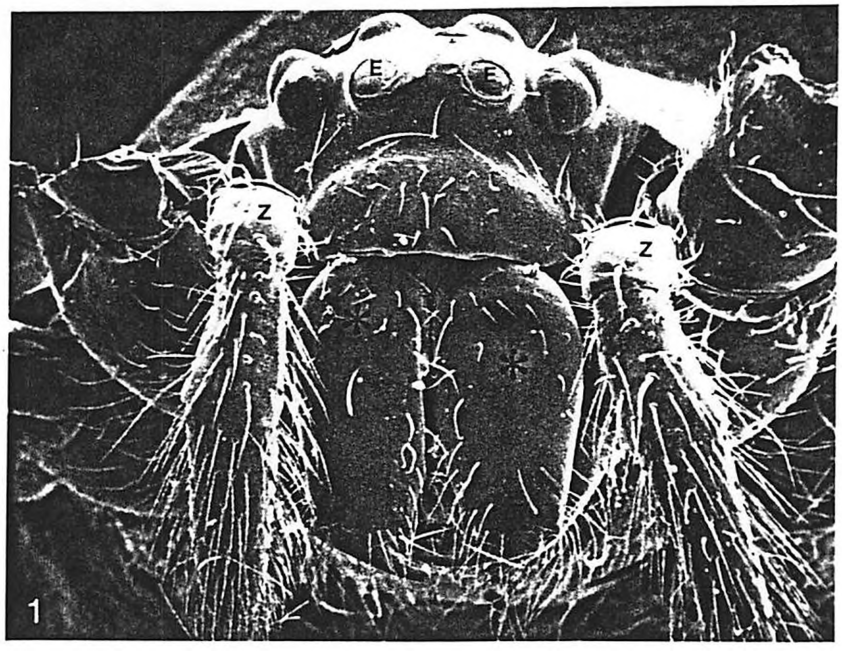

N
N
N

**FIG. 1. SPIDER #2 x55**

This micrograph shows the pedipalps (**Z**) and the chelicerae (*)as well as the 8 eyes (**E**).

**FIG.2. SPIDER #2 x122**

Four sets of spinnerets (**N**) can be seen in Fig.2.

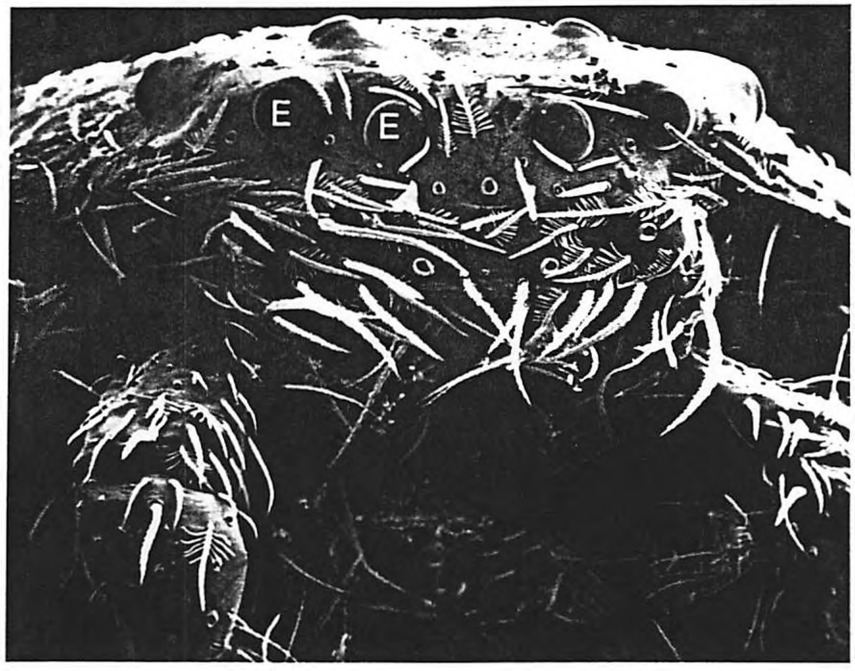

E
E
Z
Z
1


N
N
2

:32

**SPIDER #3 x147**

The eyes (**E**) are the only identifiable structures on the "face" of this spider.


E
E

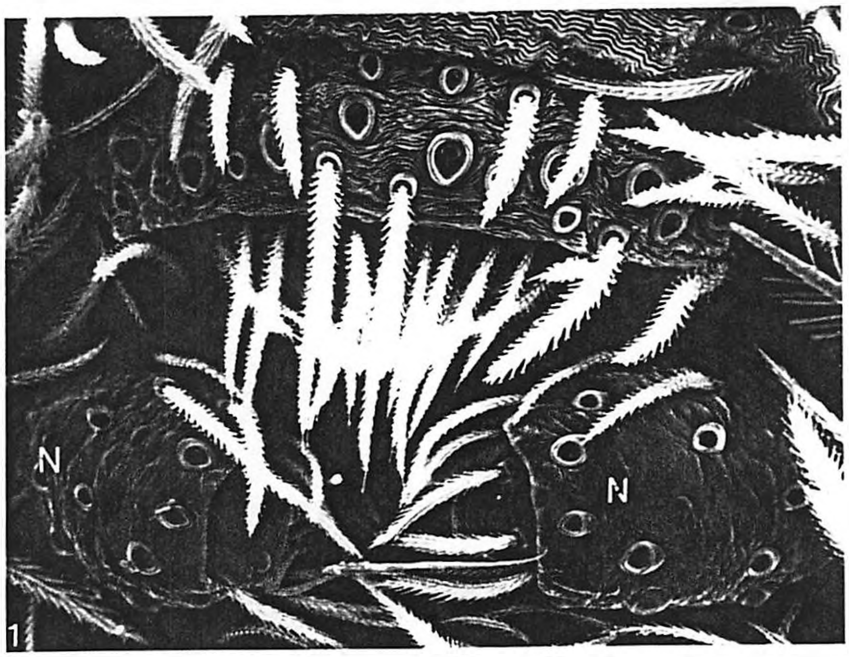
:33

**FIG. 1. SPIDER #3 x510**

The spinnerets (N) are visible on the rear of this spider and many fine white, feathery hairs.

**FIG. 2. SPIDER #3 x1,275**

An enlargement of Fig. 1. reveals the "wrinkled" texture of the tissue on the spider's body.


N
N
1


2

# WORKS CITED


Bils, Robert F. Electron Microscopy. Western publishing Co., Inc. Los Angeles. 1974.

Bloom, W., Faucett, D. A Textbook of Histology. W.B. Saunders Company, Philadelphia, Pennsylvania. 1975.

Borror, D.J., DeLong, D.W., Triplehorn, C.A. An Introduction to the Study of Insects. Holt, Rinehart, and Winston. 1976.

Bozzolla, J.J., Russel, L.D. Electron Microscopy. Jones and Bartlett Publishers, Boston. 1992.

Kessel, R.G., Kardon, R.H. Tissue and Organs: A Text Atlas of Scanning Electron Microscopy. W.H. Freeman and Company, San Francisco. 1979.

Lott, John A. Study of Green Plants. The C.V. Mosby Company. 1976

Mauseth, James D. Botany. Jones and Bartlett Publishers. 1998.

Prescott, Lansing M., et al. Microbiology. WCB/ McGraw-Hill. 1999.