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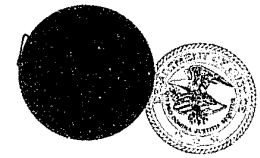

National Institute of Justice / Research Report

# Identifying Plant Food Cells in Gastric Contents for Use in Forensic Investigations:

## A Laboratory Manual

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U.S. Department of Justice
National Institute of Justice



# Identifying Plant Food Cells in Gastric Contents for Use in Forensic Investigations:

## A Laboratory Manual

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January 1988

**National Institute of Justice**

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# PREFACE

The purpose of this manual is to show you how plant cells from the stomach contents of autopsied homicide victims can be used to aid in criminal investigations. Plant cells have indigestible cell walls. Often, by means of microscopic examination, the food plant from which the cells originated can be identified. In this book, we have included almost all of the common food plants, with the exception of starches such as potato, wheat and rice. The plant cells featured in this book, because they have been subjected to chewing and swallowing in the human digestive process, are not identical with plant cells pictured in standard texts (see references at end of Chapter 1). Plant cells from gastric contents have undergone deformation to some extent; however, we have found that they are readily identifiable with a little practice.

We have found this technique to be useful in several investigations. This line of evidence has been little used in investigations before now for several reasons, we suppose. The more obvious one is that pathologists generally have had little experience in examining plant cells because their training has emphasized zoology (and animal cells), microbiology (and bacterial cells), and chemistry (no cells at all).

We are grateful for this opportunity to thank many people who helped us. Mark Norman helped develop the lab techniques and prepared many, many known food plant specimens for our work. Douglas Wray and Robert Cross skillfully printed all the photographs in the manual. George Shollenberger, our project manager at the National Institute of Justice, showed great patience with our questions and our procrastinations, and we are grateful to him. In the course of this work, we have met many law enforcement people. Without exception, they were kind, patient, and grateful when we tried to help them.

None of us had ever planned to do forensic work in our research careers until we received a phone call five years ago from Ben Galloway, M.D., of the Denver Coroner's Office and University of Colorado Medical School. He called full of questions:

"If you have some plant cells from human stomach contents, can you tell what sort of plant it comes from?"

We didn't know.

"Well, if I send you some slides of stomach contents, will you look at them, and see what you can find out?"

We looked, and identified some of what we saw.

Ben, we thank you for starting us on our investigations. You inspire us with your tireless work for the rights of victims and their survivors. Our purpose in preparing this manual is to assist those whose job it is to bring criminals to the courts and see that justice is done. If even one criminal is brought to justice because of these techniques, or one victimization prevented, we will consider this guide to have been worth every minute of the time we put into it.

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October, 1987

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# CHAPTER 1

## How Plant Cells Are Used in Investigations of Deaths

We discuss here how plant cells from stomach contents can be useful to homicide, accidental dealth, or questioned death investigations. First, we present a general discussion of how the material can be useful, and then we give some examples from our own experiences.

If the plant cells can be identified they can be used to show what a victim ate before death. Since food remains in the stomach for a rather limited amount of time before moving on to the small intestine, it is defensible to assume that the food was eaten within a couple of hours before death. Food remains in the stomach for longer periods only in exceptionable medical situations. Sometimes persons with certain sorts of ulcers or gastric diseases may retain food for a longer period, but this situation usually is discovered by the medical procedures which are part of an autopsy. Although animal-derived food material may continue to digest after death, plant cells will retain their diagnostic features for many hours after death.

We present four case histories to show how this evidence can be applied in investigations. We have changed some facts about the victims and the criminals in order to preserve the anonymity of the people involved; but we have not altered any aspects of the investigative procedures. We assume you will not encounter cases exactly like these, but we hope these examples will lead you to use this sort of evidence when your special circumstances suggest it may be useful.

Case Study 1. This case occurred early in our study of uses of plant cells in homicide investigations. A forensic scientist called us to identify cells of plant foods from the stomach contents of a young woman who had been stabbed to death. The victim's last known meal had come from a fast food chain restaurant which has

a very limited menu. We found cells of cabbage, beans, and green peppers in our examination in addition to the onions, tomato, and lettuce. The first three items were not served at the fast food restaurant. The young woman's character was such that she would not have eaten a meal with a stranger, nor would she have eaten alone on this particular evening when she was expected at her parents' home for dinner. This led investigators to conclude that the victim had known her killer, which was a useful clue in this case.

Case Study 2. The second example was a case of questioned death. In this case a young boy died, and the prosecuting attorney suspected that the child had been drowned by the parents. The parents claimed the death was accidental, and that they were loving, concerned parents. In their sworn statements,they said that just before the boy's accident they had fed him a snack of canned fruit cocktail. There was no evidence of any food having been consumed by the child near the time of his death. Further, there was no evidence that the child had consumed any sort of plant food because no plant cells were found in the stomach samples supplied by the pathologist, despite a diligent search. If the boy had eaten canned fruit cocktail we would have found evidence of such fruits as pears, peaches, cherries, grapes or pineapple. Since we found no cells in the victim's stomach contents that could in any way be identified as coming from fruit cocktail, the sworn testimony of the parents was contradicted. This raised questions about the validity of their other statements.

Case Study 3. In this case a gangland-style murder of several people took place. Prior to their deaths, the victims had been consuming pizza, and neighbors reported seeing the pizza van in the neighborhood about the time of the murder. The delivery person had delivered two orders to two apartments in the same building. He could not remember anything specific about the two groups. However, he remembered what sort of pizza each group had ordered, and he could describe the person who had received

the order in each case. He distinctly remembered one order with, and one without, onions. We examined some slides of the two victims' stomach contents, but we could find no cells which belonged to onions. The delivery person could describe the person who accepted the order for the onionless pizza. We were assured by the sheriff's office that this information was useful.

Case Study 4. This double murder remains unsolved. Two people who did know each other and who had very different lifestyles were murdered in somewhat similar fashion in a certain medium-sized town. The only common thread was that the two bore strong superficial resemblance to each other. No witnesses could be found who had ever seen the victims together, but the pathologist who conducted the autopsy suspected they might have shared a meal prior to their deaths. We carefully examined the stomach contents of the victims, and concluded that both had eaten the same plant foods prior to death. All of the plants we could identify were the sorts of foods served at more stylish oriental restaurants in the area. The investigators, in private, suggest that this was the action of a serial killer, and that someday some of this forensic detail may be useful in bringing the perpetrator to justice.

These cases show how you can use plant cells from victims' stomachs to investigate such questions as 1) had the person eaten any plant food at all shortly before death? 2) what sorts of plants were eaten? 3) what sort of restaurant provided the food? 4) to whom had food of a certain sort been served or delivered? and 5) was it likely that certain people shared a last meal? This is not an exhaustive list of possibilities; the applications of these techniques will draw heavily upon your own creativity.

This book will give you a brief introduction to plant cells, methods of staining them, what happens to them when they are digested, and how to identify them from masticated and partially digested samples. There are several books that we recommend for more detailed study of plant cells from food materials and their

identification. These are listed below Remember that the cells you will see in your investigations, which have been chewed and partially digested, may not look exactly like those pictured in these books, which are taken directly from the plants.

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# CHAPTER 2

## Introduction to Plant Cells

Many parts of plants serve as foods for humans. We may eat the seed producing parts of the plants technically known as fruits: examples of fruits are apples, tomatoes, and green beans. Or we may eat the seeds themselves: examples of seed foods include rice, pinto beans, and peanuts. In other cases, it is the vegetative organs of the plants that are consumed. The vegetative organs are the plant's roots, stems, and leaves. Root foods include carrots, turnips, and radishes; leafy foods include onions, lettuce and spinach. In some cases, combinations of plant parts are eaten. For example, cabbage is a combination of stems plus leaves. Many consumable plant parts have an outer covering layer of cells enclosing a pulpy layer. The outer layer can be epidermis, peel, or, in some cases, seed coat. Commonly, the outer covering of a stem or leaf is called epidermis, while the outer covering of a fruit or root is called peel. Seed coverings, such as the reddish husk of a peanut or kidney bean, often are called seed coats. Both the outer layers and the pulp may be used in identifying the plant; or, in the case of peeled fruits and vegetables, only the pulp will be present. In rare cases, such as the fleshy part of the green bean, the epidermis is so conspicuous that in this guide we ignore the few pulp cells that may be present.

Most food plants are classified as flowering plants (Kingdom Plantae). A few are mushrooms or fungi and belong to the Kingdom Fungi. The cells of the food plants have characteristics that distinguish them from the cells of all other organisms. Their most important trait is the cell wall of the plant cell. The cell wall itself is not living, but it serves as a container for the living parts of the cell (Fig. 1.1). A highly indigestible (by humans) substance called cellulose is laid down in one or more layers to form the cell wall. It will not be necessary for you to distinguish the numbers of layers an individual cell wall possesses, although overall thickness

characteristic in the identification process. Both the thickness of the cell wall and its shape can reflect the function of the cells. For example, cells which are specialized for transporting water or other liquids throughout the plant are often long, fairly thin, and hollow. They are hollow because the living contents have disintegrated after the cell assumed its functional shape. Often these cells have distinctive thickening patterns on their inside walls. These thickenings commonly are laid down as rings, spirals, or strips (fig. 2.2). Plants also may possess cells which allow the stem or leaf to be pliable but not break in a strong wind. Such cells are called fibers. They are much longer than wide and have thick walls.

Many people who study plant cells divide them into cell types based upon their shapes and wall thicknesses. Here are some of the more common ones to be seen in food plants.

Cell type 1: Parenchyma Cells (pronounced par-en'-ki-muh). These are thin-walled and more or less equal in all dimensions. Cubes (fig. 2.3) or spheres (fig. 2.4) are common examples of the shape of parenchyma cells, but the shape of many parenchyma cells may be irregular modifications of these shapes (figs. 2.5 and 2.6). Parenchyma tissue is found in many places in the plant, often making up the bulk of the edible portions of food plants. It is especially common in pulp.

Cell type 2. Epidermal Cells. Several distinctive cell types are associated with the epidermis of edible plants. Sometimes epidermal cells are shaped like jigsaw puzzle pieces (figs. 2.7 and 2.8). In other cases, epidermal cells may be more or less regular in shape (fig. 2.9). Sometimes, there are small pores called stomates in the epidermis of plants, especially in leaves and stems. These allow the exchange of gases and water vapor between the cells of the plant and the external environment. The stomates are framed by special cells called guard cells which function to enlarge or close the stomate (figs. 2.7 and 2.10). The presence of stomates on a piece of plant material can be very useful in identification, because they differ from plant food to plant food in size and other features.

Also, plants may have structures known as plant hairs growing from the epidermis. Sometimes these hairs are branched

(fig. 2.11) or in other cases they may be unbranched hairs (fig. 2.12). These hairs may densely cover the surface of a leaf, fruit, or stem, or they may be present, but rare. Sometimes special hairs, called root hairs, can be seen on the very fresh, young roots of plant foods such as alfalfa sprouts and bean sprouts.

**Cell type 3: Conducting Cells.** Conducting cells are conspicuous and can be found in all seed plants and in most plant tissues. Tracheary (pronounced trak-ee'-air-ee) elements (fig. 2.2) are the cells which conduct water from the ground up through the plant. Only the walls of these cells remain when they function as water transporters. The intact conducting cells are grouped together in the plant, and form a network of tubes for conducting water and nutrients throughout the plant. These groupings of conducting cells are referred to as veins when the conducting cells are seen intact in many plant foods (figs. 2.13 and 2.14).

**Cell type 4: Sclerenchyma Cells** (pronounced sklar-en'-ki-muh). Sclerenchyma cells provide great strength to stems and other structures of the plant because their cell walls are reinforced with a compound called lignin. They occur in many sizes and shapes, but these cells all have very thick cell walls with very small cell cavities. Some, the fibers, are much longer than wide, while others, the stone cells (fig. 2.15), are more nearly equal-sided. It is stone cells that give pear pulp its gritty texture. Some sclerenchyma cells have very odd shapes. They can look like bones or many-pointed stars. Because of the very coarse and tough nature of these cells, they are rarely found in food materials from plants.

**Cell type 5: Inclusions.** Strictly speaking, inclusions are not types of cells, but instead are distinctive objects found inside cells. They may or may not still be inside a cell when you see them because the processes of chewing and early digestion may have disrupted the cell walls so that the inclusions have been removed from the cells in which they were formed.

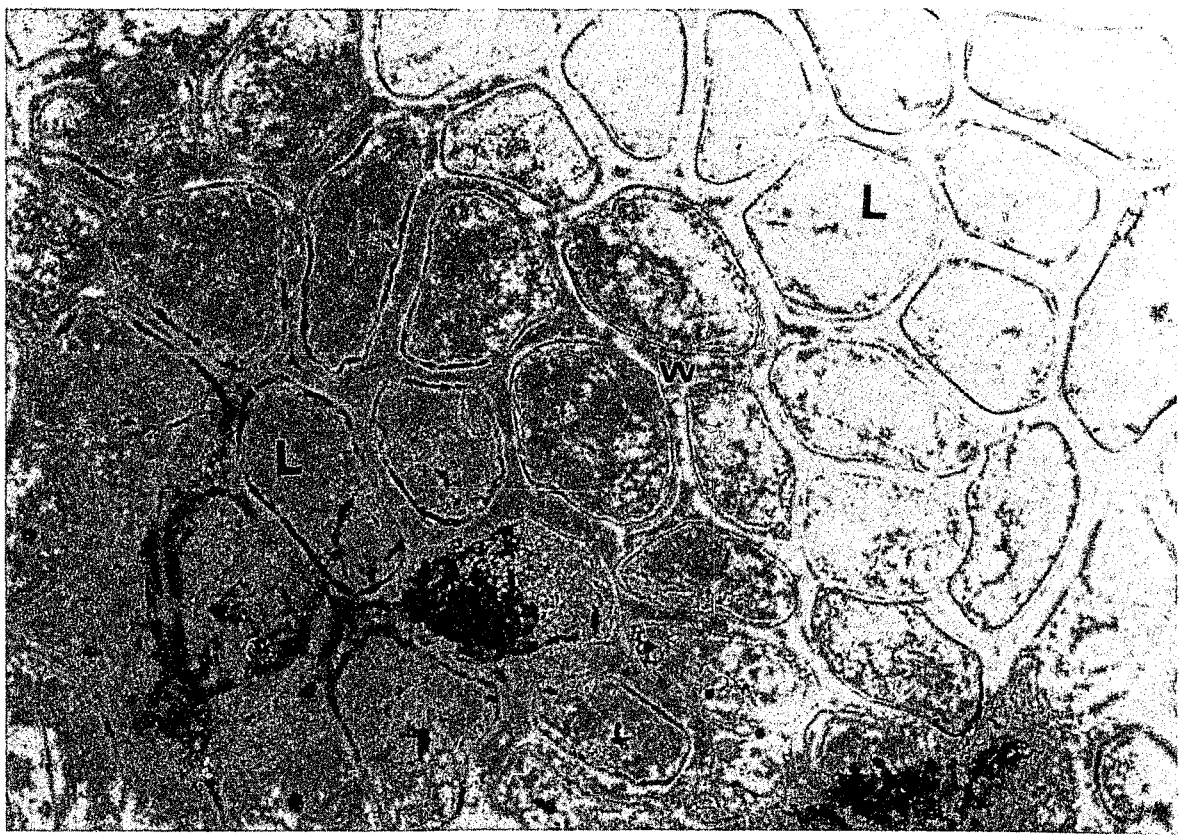
The inclusions most useful in this work are crystals. Crystals occur in characteristic forms within particular kinds of food plants. Therefore, it is necessary to distinguish among and give technical

names to the common forms that may be encountered. These are: prisms (fig. 2.16), which can be elongate or square and box-like in shape; raphides, which are needle-like crystals (fig. 2.17), and druses, which are globular clusters of crystals (figs. 2.18 and 2.19).

Another very important type of inclusion is the starch grain. Generally, the starch grains found in investigations of plant foods are rounded or oval in shape and opaque (fig. 2.20). Starch is a common inclusion in the cells of rice, potatoes, wheat and corn, so starch grains can be useful in identifying the presence of French fries, crackers, biscuits, bread, tortillas, or other common "starchy" foods in stomach contents. The starches of each kind of food plant usually are distinctive in shape and optical properties, but exact interpretations are beyond the scope of this book. See Chapter 5 for a method of testing for the presence of starch.

Droplets of vegetable oil or fat may also be included in the cells of some food plants. These droplets are slow to digest, and some will still cling to the cellular material of such foods as olives (see figs. 8.46 and 9.33) and sesame (see fig. 9.47) even after the food has been in the stomach for some time.

Cell type 6: Fungal Cells. Fungi are not plants, nor are they closely related to the flowering plants that provide us with most plant foods. However, in this work you may encounter fungal cells from two sources: mushrooms (we are eating only fungus when we eat a mushroom), and cells which result from a fungal infection of a food plant (we are eating food plant pulp and fungus when we eat papaya). The fungal cells of interest occur in two forms: spores and hyphae. Fungal spores are round, small cells that function as reproductive units (analogous to seeds) for the fungi. The other sort of fungal cell is the hypha (plural: hyphae). Hyphae grow intertwined to make up the body of the mushroom or through the pulp of the fruit or vegetable, such as in papaya (fig. 2.21). Hyphae are long, tubular cells that lack crosswalls (fig. 2.22). This lack of crosswalls distinguishes fungal cells from those of the flowering plants.



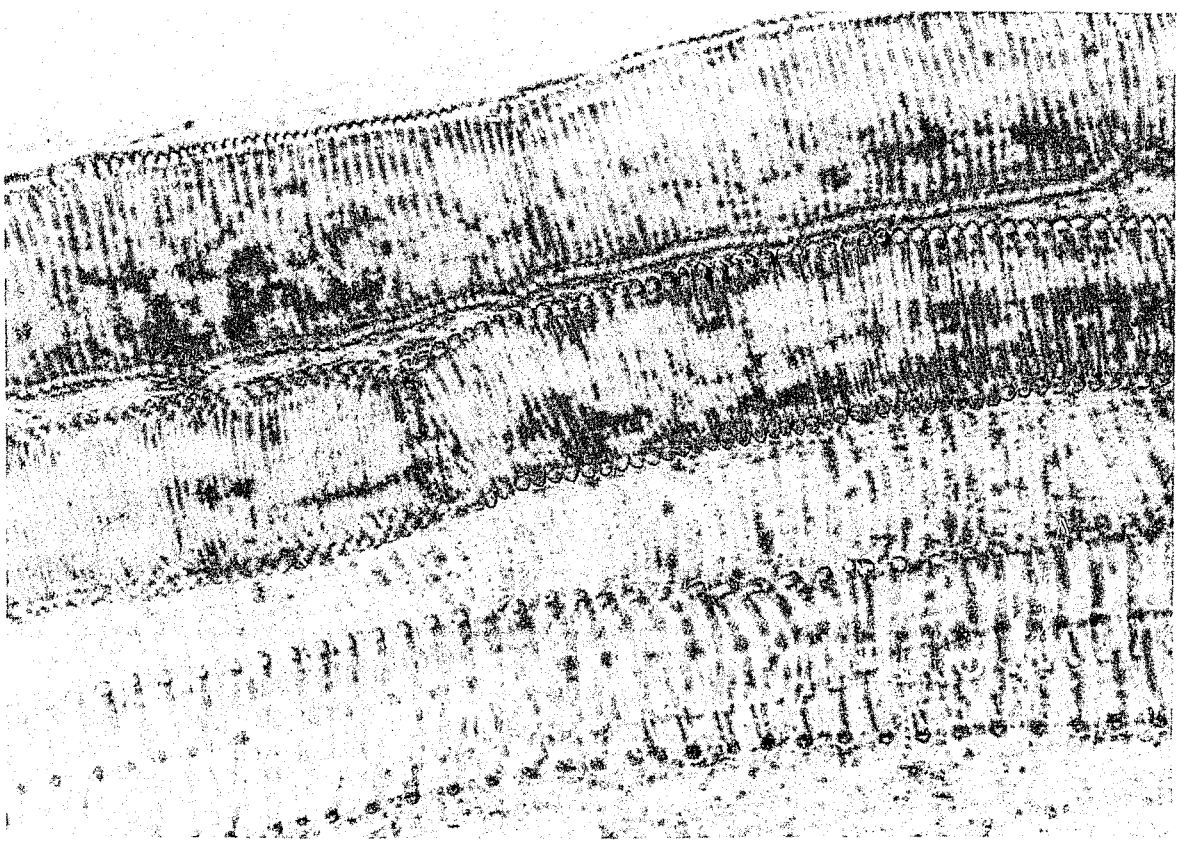
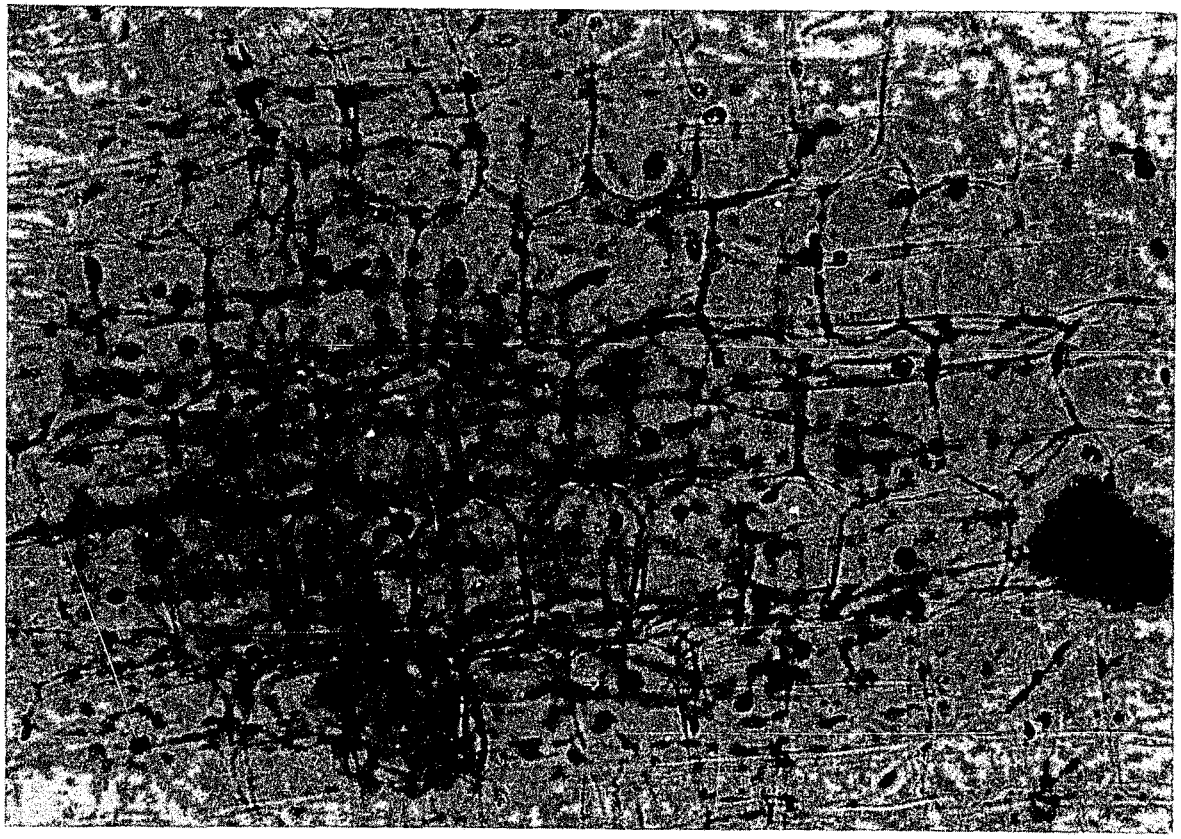



Figure 2.1 The plant cell (example from cherry skin in Mounting Medium B, X560 taken at 25X). L = living portion, W = the non-living cell wall.

Figure 2.2 Conducting cells (example from squash, Mounting Medium A, X800 taken at 25X). T = thickenings on the cell walls.



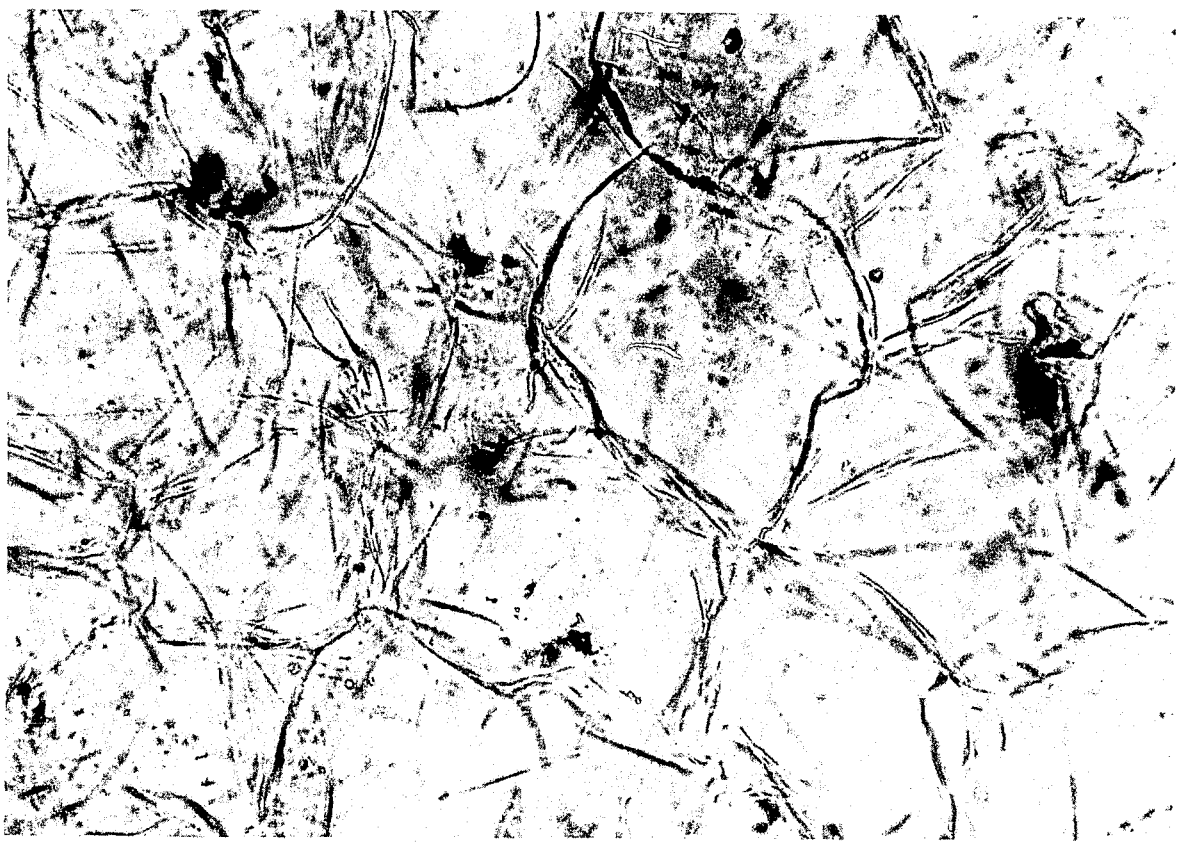
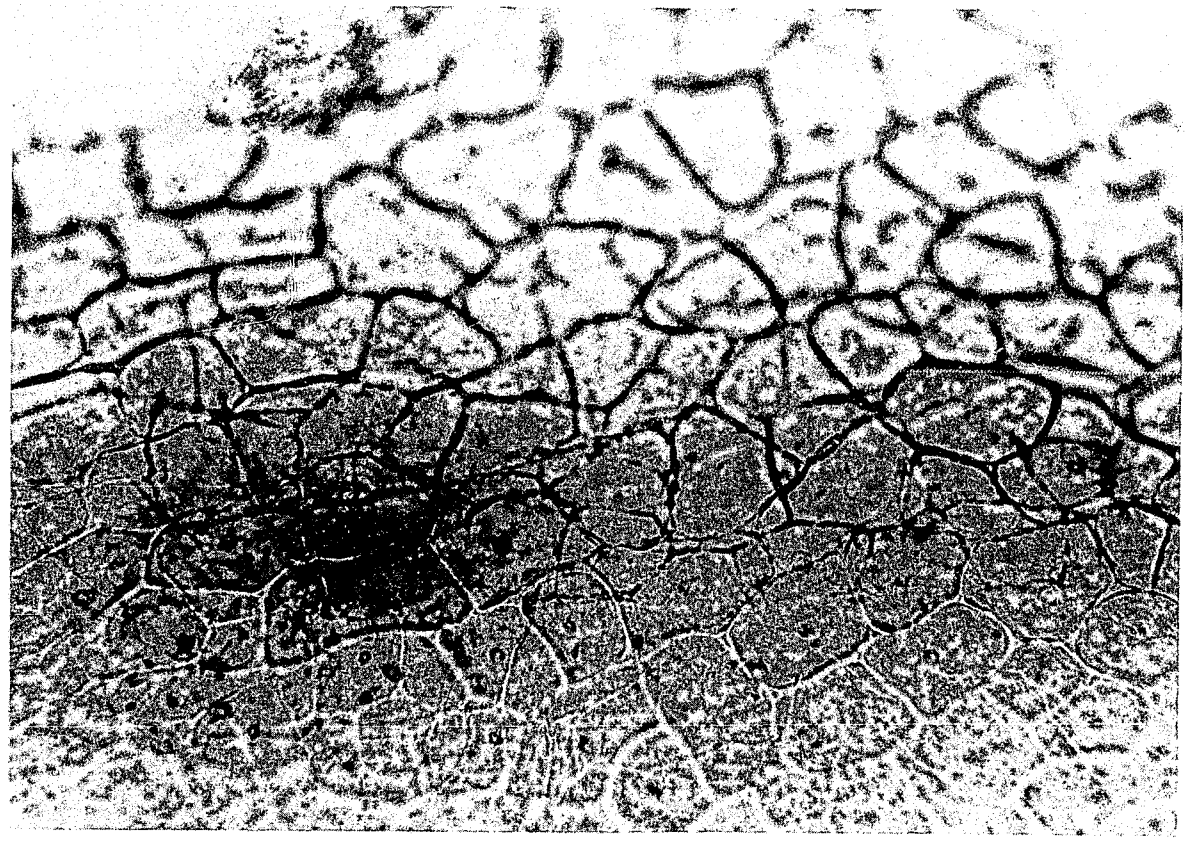


Figure 2.3 Parenchyma cells (example from onion in Mounting Medium B, X224 taken at 10X). Note the cubical shape of these cells. Some elongate epidermal cells can be seen in the background.

Figure 2.4 Parenchyma cells (example from apple pulp in Mounting Medium A, X224 taken at 10X). Note that these cells are large, relatively sperical, and thin-walled.



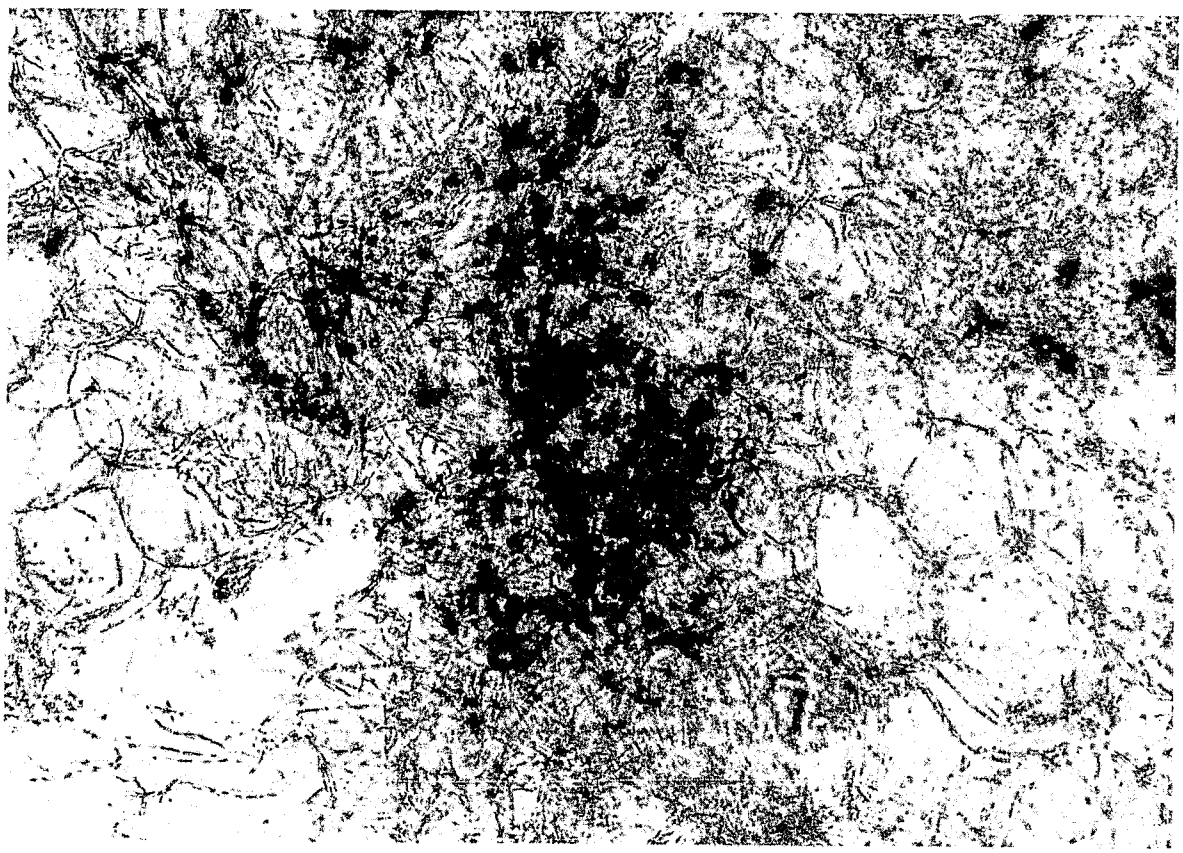


Figure 2.5 Parenchyma cells (example from green pepper pulp in Mounting Medium B, X224 taken at 10X). Note the irregular, somewhat angular, shape of these cells.

Figure 2.6 Parenchyma cells (example from cabbage pulp in Mounting Medium B, X224 taken at 10X). Note that these thin-walled, relatively spherical cells resemble those of apple pulp (fig. 2.4), but are much smaller.

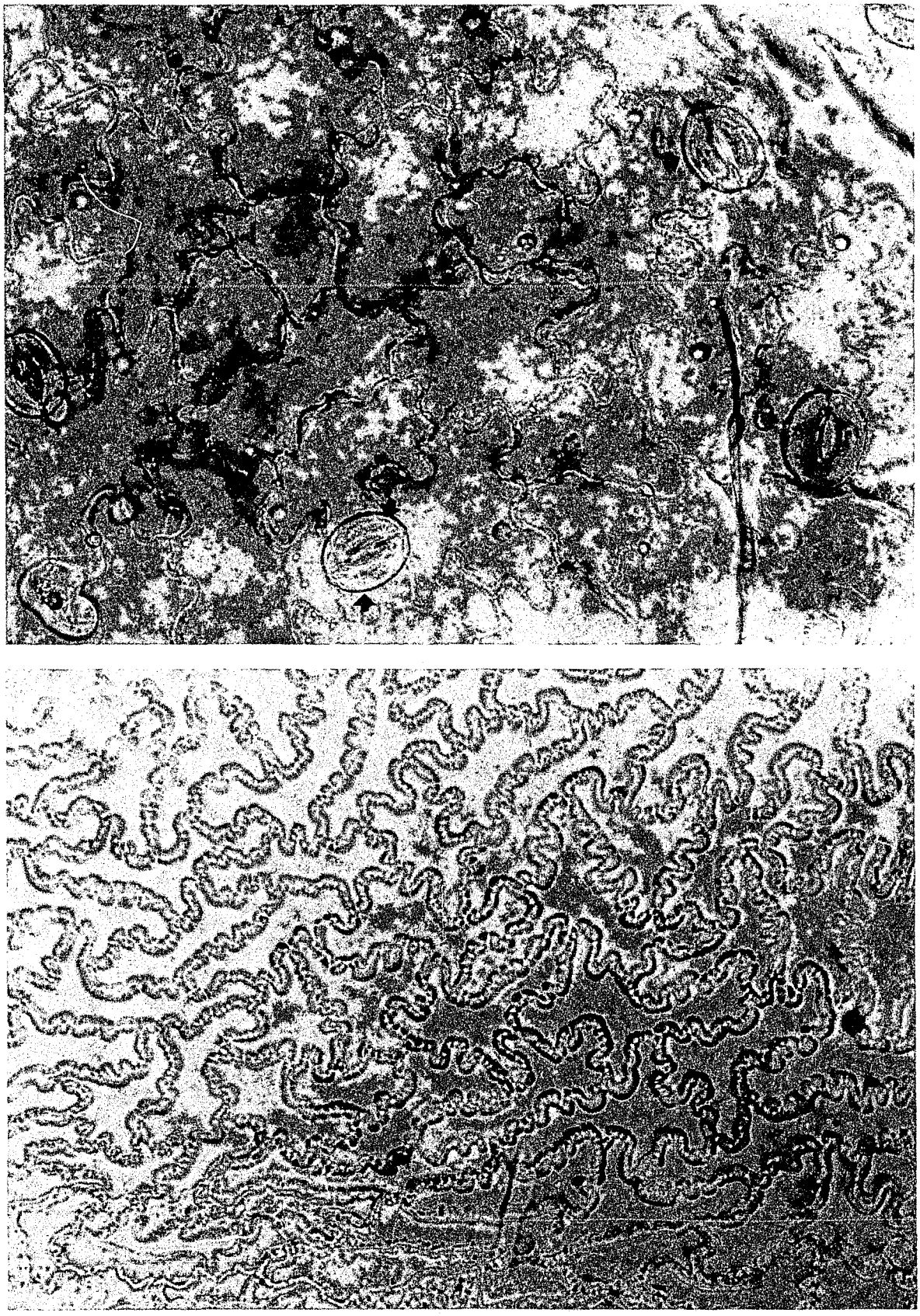
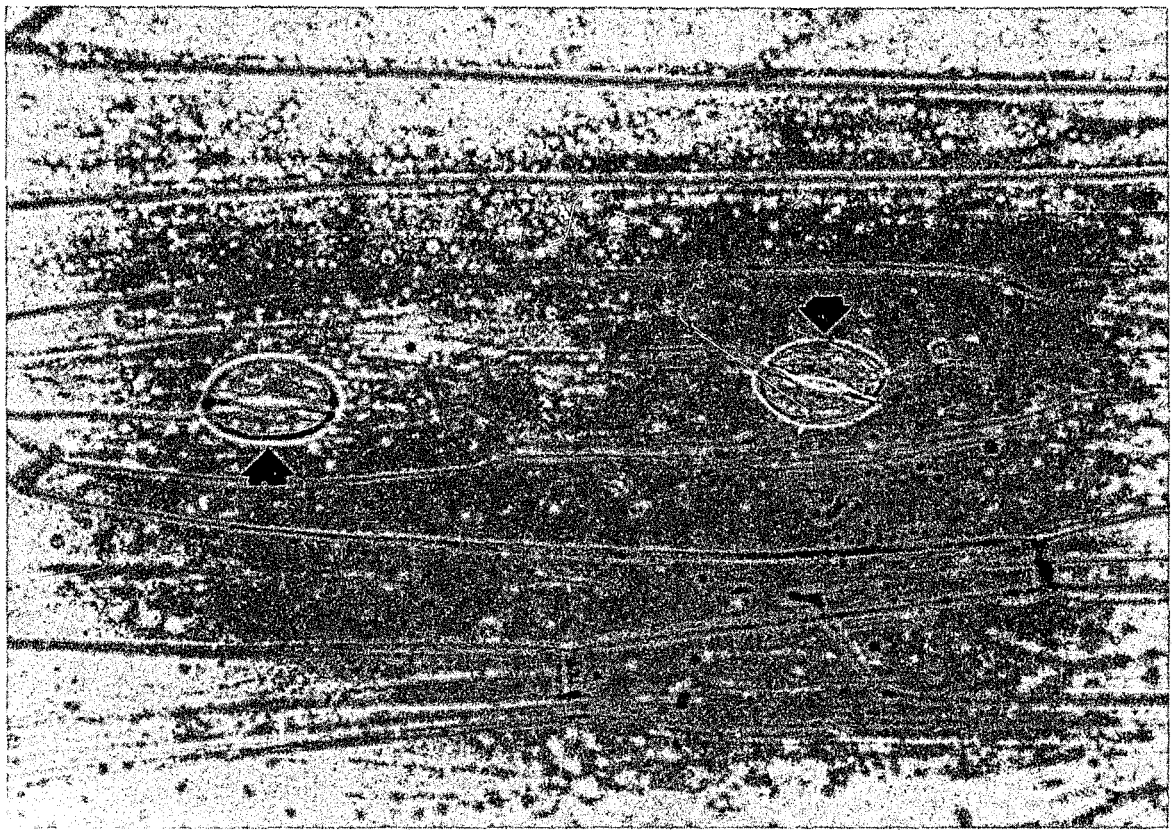


Figure 2.7 Epidermal cells shaped like jigsaw-puzzle pieces with thin walls (example from lettuce in Mounting Medium B, X560 taken at 25X). Note also the stomates, framed by their guard cells (arrows) scattered on the epidermal surface.

Figure 2.8 Epidermal cells shaped like jigsaw-puzzle pieces with thick walls (example from green pepper in Mounting Medium A, X800 taken at 25X).



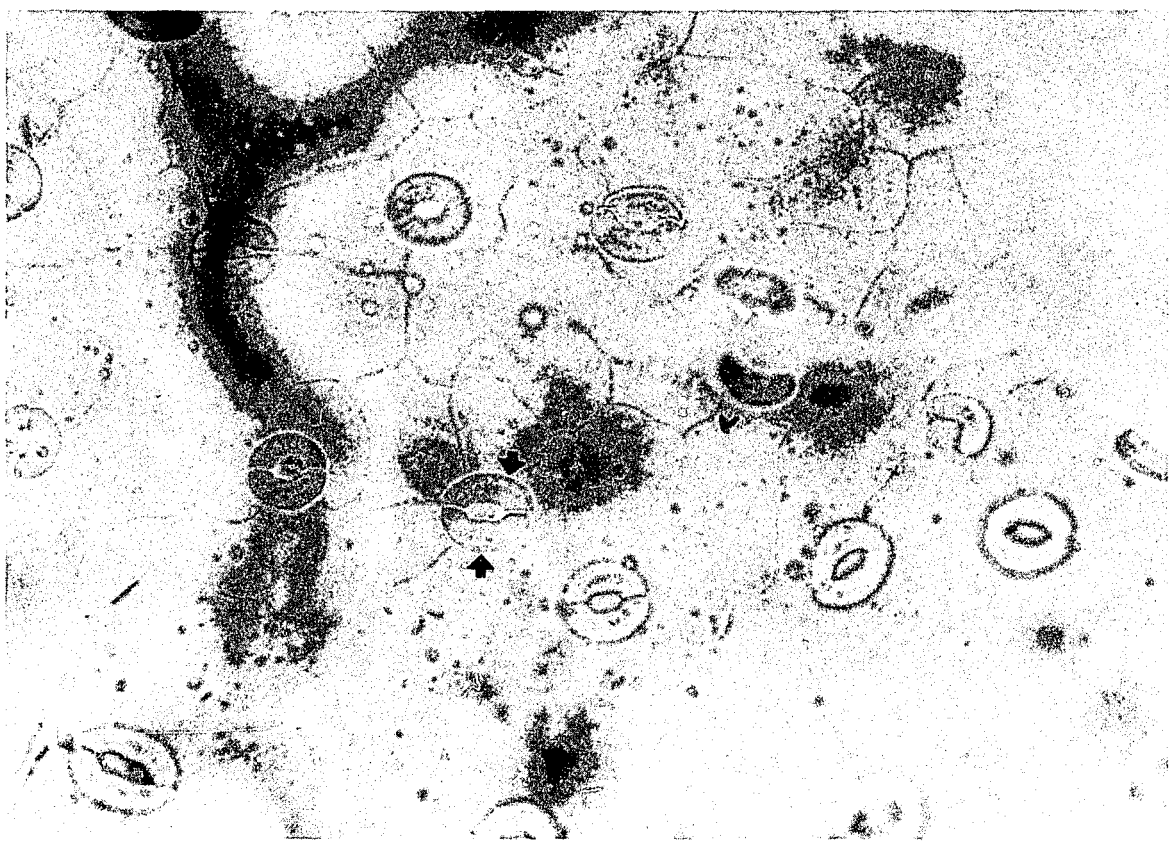


Figure 2.9 Epidermal cells regular in shape (example from mustard greens in Mounting Medium B, X560 taken at 25X). Note the stomates (arrows).

Figure 2.10 Stomates with guard cells (arrows) (example from underside of spinach leaf in Mounting Medium A, X560 taken at 25X). Compare these stomates and guard cells to the larger ones indicated in Figures 2.7 and 2.9.

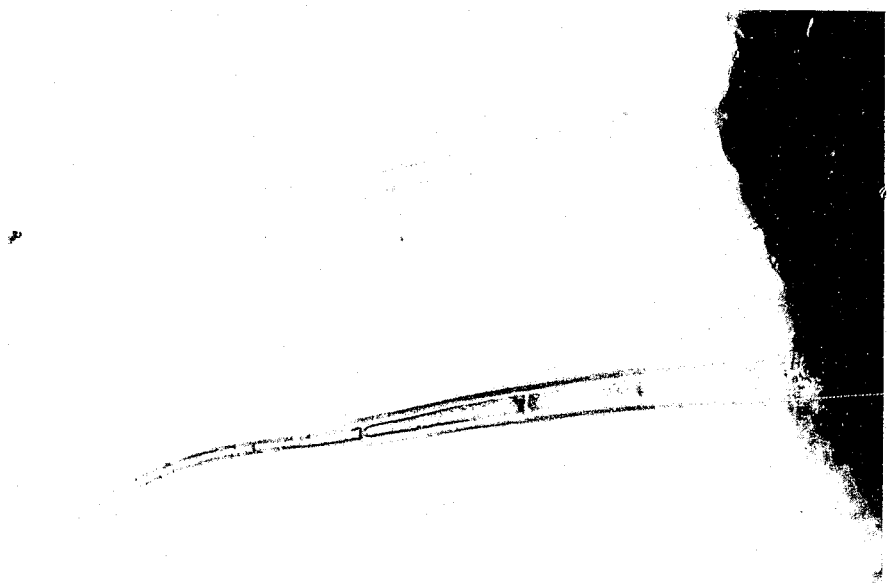
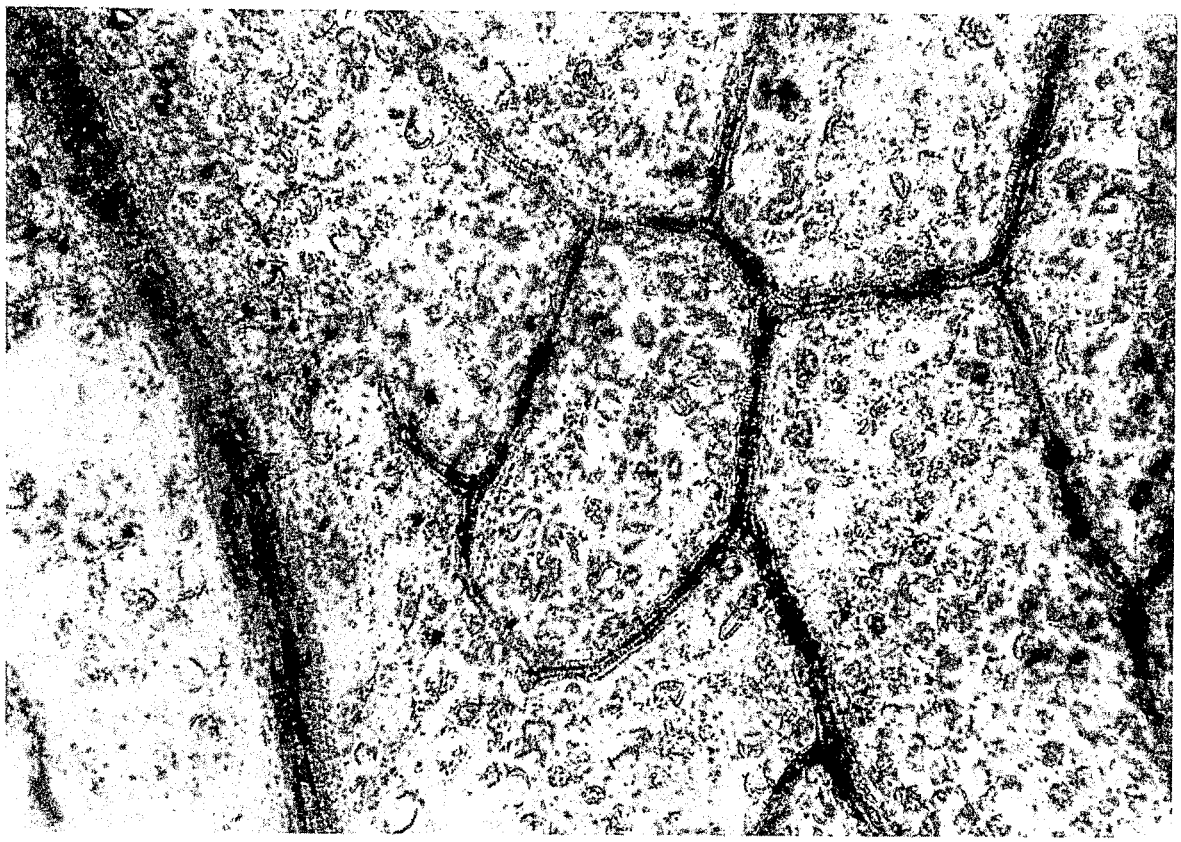


Figure 2.11 Branched hairs (arrow) (example from rosemary in Mounting Medium B, X320 taken at 10X).

Figure 2.12 Unbranched hairs (example from oregano in Mounting Medium A, X224 taken at 10X).



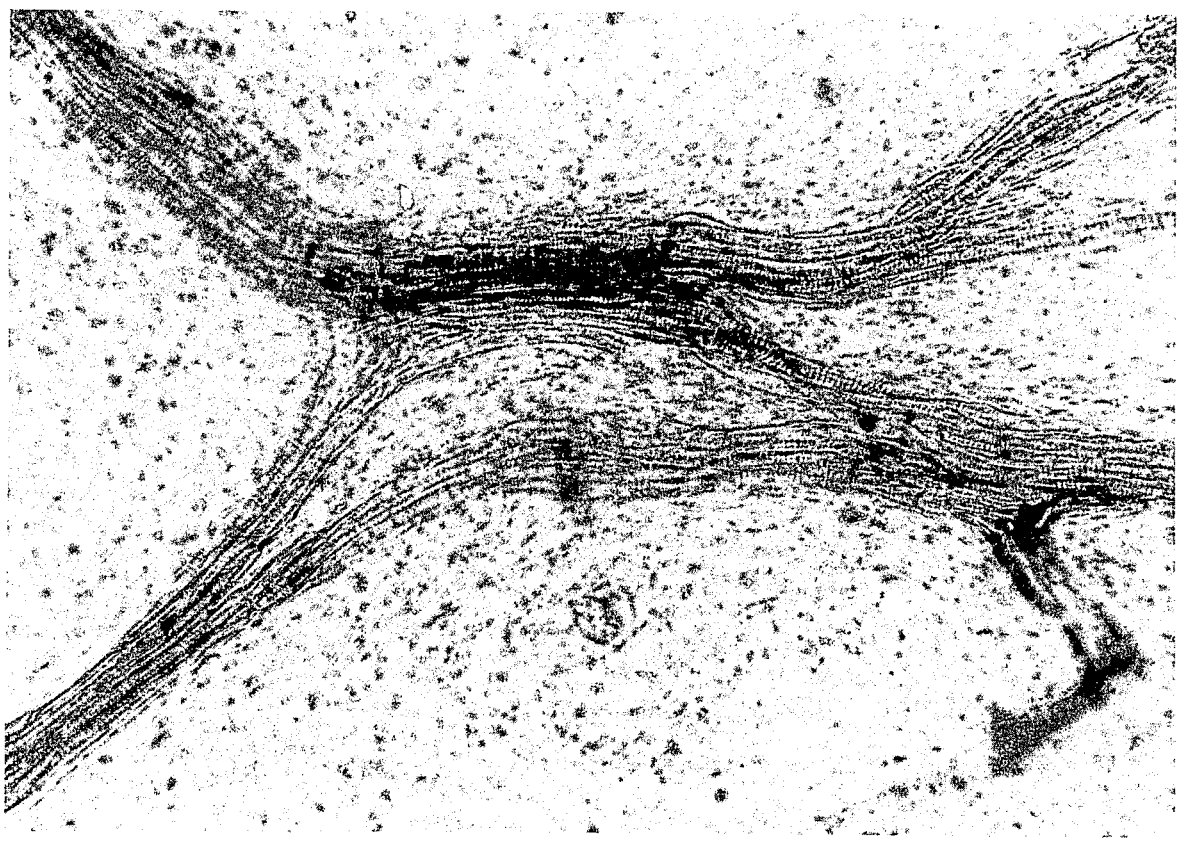


Figure 2.13 Network of veins (groups of conducting cells) (example from mustard green leaf in Mounting Medium A, X230 taken at 10X). Similar patterns occur in the leaves of other plant foods including lettuce, cabbage and parsley.

Figure 2.14 Vein pattern occurring in pulp (example from citrus in Mounting Medium A, X224 taken at 10X).

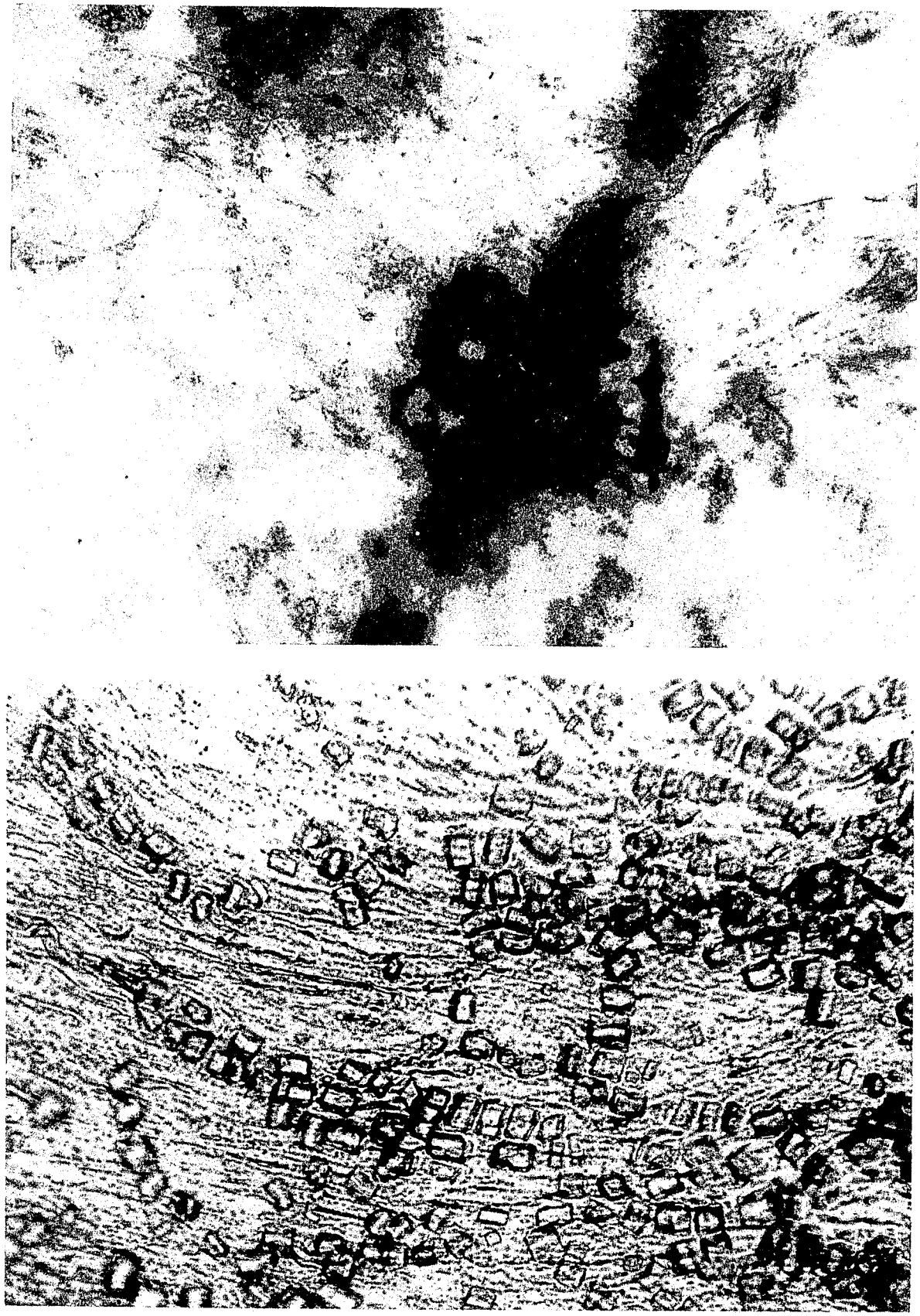
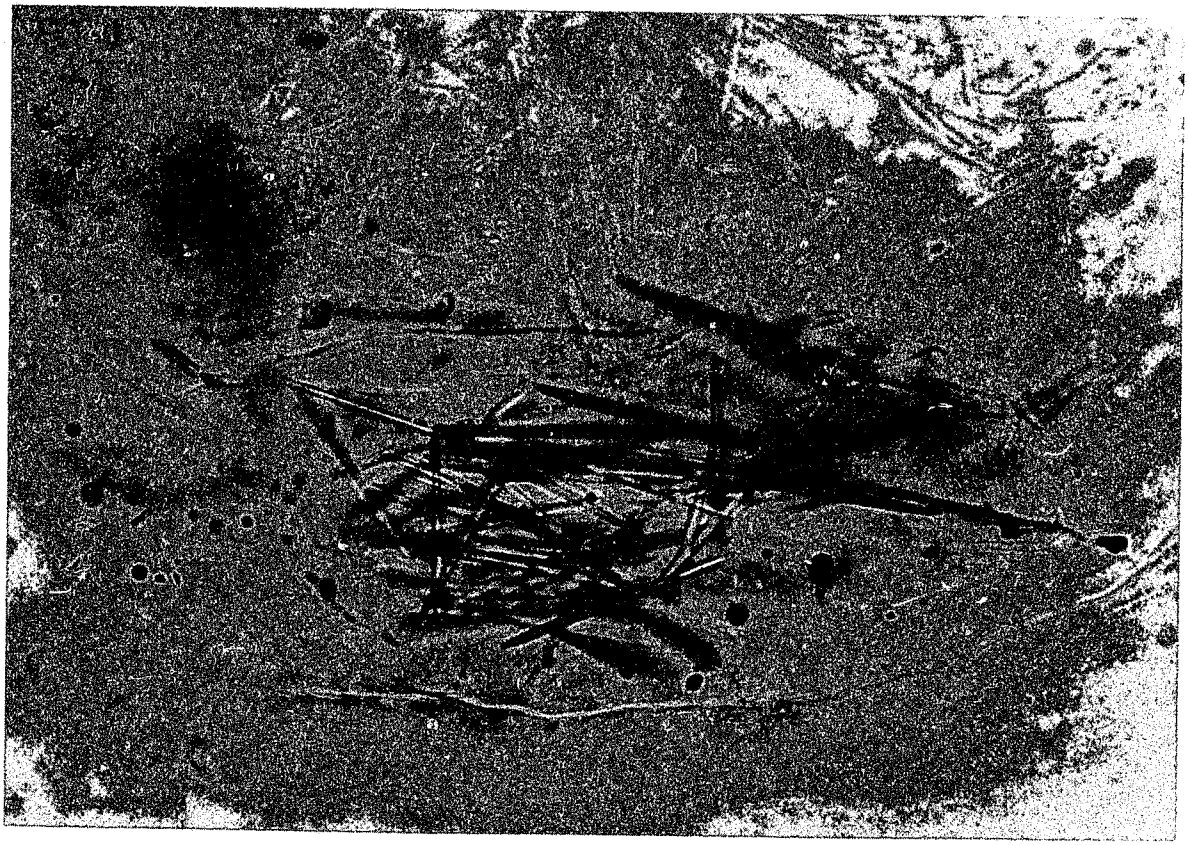


Figure 2.15 Stone cells (sclerenchyma) (example from pear pulp in Mounting Medium A, X224 taken at 10X).

Figure 2.16 Crystals: prism type (example from okra pulp in Mounting Medium A, X320 taken at 10X).



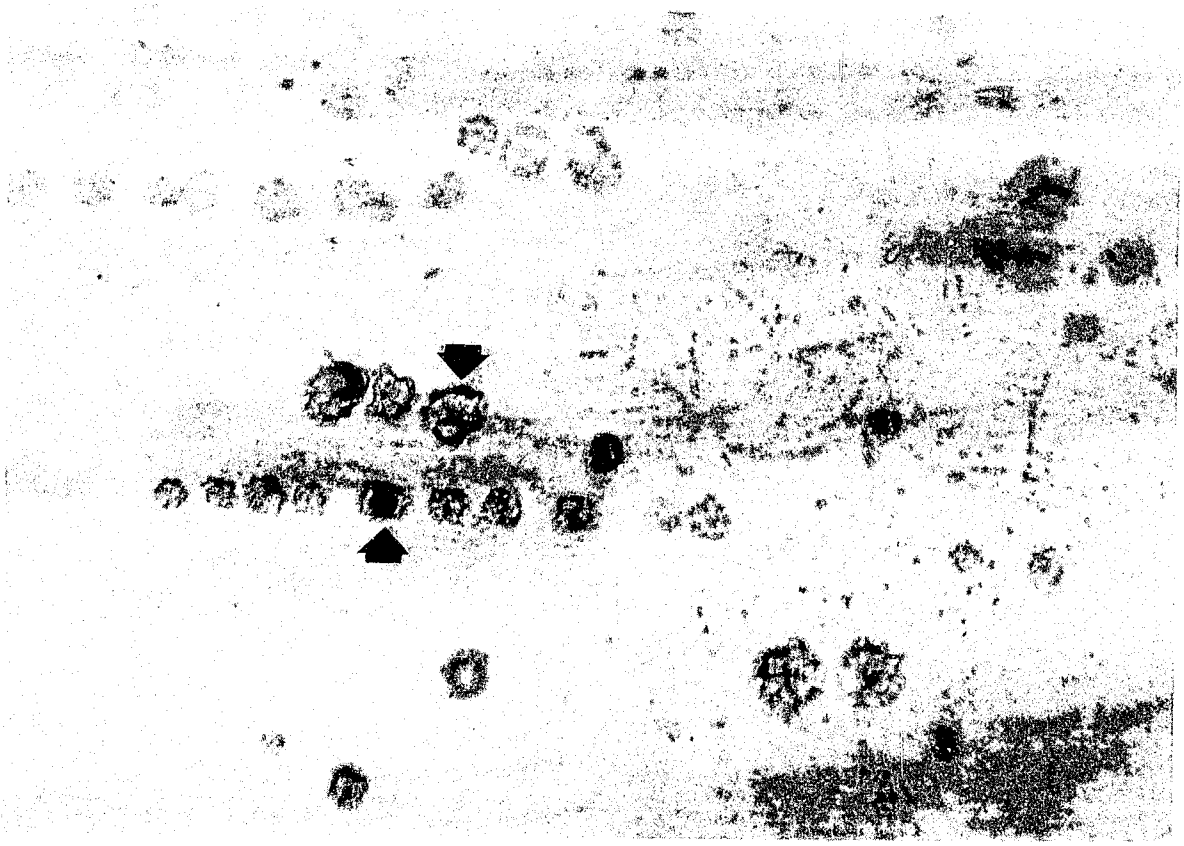
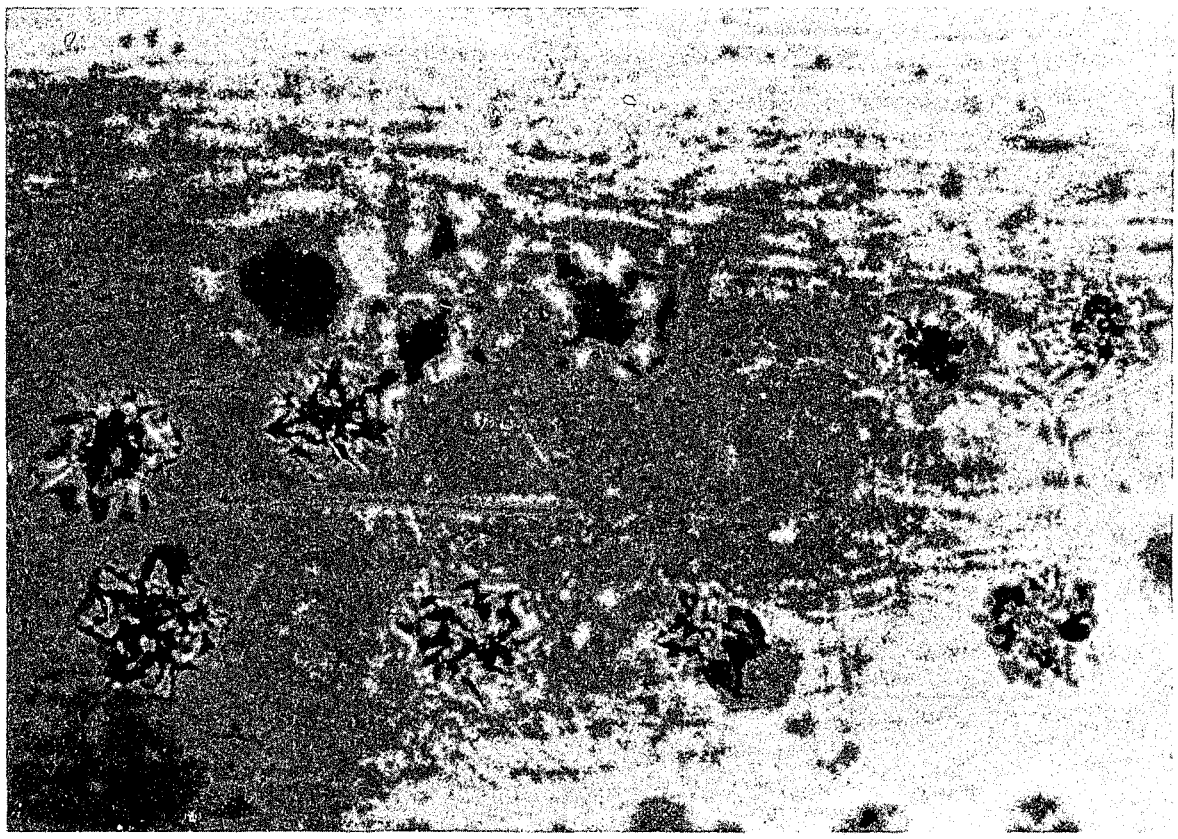


Figure 2.17 Crystals: raphide type (example from pineapple in Mounting Medium A, X560 taken at 25X).

Figure 2.18 Crystals: druse type (example from okra pulp in Mounting Medium A, X320 taken at 10X). These druses (arrow) are found in rows because they are aligned along the veins, although this is not the case in all food plants with druses.



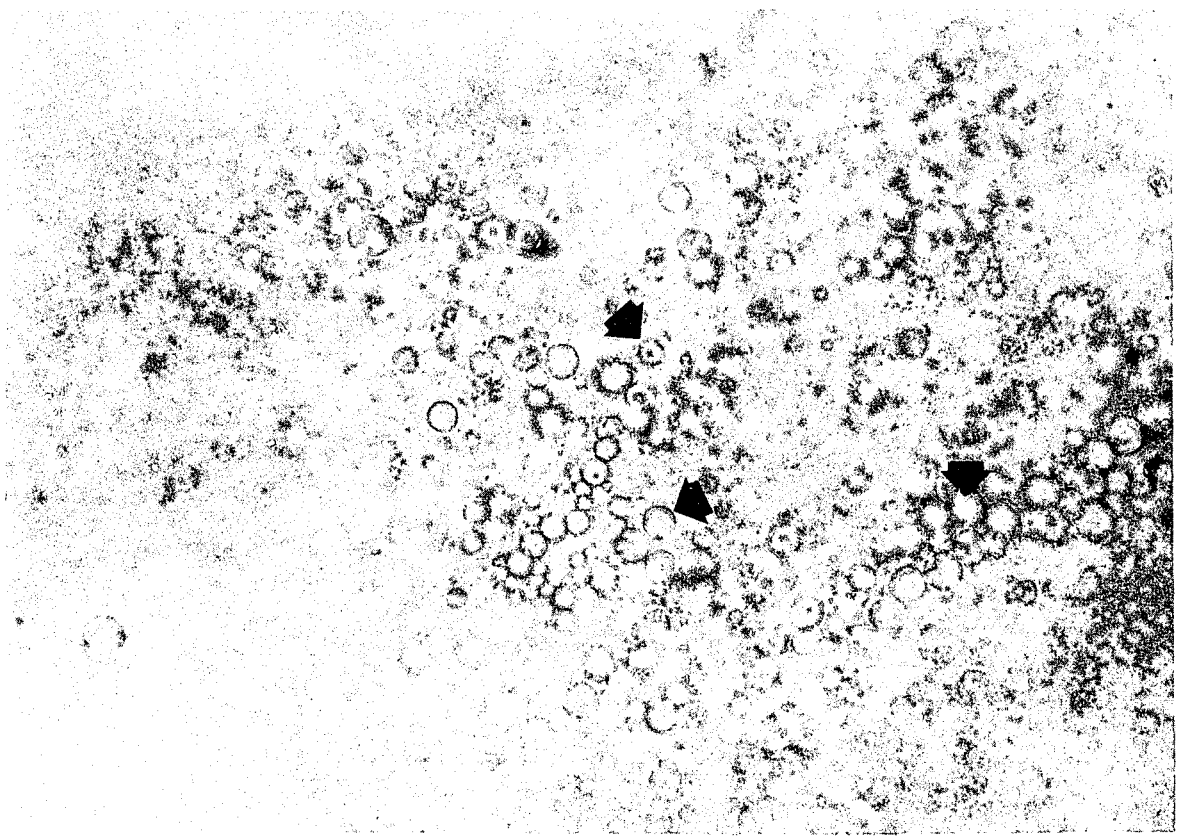
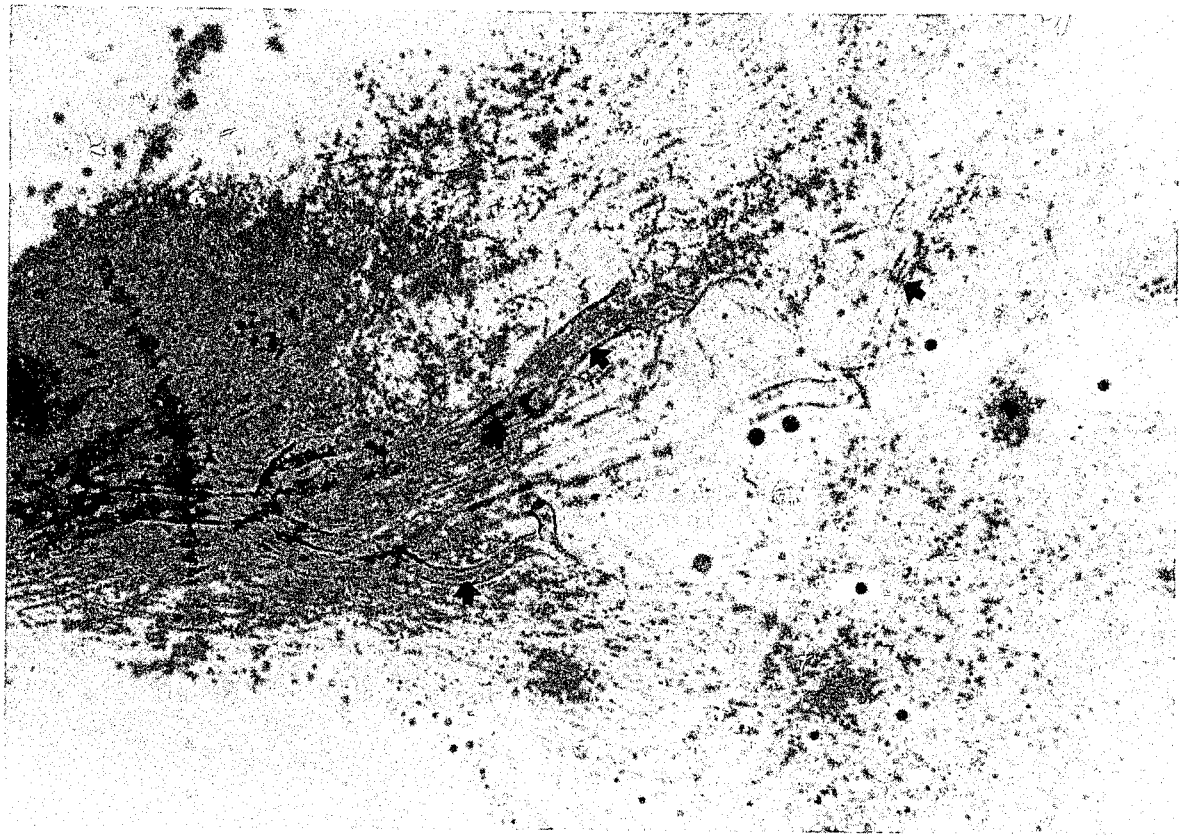


Figure 2.19 Crystals: druse type (example from okra pulp in Mounting Medium A, X560 taken at 25X). Note the "mace" shape of the crystals.

Figure 2.20 Starch grains (arrows) (example from squash in Mounting Medium A, X560 taken at 25X).



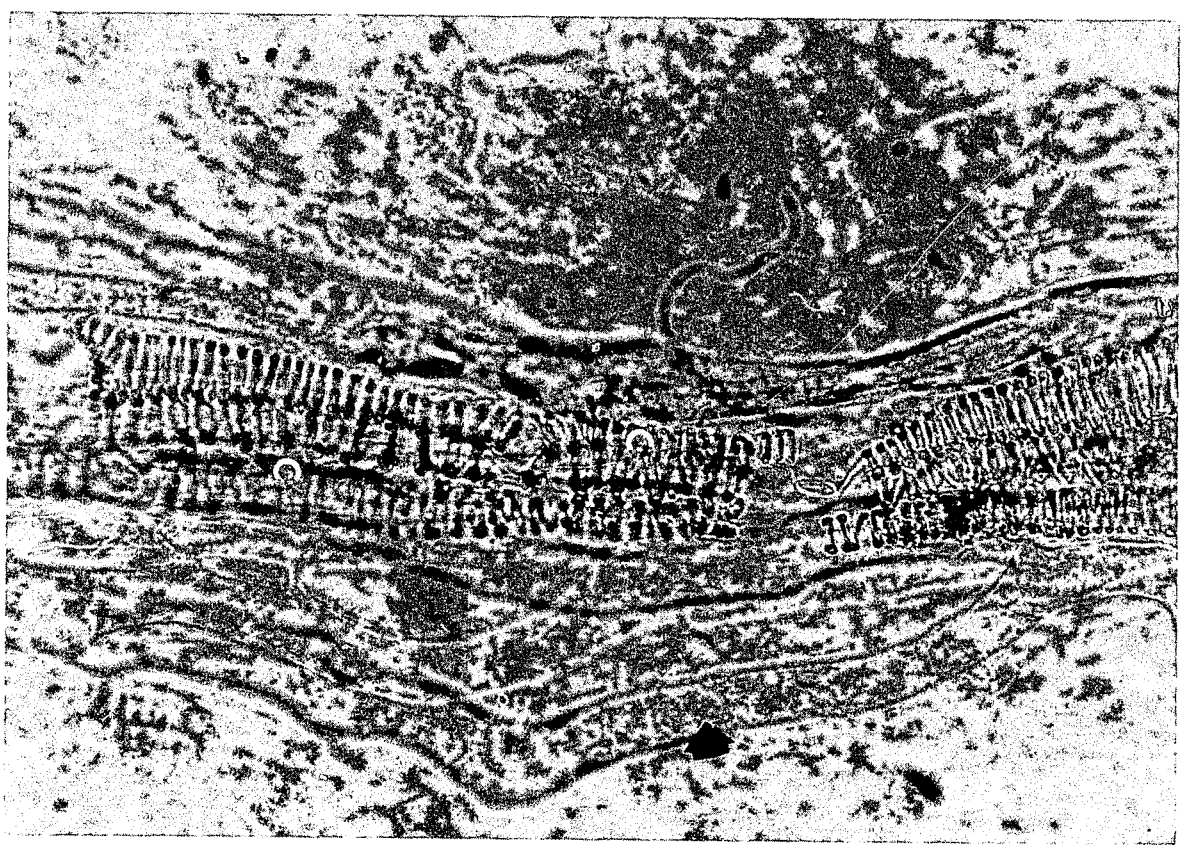


Figure 2.21 Fungal hyphae (arrows) in fruit pulp (example from papaya in Mounting Medium B, X224 taken at 10X). Note the lack of cross-walls and the branching pattern of the hyphae.

Figure 2.22 Fungal hyphae (arrow) in fruit pulp, and conducting cells (C) of the fruit pulp (example from papaya in Mounting Medium A, X560 taken at 25X).

# CHAPTER 3

## Introduction to the Digestion of Plant Cells

The digestive process begins with the chewing (mastication) of food and the mixing of it with saliva to form a mass or bolus that can be swallowed. Carbohydrate digestion begins in the mouth through the action of an enzyme known as salivary amylase, which attacks starches and some other complex polysaccharides. When the food bolus reaches the stomach, it is mixed by the churning action of the stomach muscles with hydrochloric acid (HCl) and the protein-digesting enzyme pepsin, which are both secreted by the stomach lining.

Generally, the events initiated in the mouth and completed in the stomach destroy animal cells from meat, chicken or fish very quickly. However, plant cells have rigid cell walls (see Chapter 1) composed of virtually indigestible materials, such as cellulose, and retain most of their external appearance although the cellular contents may be digested wholly or in part. Furthermore, each plant species consists of one or more distinctive cell type with recognizable external features that make it possible to identify the plant species by examining undigested fragments. Thus, it is possible to identify these cells with the aid of the compound microscope hours after they have entered the stomach. It also would be possible to make similar identifications from vomitus, intestinal contents or even feces. In many instances it has been possible for anthropologists to reconstruct diets of primitive human populations on the basis of plant cell analyses of petrified fecal remains. Indeed, the diets of northern Europeans of 2000 years ago have been discerned from the presence of plant material in the stomach contents of corpses that had been preserved for centuries in acid bogs.

In a healthy person, virtually all food leaves the stomach via a muscle-controlled valve, the pyloric valve, and enters the intestine for the completion of digestion. Normally, this takes place within four hours

after eating, even when a large meal is consumed. For a small meal or snack, the stomach often empties within a couple hours of eating.

# CHAPTER 4

## Supplies Needed

The supplies you will need to perform the analysis of stomach contents are listed in Appendix 1. We have designed a "Plant Cell Analysis Kit," which contains the supplies you need to perform the procedures we give below. This Kit is available from Carolina Biological Supply (address given in Appendix 1).

These supplies also can be obtained from vendors of scientific supplies and equipment. In Appendix 1, we have provided the names, addresses and telephone numbers of several vendors. Although this is not an exhaustive list of such vendors in the United States, under no circumstances do we intend to indicate any favoritism. Vendors in your area can be located through the classified pages of telephone books, usually under the heading "scientific apparatus and instruments."

You will need a compound microscope (often called simply a "light microscope") with 4X and/or 10X objective. A 25X or a 40X objective would be helpful, but is not imperative (see Chapter 6). An ocular micrometer and a stage micrometer are highly recommended, but the work can be done without them. A dissecting microscope with a total magnification of 8 to 10 X also would be helpful but is not required.

In Chapter 9, we present photographs of most of the plant foods in the Key (Chapter 8) taken on a scanning electron microscope (SEM). Though our Key relies only on light microscope observations (Chapter 6), you might wish to use SEM photographs to confirm your identifications. If you have the monetary resources, and an SEM laboratory in your area (most Universities have at least one), you can have an electron microscopist prepare samples of your materials according to the techniques described in Chapter 9. The photographs that the microscopist can provide you can then be used to compare to those in figures 9.1 through 9.56.

# CHAPTER 5

## How to Prepare Slides

The procedures we present here have been developed for the preparation of fresh chewed or previously preserved stomach contents. If you receive samples of stomach contents from an autopsy, you can assume they have been "previously preserved." The purpose of preparing slides from plant materials you (or an obliging co-worker) have chewed is to assure yourself of your identification of plant materials. If you think you might have a plant food in your sample that we have not included in this guide, or if your sample does not match well with one of our photographs, we strongly urge you to make your own comparison specimen(s). If you are checking more than one type of food, be sure to do each type individually. Use this (these) prepared sample(s) to check the identification of the unknown material. Also, always make several slides of each sample or "test food;" never rely on a single slide to show you all the features used in identification.

The solutions in the "Plant Cell Analysis Kit" are labeled with the names used in this Chapter. They are described in detail in Appendix 1 (see Chapter 4).

For all slides to be prepared, follow these first three steps:

STEP I

1. A. FOR FRESHLY CHEWED TISSUE. Chew the raw and/or cooked test food material thoroughly, about 40 times, (Resist the temptation to swallow!) and spit it into a sample vial. Fill the vial with FAA preservative and cap the vial tightly. Let stand for at least 24 hours.

or

1. B. FOR PREVIOUSLY PRESERVED STOMACH CONTENTS

Remove a **subsample** of the stomach contents material you received from the autopsy and place it in a **sample vial**. Add **70% ethanol** to fill the vial. Cap the **vial** tightly. Leave it there for at least 24 hours before proceeding further.

STEP II

1. For either treatment described under Step I, drain away as much of the liquid applied in Step I as you can without losing your subsample. Add enough drops of **Safranin 'O' Stain** to cover the sample. Usually one or two drops should be sufficient. The stain should remain in place for 15 minutes to one hour, depending upon the nature of the sample. If the material to be stained is thick or in clearly defined pieces, stain it for a longer time. If the material is fine-textured, proceed to the next step after 15 minutes of staining time. **Practice on your sample tissues to determine optimal staining time before proceeding to the stomach samples.**

STEP III

1. Add **picric acid solution** to the vial containing the subsample and the stain, and let this stand for 5 minutes.

2. After the 5 minutes, draw off the liquid in the vial with an **eyedropper** and dispose of it in a waste container appropriately labelled for picric acid disposal. Follow your Health Department rules to dispose of picric acid safely.

3. Add enough distilled water (or tap water if you do not have access to distilled water) to cover the subsample in the sample vial, and let it stand for 3 minutes.

4. Drain off the water and proceed quickly to the procedures below.

Now, we recommend that you divide your processed subsample into two portions. Follow the procedure for **Mounting Medium A** with one portion and for **Mounting Medium B** with the other portion. The two mounting media produce different results on some food materials, and it is often necessary to check the results from both in order to identify the material correctly.

For **Mounting Medium A** (Modified Hoyer's Medium) follow these steps (see below for Mounting Medium B):

STEP IV

1. Lay out the number of **microscope slides** you wish to prepare on your workspace.

2. Remove tissue pieces from the **sample vial** with **forceps**, and distribute them thinly (so that they will transmit light, see Chapter 6) on the slide's surface in an area a little smaller than the area of the **coverslip** you will use.

3. You should do this process quickly to keep the tissue pieces from drying out! Put just enough of **Mounting Medium A** on the sample to cover it.

STEP V

1. Place a **coverslip** carefully over the sample in the following manner. Lay one edge of a coverslip alongside the Mounting Medium, and let the coverslip fall slowly into place as a cover over the tissue pieces.

2. Gently press the coverslip into place with a pencil eraser to

complete the contact between the coverslip and the specimen. If you see air bubbles under the coverslip, put a piece of blotting paper or paper towel over the coverslip and tap the coverslip, through its paper covering, several times with the eraser. Be sure to remove the blotter after you have tapped the into place, or it will be glued to the slide by the Mounting Medium as it dries.

3. Carefully attach to the slide a **label**, which tells what it is and when it was prepared.

4. Allow the slide to dry flat on a table for several hours or overnight.

5. Now, you are ready to examine the slide under the microscope.

For **Mounting Medium B (Permount)**, **follow these steps:**

STEP IV

1. Fill the vial with **70% ethanol**, and let it stand for 3 minutes.

2. Drain the vial, fill it with **95% ethanol**, and let it stand for 3 minutes.

3. Drain the vial, fill it with with **100% ethanol**, and let it stand for 3 minutes.

STEP V

1. Replace the **100% ethanol** with fresh **100% ethanol**, and add an equal amount of **clearing agent** to the vial. We recommend using a clearing agent with low toxicity. Take great care to avoid inhaling the **clearing agent**. If your

lab has a fume hood, perform this step under the hood.

STEP VI You should do this process quickly to prevent the tissue pieces from drying out!

1. Lay out the number of **microscope slides** you wish to prepare on your workspace.

2. Remove tissue pieces from the **sample vial** with **forceps**, and distribute them thinly (so that they will transmit light, see Chapter 6) on the slide's surface in an area a little smaller than the area of the **coverslip** you will use.

3. Put just enough of **Mounting Medium B** on the sample to cover it.

STEP VII

1. Place a **coverslip** carefully over the sample in the following manner. Lay one edge of a coverslip alongside the sample, and let the coverslip fall slowly into place as a cover over the sample.

2. Gently press the **coverslip** into place with a pencil eraser to complete the contact between the coverslip and the specimen. If you see air bubbles under the coverslip, put a piece of blotting paper or paper towel over the coverslip and tap the coverslip, through its paper covering, several times with the eraser. Be sure to remove the blotter after you have tapped the coverslip into place, or it will be glued to the slide by the Mounting Medium as it dries.

3. Carefully attach to the slide a **label**, which tells what it is and when it was prepared.

4. Allow the slide to dry flat on a table for several hours or

overnight.

5. Now you are ready to examine the slide under the microscope.

## Performing a Starch Test

Starch can be identified by putting a drop of **potassium iodide solution** on the material you think contains starch. Starch grains stain black in the presence of potassium iodide. You can make a slide by placing a drop of the original stomach sample on the slide, adding a drop of potassium iodide, and then adding a coverslip. Observe the slide under the microscope. Individual starch grains will be stained black. You also can perform the starch test by adding a drop of potassium iodide to material removed from the sample and placed in a small dish. If it turns black, then starch is present.

If you feel that it is necessary to your investigation to know exactly what type of starch grains you might have (i.e. whether it is potato starch or wheat starch or rice starch, etc.), take some of your sample to a botanist at a college or university who has a polarizing light microscope. Different sorts of starch grains have different patterns under polarized light, but the exact identification of them is well beyond the scope of this book.

# CHAPTER 6

## Microscopic Examination of Slides

### Parts of a Light Microscope and How They Work

A microscope is a system of lenses which focuses a beam of light on a narrow area, and then transmits that light beam through additional lenses to the eye of an observer. Magnification is accomplished during this process by the physical properties of the lenses. By placing a specimen in this light path, characteristics of it that are too small to see with the unaided eye become magnified and therefore observable. Visibility depends on the ability of every surface in the light path to transmit the light. If the specimen is too thick or otherwise opaque, observation will be impaired. Remember this when preparing slides (see Chapter 5). Likewise, the lens surfaces must be free of any dust or film that might limit the amount of light transmitted through them.

The important components of the microscope are the **light source**, the **condenser**, the **stage**, the **objective lenses**, and the **ocular lenses**, which are also called eyepieces (figs. 6.1 and 6.2). The light source may be a **built-in illuminator** (fig. 6.1), or a **mirror** (fig. 6.2) positioned so that light from a lamp or window is reflected at an angle to the condenser. The condenser is so-called because it narrows the beam of light coming from the illuminator into a beam that just fills the area of the objective lens, which is usually on the order of 2 mm or so wide. The stage is the platform on which the specimen slide is placed. There is a hole in the stage which is directly in line with the condenser and with the objective lens. When the slide is positioned properly, the light transmitted by the condenser will pass through the slide, the specimen, and into the objective. Within the objective is the first of the magnifying lenses. Different objectives (4X, 10X, 40X, etc.) have different combinations of lenses to provide the different amounts of magnification. The light passes through the objective and into the

ocular, where one additional magnifying lens (usually 10X) is located. The final magnification of the specimen that is the amount of magnification provided by the objective times the amount of magnification of the ocular. Therefore, if you are using a 4X objective, the specimen is magnified 40 times to your eye, or 100 times if you are using a 10X objective.

One of the characteristics of magnifying lenses is that the image of the specimen is rotated 180° with respect to the actual object being observed. Thus, when you look at a specimen through the microscope, it will appear upside down and backwards in relation to the actual position of the specimen. This will present no problems to you in identifying plant cells with the Key in Chapter 8, but you may need to practice with the microscope to accustom yourself to the direction objects appear to move when you move the slide about on the stage so that you can examine all parts of your specimen.

When you look through the microscope, the circular, illuminated area that you see is called the **field of view** or just **field**. Also, microscopists must consider a phenomenon called **depth of field**. The depth of field is dependent upon the **focal length** of the **objective lens** that you are using. The focal length is the distance between the lens and the object being viewed through it, within which the object is "in focus" to the observer. If an object is in focus, it is within the **focal plane** of the lens. A low magnification lens has a greater focal length and therefore a deeper focal plane and therefore greater depth of field than does a high magnification lens. Rarely are all parts of a specimen in the focal plane at once. Microscopists must therefore adjust the focus carefully and frequently to see all the features of the specimen being studied.

It is difficult to see differences among dark objects on a dark night, or white objects against a white background. It is much easier to see dark objects on a light background, because the object and background contrast one another. Almost no biological tissue being examined under the microscope will have contrast among its parts unless it is stained with a dye that stains some features of

the tissue and not others. There are many stains that have been developed for use with microscopy, but we are recommending in this guide that you use only one: Safranin O (see Appendix 1 above for vendors of Safranin O), which is readily available and easy to use. Safranin O is an excellent stain for plant tissues because it will differentiate between lignin, which takes up safranin, and cellulose, which does not take up safranin), and among other features of the cells and tissues.

## How to Use the Microscope

Before beginning any microscope work, always make sure that your instrument is clean. Use lens paper (NEVER use facial tissue, Kimwipes, etc. because they are rough and can scratch the glass) to clean all the glass surfaces, including the surface of the light source (mirror or illuminator), the condenser lens, and the objective and ocular lenses (see figs. 6.1 and 6.2). Rarely is liquid necessary to clean lenses, but should it be, use a lens cleaner such as those used for cameras or other optical equipment (do NOT use alcohol or xylene, etc.). Wearers of mascara should remove it before using the microscope.

When you are ready to examine your slides (see Chapter 5 on slide preparation), make sure that the lowest power objective (the shortest one) is in place for viewing. Place the slide on the stage carefully, so that you do not touch the surfaces of any of the objective lenses with the slide. Turn on the built-in illuminator, or position the mirror, so that the field of view through the ocular(s) is just filled with light. Position the condenser (if it is movable) as high as it will go. This should mean that it is right under the slide. Then drop it down about the same distance below the stage as the objective is above the stage, and adjust it to give the maximum brightness. Bring the specimen to be examined into focus by turning the coarse adjustment. After you have done that at low power, always keep a hand on the fine adjustment, and focus up and down continually because you are looking at three-dimensional objects which are seen only in two dimensions at any

one level of focus. Never use the coarse adjustment for focusing except at the lowest powers (4X to 10X) because you can actually hit the slide with the high power lenses if you use the coarse adjustment when one of them is in place. This can, and probably will, damage both your slide and the lens.

For the observations you will make while using this book, you will probably need only the 4X or 10X objective for most of your observations, and rarely you may need a 25X or 40X objective. If you have a 25X objective, you will find it an adequate higher magnification for making identifications, yet it is short enough to minimize the possibility of damaging the slide or the objective. Should you need the higher magnification, make sure your specimen is in focus at low power (4X or 10X), before switching the 25X or 40X objective into place. Your specimen should still be nearly in focus, and you can use the fine adjustment to fine-tune the focus. Remember, however, that the high power lens will have a shallower depth of field than the low power lens. This means that adjusting the focus continually and carefully will be even more important.

When you have finished using the microscope, make sure that it is clean -- don't leave drops of water or mounting media on any part of the microscope. Cover the microscope between uses with a clean plastic bag to protect it from dust. Taking these precautions will simplify the cleaning you need to do before you use the microscope the next time.

## Measuring on the Microscope

You will find it necessary in certain cases to make comparisons of cell sizes when using the dichotomous Key in Chapter 8 to identify and verify the correct plant. In order to estimate the size of an object that you are viewing through the microscope, you must first determine the magnified width of the whole area (the "field") that you see through the eyepiece(s). By placing a **clear plastic millimeter ruler** (see Appendix 1) on an empty slide on the microscope stage and looking at it in focus

through the microscope on lowest power (typically 4X or 10X), you can approximate the diameter of the field. Follow these steps:

1. Determine the width of the field in millimeters (mm) by counting the number of lines on the ruler that are visible through the microscope at low power. Write this number down.

2. Divide the magnification of the next higher power objective lens of your microscope by the magnification power of the lowest power lens (the one that you used to view the millimeter ruler). For example, if your lowest power lens was 4X, and your next highest power is 40X, the number that you would derive from doing this step is 10 (40 divided by 4). Write this number down.

3. Now divide the field diameter in millimeters (mm) that you derived in step 1 by the number that you derived in step 2. This will give you the approximate field size for the higher of the two magnifications. Write this number down.

4. When you are using the Key (see instructions on use of a key, Chapter 7), and it asks you for information on sizes of objects, estimate the length and width of an object in number of millimeters by estimating what proportion of the total field size (for the magnification you are using) that the object takes up. For example, if your field size is 3.5 mm, and the object is one-half as long as the field is wide, then the object's length is approximately 1.75 mm.

A more accurate way to determine the diameter of the field is to use a stage micrometer, which is a finely subdivided millimeter ruler embedded in a glass slide (fig. 6.3). This is available from a variety of commercial suppliers (see Appendix 1). It is used in exactly the same way as the millimeter ruler is used,

except that the field diameter for each magnification is measured directly, rather than by estimate. Instructions are provided with the micrometer.

For precise measurements, it would be best to have an **ocular (eyepiece) micrometer** as well as a **stage micrometer**. The ocular micrometer has a small scale or grid etched into a circular piece of glass that can be inserted into the ocular of the microscope (or into one of the two oculars if you have a binocular microscope). By focusing on the stage micrometer with the ocular micrometer in place, you can accurately determine how many fractions of a millimeter are represented by each of the arbitrary units on the ocular micrometer. Once this calibration is done for each magnification on the microscope, one can easily determine the measurements of any object viewed at any magnification by "measuring" it with the ocular micrometer, and multiplying the number of arbitrary units by the fraction of millimeters that each arbitrary unit represents at the particular magnification that you are using. Use of the ocular micrometer is recommended even where the ruler technique is used to estimate the field diameter. Instructions for this are provided with an ocular micrometer, should you purchase one.

## Indicating the magnification used

When you are writing or speaking about the magnification used in microscopic work, there are two conventions that you should follow. We have followed these in the figure captions for all of the microscopic photographs included in this book.

Convention 1: When referring to the degree of magnification used when you were actually looking through the microscope (that is, which objective lens you were using), you write the number first, and then add an "X" for "times" as in "...photograph taken using a 10X objective."

Convention 2: When referring to a photograph taken through the microscope and then printed in a book such as this one, the total number of times that the final image is bigger than the actual object is written after the "X," as in "... X224" or "X560."

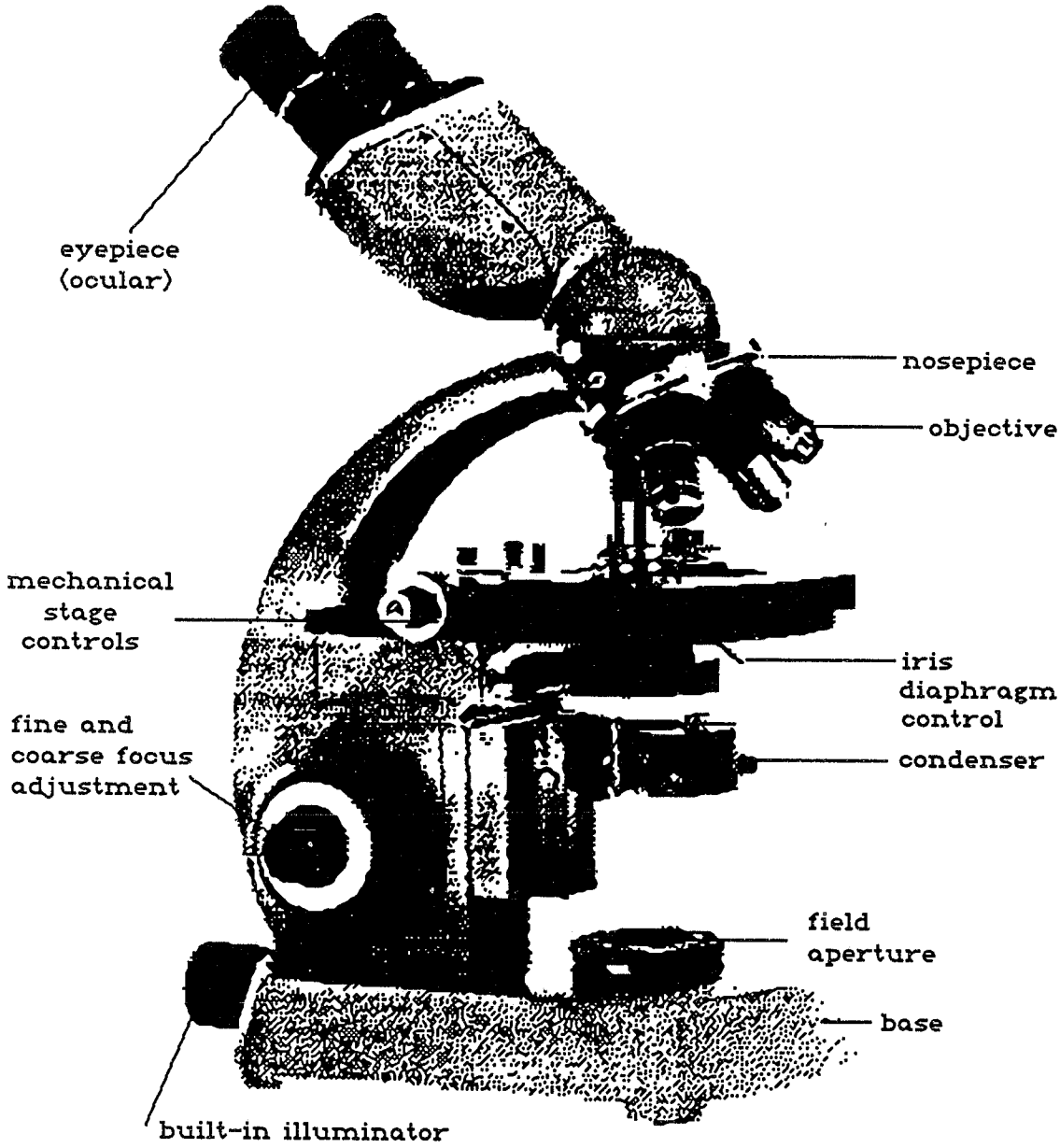



Figure 6.1 Diagram of a binocular compound microscope with built-in illuminator and mechanical stage.

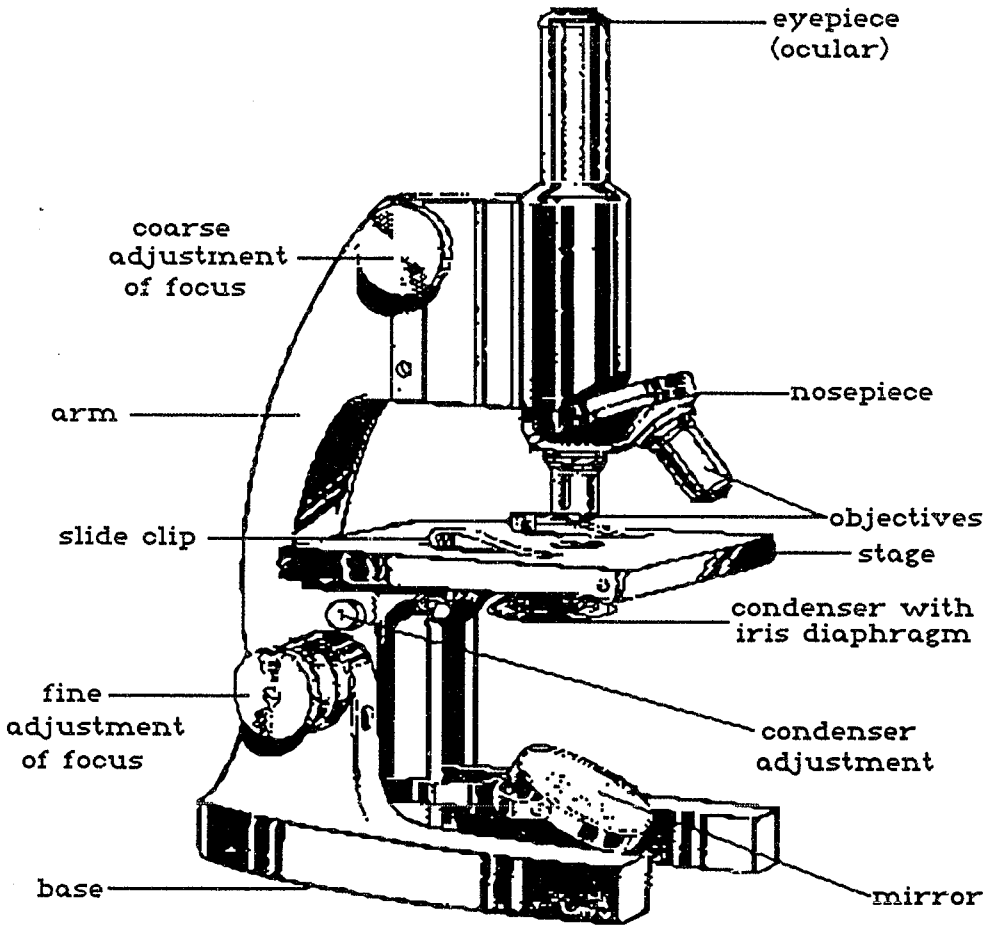



Figure 6.2 Diagram of a monocular compound microscope with mirror and standard stage with slide clips.

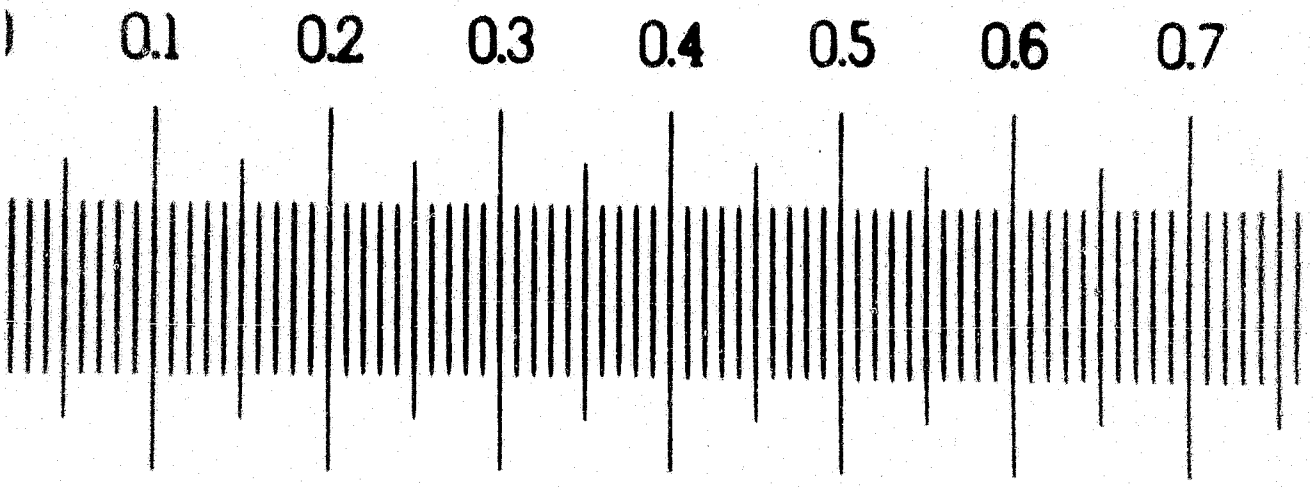



Figure 6.3 Photograph (X224) of a stage micrometer taken through a 10X objective.

# CHAPTER 7

## Using a Dichotomous Key to Identify Materials

"Dichotomous" means "branching in two." A **dichotomous key** is so-named because it consists of a series of two-branched choices. You use a key by choosing one of the two branches each time a question is raised, until the "answer" (the identity of the object being considered) is reached.

The key that we present in this guide (Chapter 8) is called a **yoked key** because the two branches of each choice are presented together, i.e. yoked. The two branches are parts of a **couplet**, and will be given the same number. Example:

1. Fruit spherical, skin usually slightly lumpy and orange in color.....Orange

1. Fruit basically spherical but indented top and bottom, skin smooth and red, yellow or green.....2

2. Skin red.....Red Delicious Apple

2. Skin yellow or green.....Golden Delicious Apple

The two statements which are numbered "1" are the two branches of couplet number 1. The two statements that are numbered "2" are the two branches of couplet number 2. If you have in your lunchbox a round fruit that is smooth-skinned and red, you would determine its identity by using the example key presented above (please bear with this simple example) as follows:

Observe the object that you wish to identify, noting its obvious characteristics (basically spherical, but not quite; smooth; red-colored). Read **both** statements of the first couplet, which is asking you if your fruit is orange or some other color and whether it is or is not spherical and smooth. The object under consideration does not match the characteristics listed in the first statement of the first couplet, but it does agree with those listed in the second

statement of the first couplet. Therefore, you take that branch of the "road" by continuing on to couplet number two, as the statement that you chose directs you to do. You then read both the statements of couplet number two. The object you hold has red rather than green or yellow skin, so you choose the first "branch" of the couplet, which tells you the fruit in your lunch is a Red Delicious Apple.

This is an extremely simple example, but it does illustrate the method for using a key: 1) always read both the statements of a couplet before making a choice between them, and 2) follow the instructions given you by the statement you chose. These instructions will either be to attach a name (given at the end of the statement) to your object of consideration, or to proceed to another couplet within the key. In a long key such as that in Chapter 8, this couplet most likely will not come next on the page, but may in fact be some distance away along the branching path. You find it by following the numbering system. Go immediately to the couplet indicated by the answer to a particular couplet. If the statement of couplet number 1 you choose tells you to go to number 23 (as does the one in Chapter 8), skip over couplets 2 through 22.

Because there are always two (and only two) statements per couplet, you need only make a choice between two possibilities at any one time. If you proceed through a key and arrive at an answer (a name) that seems incorrect, or come to a couplet that presents no statement that seems correct, you can backtrack through the key to the couplet at which you may have taken a wrong turn. Then work forward again, checking your choices carefully as you go. Once you believe you have identified the specimen according to the key, read the detailed description and examine the photographs provided here to verify your identification by comparison.

You could identify your object by comparing it with every photograph in this guide until you find a match, but that is extremely time-consuming. Using the key is a much faster way of arriving at a possible name. Checking your material against the

photograph suggested by the key is a much quicker way to verify your identification.

If you think you might have a plant food in your sample we have not included here, or if your sample does not entirely match one of our photographs, we strongly urge you to make your own comparison specimen(s). Do this by preparing samples, according to the directions in Chapter 5, of the plant food(s) that you think your sample might be. If you are checking more than one type of food, be sure to do each one individually. Use this (these) known, prepared sample(s) to compare with the identification of your unknown material.

# CHAPTER 8

## Key to Plant Foods Commonly Found in Stomach Contents

1. Plant material containing inclusions or distinctive cell types such as oil droplets, hairs, spores, fibers or crystals ..... 2

1. Plant material consisting of cells only, without inclusions or distinctive cell types ..... 23

2. Parenchyma (thin-walled, balloon-like) cells a prominent feature of the material ..... 3

2. Epidermal or peel (thick-walled, closely packed) cells a prominent feature of the material ..... 19

3. Conducting cells a prominent feature of the material ..... 4

3. Conducting cells an uncommon feature of the material ..... 12

4. Hairs present in the material ..... 5

4. Hairs absent in the material ..... 7

5. Crystals (druses approximately 0.02-0.035 mm in diameter and rectangular prisms 0.01-0.02 mm long) present in the material ... **okra**

5. Hairs but no crystals present in the material ..... 6

6. Hairs dense, short, 0.1--0.15 mm long, 0.01 mm diameter ..... **peach**

6. Hairs scattered, to 0.5 mm or more long ..... **strawberry**

7. Starch grains present in the pulp, no other inclusions present .... **squash** (including **zucchini**, **crookneck**, **acorn**, etc. and **pumpkin**)

7. Inclusions present in the pulp, starch grains not prominent ..... 8

8. Crystals present, associated with connecting tissue ..... 9

8. Crystals present, occurring away from connecting tissue ..... 11

9. Druses occurring singly ..... **fig**

9. Druses occurring in clusters or rows ..... 10

10. Druses 0.03-0.10 by 0.06-0.20 mm, single, or in rows to 0.4 mm long; pulp sacs, 0.03 mm wide, 0.2--0.6 mm long, possibly also present ..... citrus (including lemon, lime, and orange)

10. Druses very small, many contained within ovoid cells which are 0.02-0.08 mm wide, 0.08-0.2 mm long ..... beets

11. Druse crystals present ..... spinach

11. Raphide crystals present, occurring individually or in clusters ..... pineapple

12. A thin epidermis (skin, seed coat) present in the material ..... 13

12. An epidermis absent, or so thick that it cannot be observed with the microscope ..... 16

13. Starch grains included in the material ..... 14

13. Other inclusions (but no starch grains) present in the material ..... 15

14. Epidermal cells rounded, approximately 0.01 mm in diameter, closely packed ..... beans (including peas, garbanzo beans, lima beans, pinto beans, and the bean portion of green beans)

14. Epidermal cells elongate (0.02 mm wide and 0.4 mm long) or angular and approximately equal in all dimensions ..... corn

15. Oil droplets or deposits present ..... olive

15. Druse crystals present, 0.01 mm in diameter ..... plum and prune

16. Large, thick-walled, darkly staining conducting cells, 0.1 mm in diameter and varying in length, present in pulp ..... banana

16. Rounded, ellipsoid or irregular distinctive, darkly-staining objects present in pulp ..... 17

17. Objects in pulp ellipsoid, 0.3--0.7 mm long, scattered among pulp cells that have a netlike appearance ..... kiwi

17. Objects in pulp rounded or irregular, found scattered or in rows ..... 18

18. Large, irregular, many-angled cells (stone cells) scattered in the pulp ..... pear

18. Small, rounded cells, which appear as opaque dots contained within clustered, thin-walled, cylindrical cells without crosswalls occurring in the pulp ..... mushrooms

19. Epidermis with branched hairs.....rosemary

19. Epidermis with unbranched hairs.....20

20. Epidermal cells interlocking, like jigsaw-puzzle pieces.....oregano

20. Epidermal cells angular.....21

21. Hairs on epidermis very dense, druse crystals present in addition to hairs.....apricot

21. Hairs on epidermis scattered and few, no crystals present.....22

22. Stomates, but not veins, prominent in the material.....pod portion of green beans

22. Veins, but not stomates, present in the material.....sweet basil

23. Conducting cells a prominent feature of the material.....24

23. Pulp or epidermal cells a prominent feature of the material.....32

24. Epidermis or peel present in the material.....25

24. No epidermis or peel present in the material.....31

25. Epidermis or peel cells elongate, linear or rectangular.....26

25. Epidermis or peel cells rounded or irregular.....27

26. Epidermal cells linear, veins extremely prominent.....celery

26. Epidermal cells rectangular.....onions
(including green, red, white, and yellow onions, garlic and scallions)

27. Epidermis composed of thick-walled cells.....28

27. Epidermis composed of thin-walled cells.....30

28. Epidermal cells rounded, very thick-walled.....cherry

28. Epidermal cells with sharp corners.....29

29. Pulp cells many shapes and sizes.....cucumber

29. Pulp cells irregular, with rounded corners, 0.03--0.1 mm in diameter.....grapes and raisins

30. Epidermal cells with wavy edges, jigsaw puzzle-like in outline...lettuce

30. Epidermal cells with smooth edges, irregularly box-like in outline.....cabbage

31. Pulp cells thick-walled, at right angles to conducting tissue, ends tapering ..... caraway

31. Pulp cells thin-walled, pulp including fungal hyphae (wandering, branching tubes without cross-walls) up to 0.02 mm in diameter ..... papaya

32. Skin, peel, or epidermis present in the material ..... 33

32. No skin, peel, or epidermis present in the material ..... 38

33. Pulp cells larger than 0.15 mm in diameter ..... 34

33. Pulp cells smaller than 0.15 mm in diameter ..... 35

34. Epidermis or peel cells blocky, with rounded corners, several layers thick ..... apple

34. Epidermis or peel cells regularly 4- or 5-sided, angular, usually only one cell layers thick ..... tomato

35. Epidermal or skin cells brick-like, angular ..... 36

35. Epidermal or skin cells round or jigsaw-puzzle like ..... 37

36. Epidermal cells very dark-staining, arranged in brick-like rows, two to three times as long as wide, ends tapered; pulp not prominent ..... poppy seed

36. Epidermal cells delicate, not arranged in rows, approximately equal in all dimensions; conducting cells approximately 0.15 mm long and 0 03 mm wide scattered in pulp ..... radish

37. Epidermal cells rounded in outline, with sculptured surface ..... sesame

37. Epidermal cells interlocking, jigsaw-puzzle like, surfaces smooth ..... peppers (including bell, chili, etc.)

38. Pulp composed of rounded parenchyma cells ..... 39

38. Pulp composed of brick-like cells in rows ..... carrot

39. Conducting cells found only within veins ..... water chestnut

39. Conducting cells not only found in veins, but also scattered in pulp ..... turnip

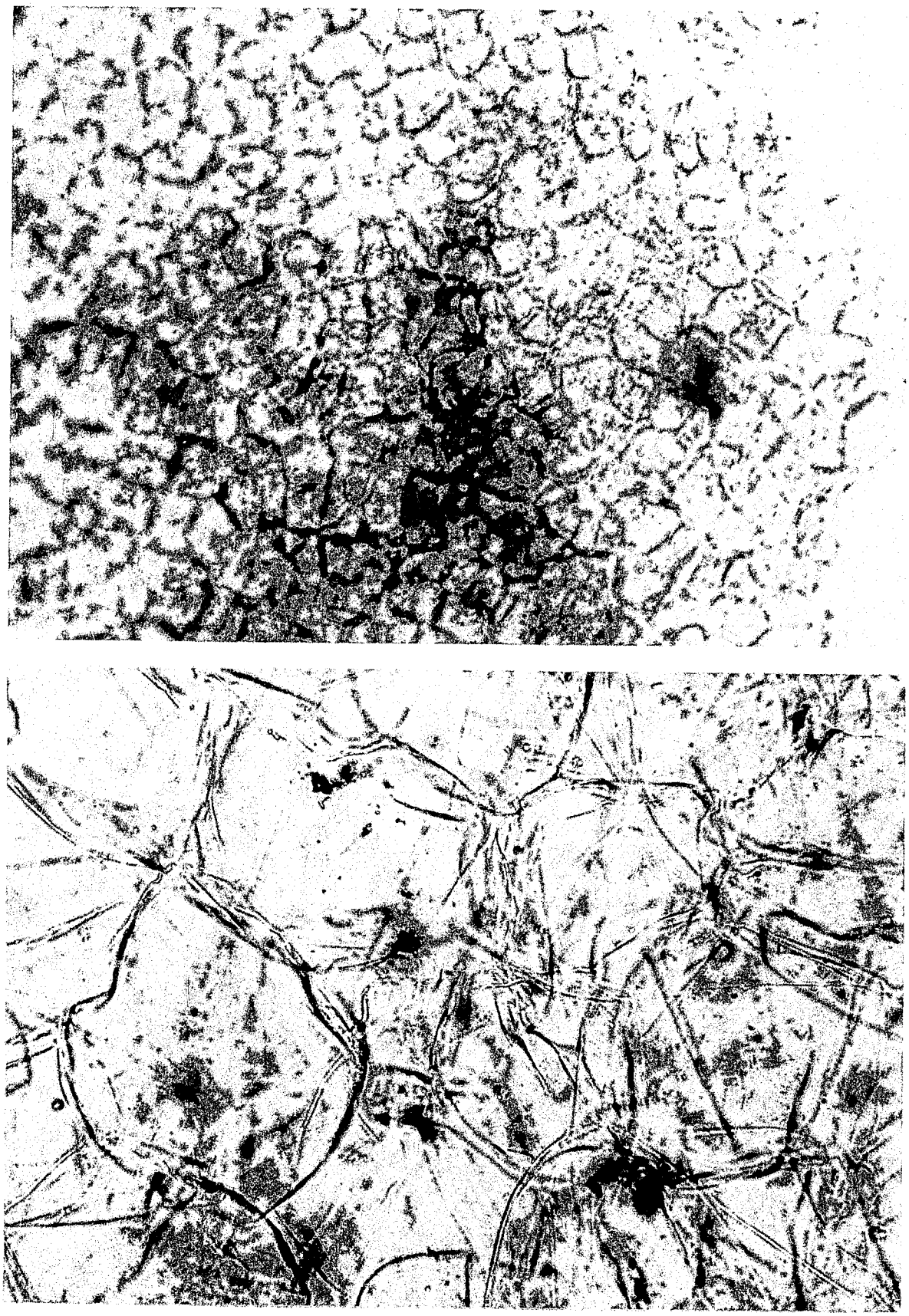
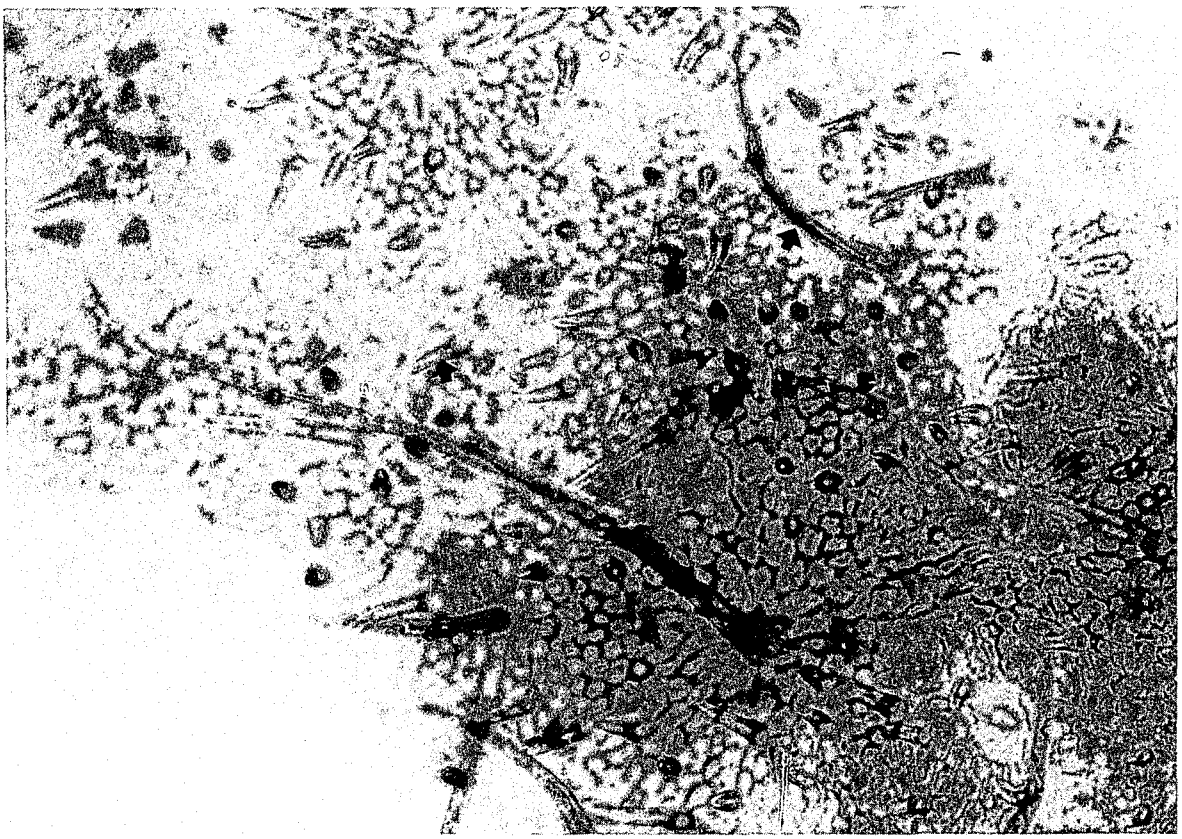


Figure 8.1 Apple peel (X292, taken at 10X) in Mounting Medium B. Cells blocky, corners rounded, several layers thick. Compare to cherry, plum or prune, and tomato. Also see fig. 9.1.

Figure 8.2 Apple pulp or applesauce (X224, taken at 10X) in Mounting Medium A. Cells thin-walled, larger than 0.15 mm in diameter. Compare to cherry, fig, grape or raisin, okra, pear, plum or prune, radish, strawberry, tomato, turnip, and water chestnut. Also see fig. 9.2.



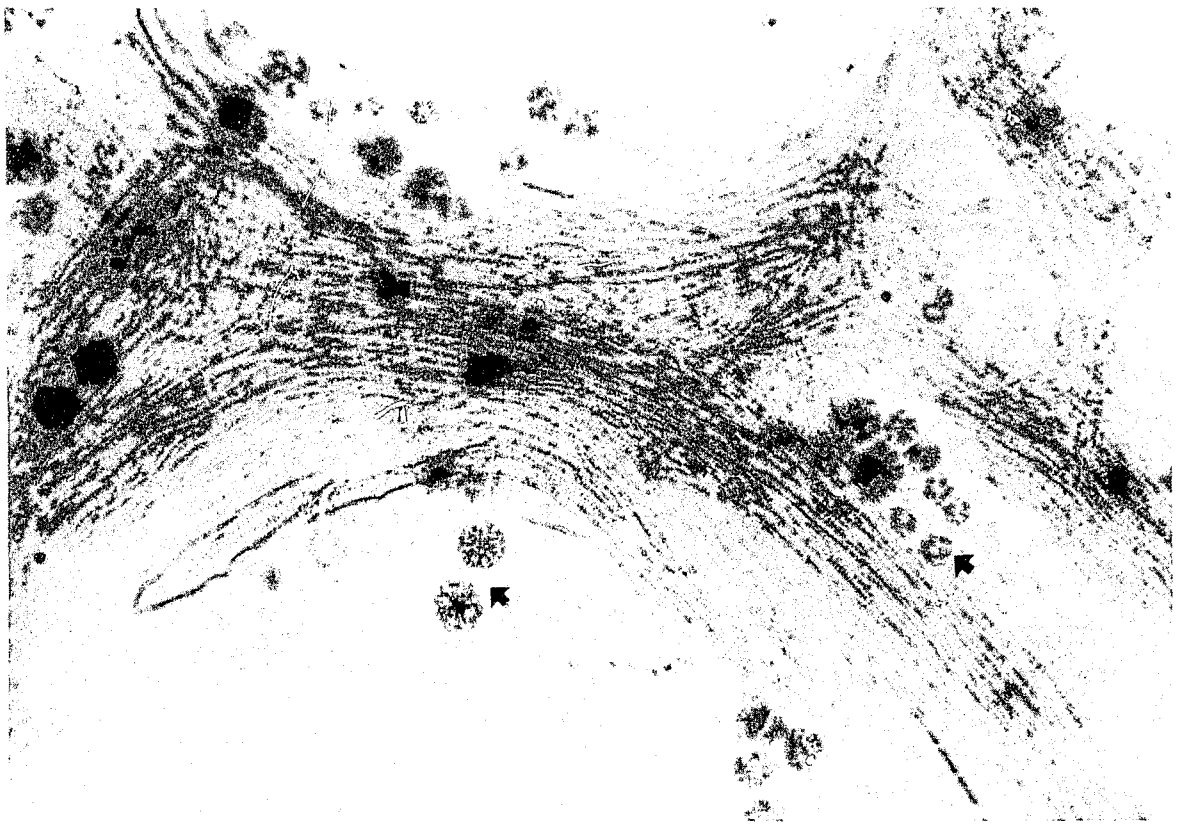
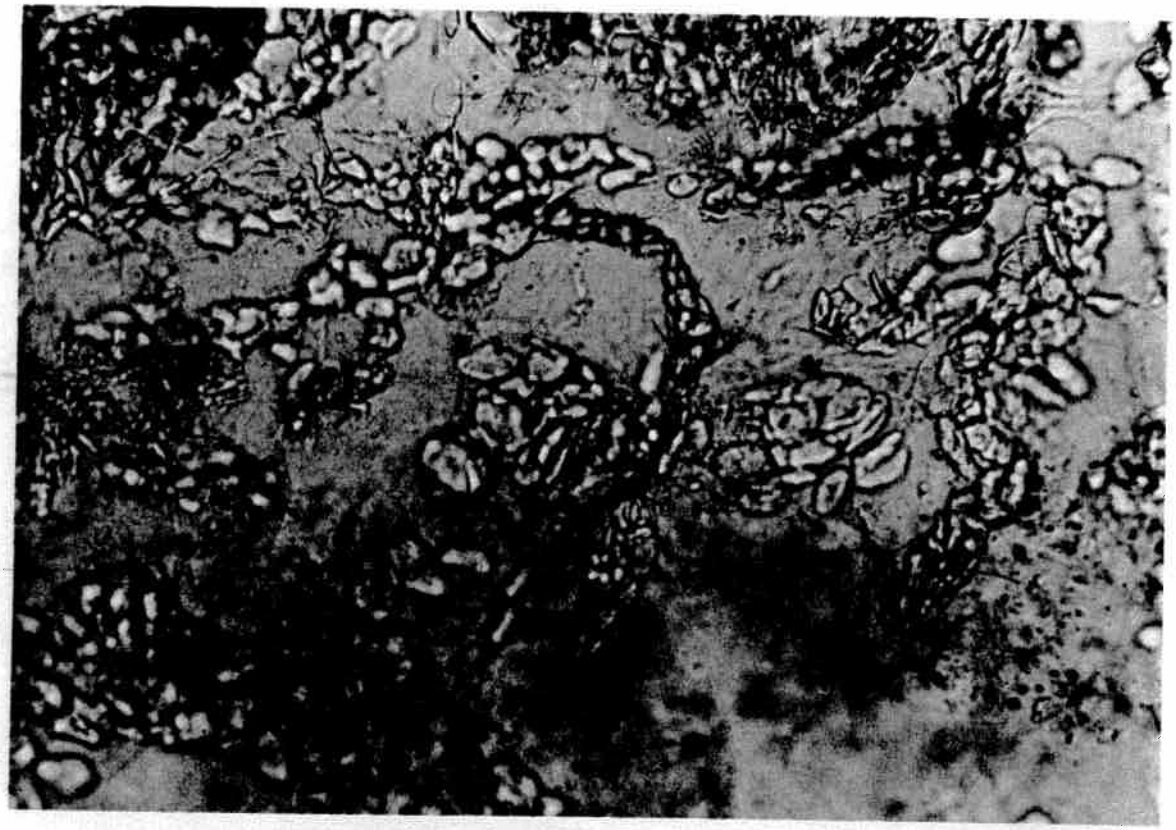


Figure 8.3 Apricot skin (X226, taken at 10X) in Mounting Medium B. Note unbranched epidermal hairs (arrow). Compare to green bean pod, okra, oregano, peach, rosemary, strawberry, and sweet basil. Also see fig. 9.3.

Figure 8.4 Apricot pulp (X226, taken at 10X) in Mounting Medium B. Note druse crystals (arrow) near conducting tissue. Compare to fig pulp (fig. 8.29). Also see fig. 9.3.



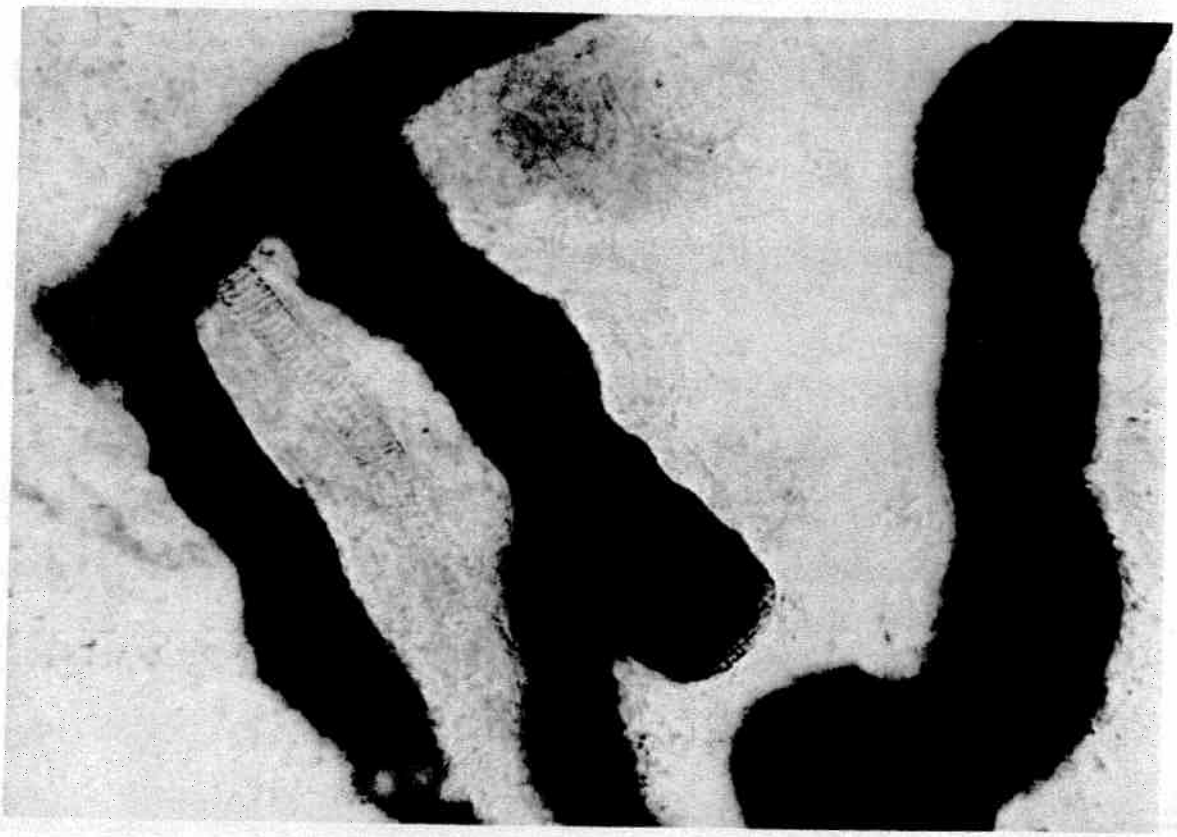


Figure 8.5 Banana pulp (X560, taken at 10X) in Mounting Medium A. Note numerous irregularly shaped starch grains (arrow).

Figure 8.6 Banana pulp (X224, taken at 10X) in Mounting Medium B. Distinctive, thick-walled, irregularly barrel-shaped conducting cells. Also see fig. 9.4.

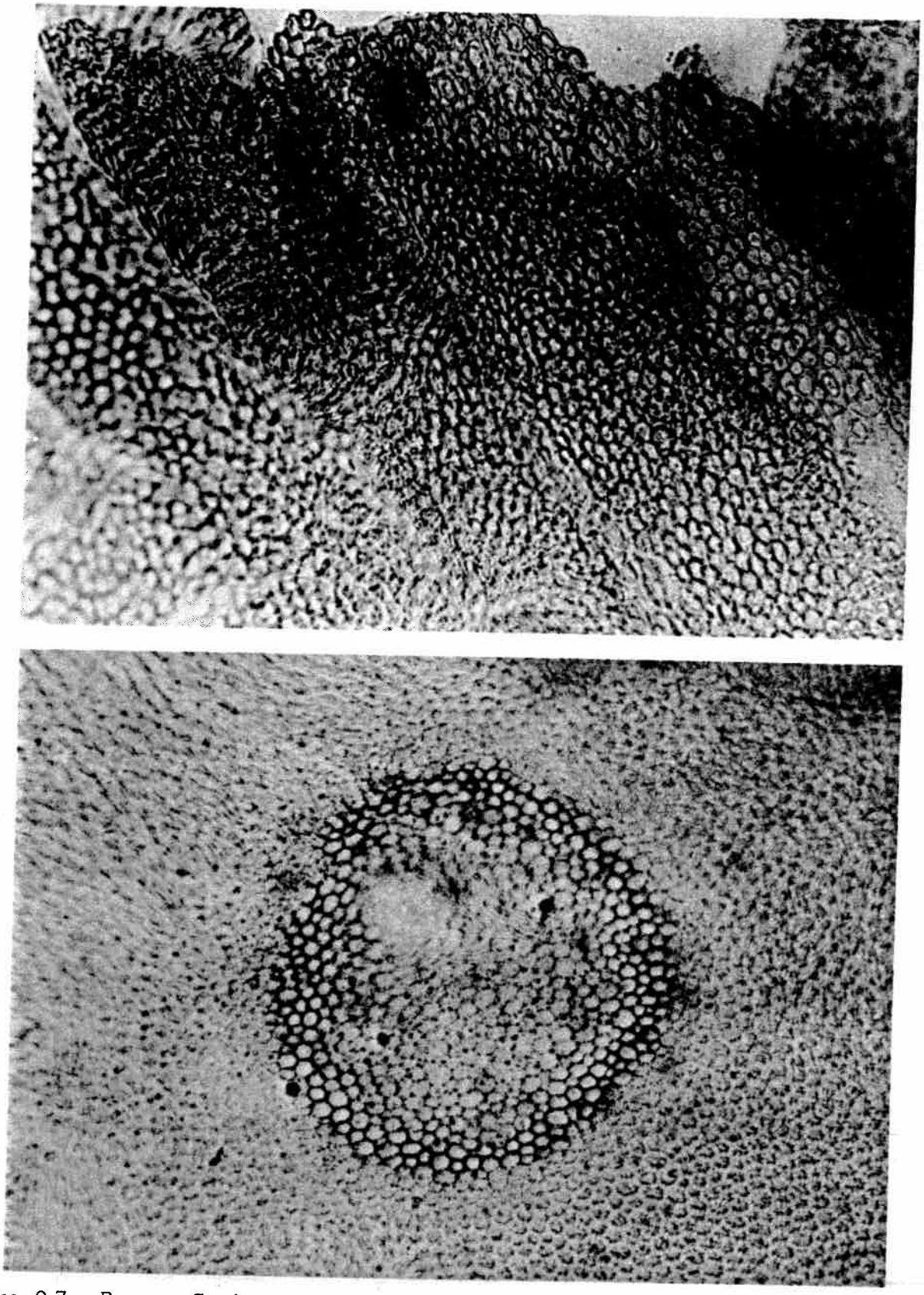


Figure 8.7 Bean. Seed coat of garbanzo bean (X246, taken at 10X) in Mounting Medium B. Note tightly packed, angular cells. Also see fig. 9.6.

Figure 8.8 Bean. Seed coat of pea (X276, taken at 10X) in Mounting Medium B. Also see fig. 9.8.

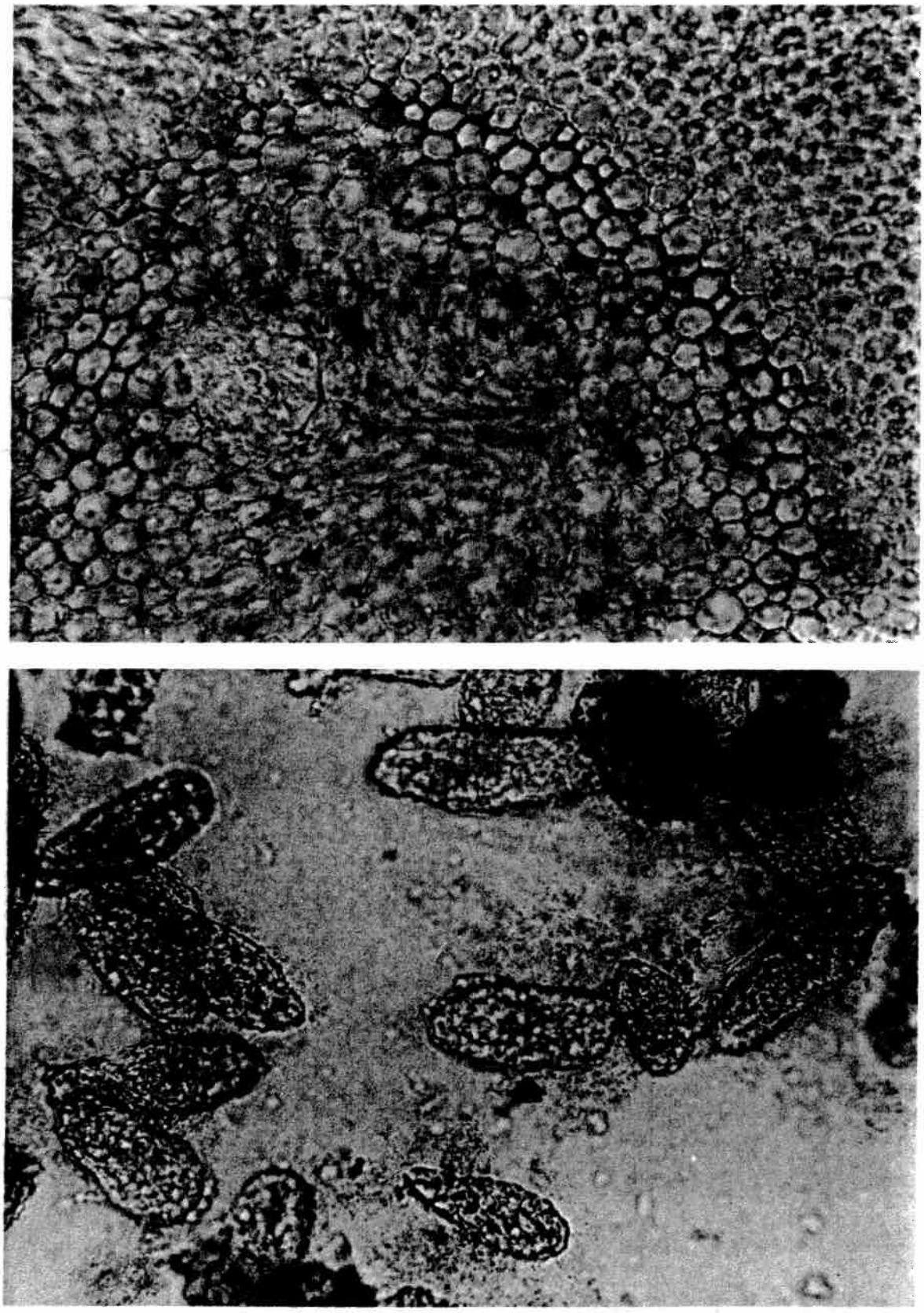


Figure 8.9 Bean. Seed coat of pea (X610, taken at 25X) in Mounting Medium B. Note tightly packed, angular cells. Also see fig. 9.8.

Figure 8.10 Bean. Pulp of garbanzo bean (X246, taken at 10X) in Mounting Medium A. Note elongate cells containing many starch grains (arrow). Also see fig. 9.5.

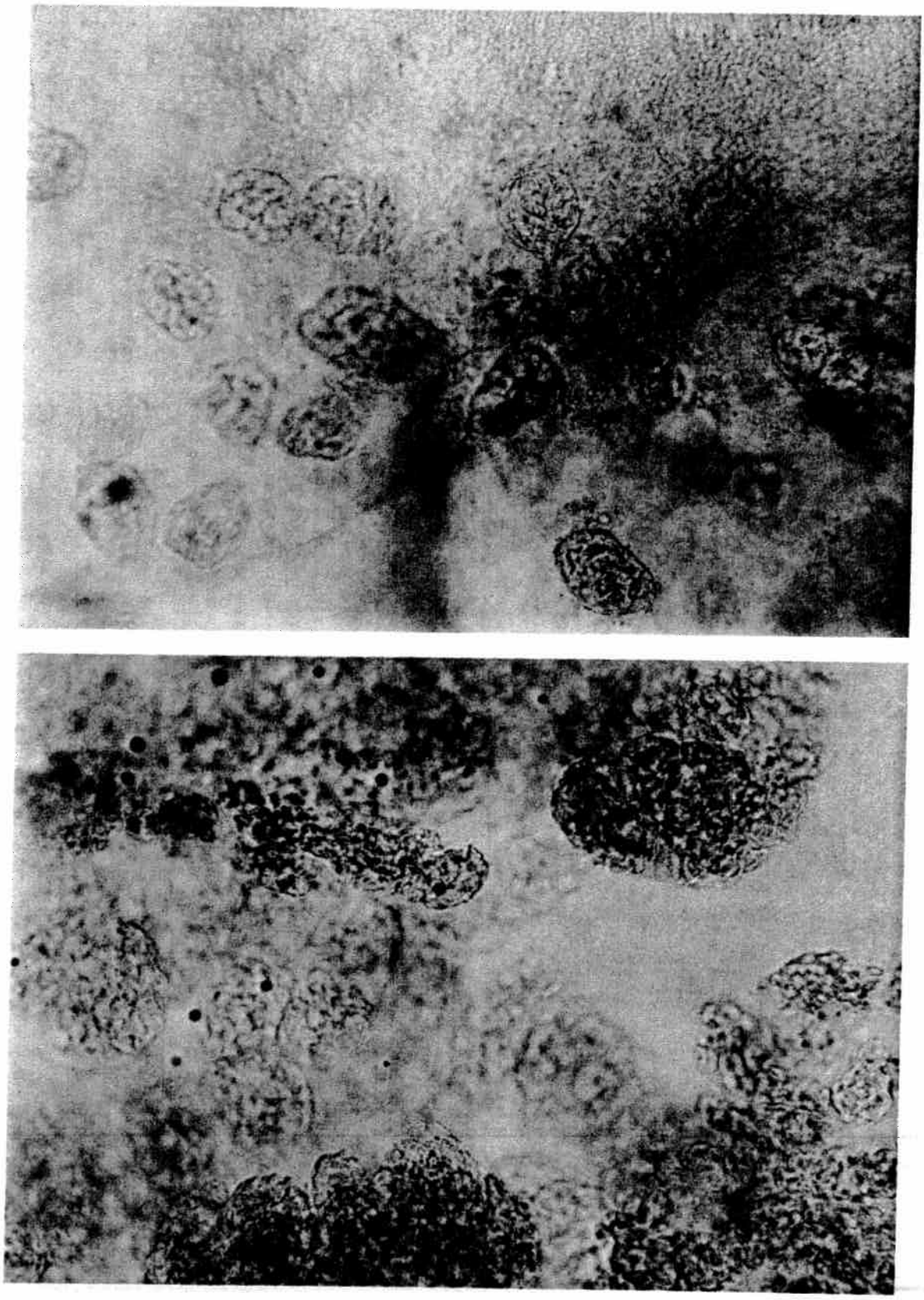


Figure 8.11 Bean. Pulp of lima bean (X226, taken at 10X) in Mounting Medium B. Note elongate cells containing many starch grains.

Figure 8.12 Bean. Pulp of pea (X244, taken at 10X) in Mounting Medium B. Note elongate cells containing many starch grains.

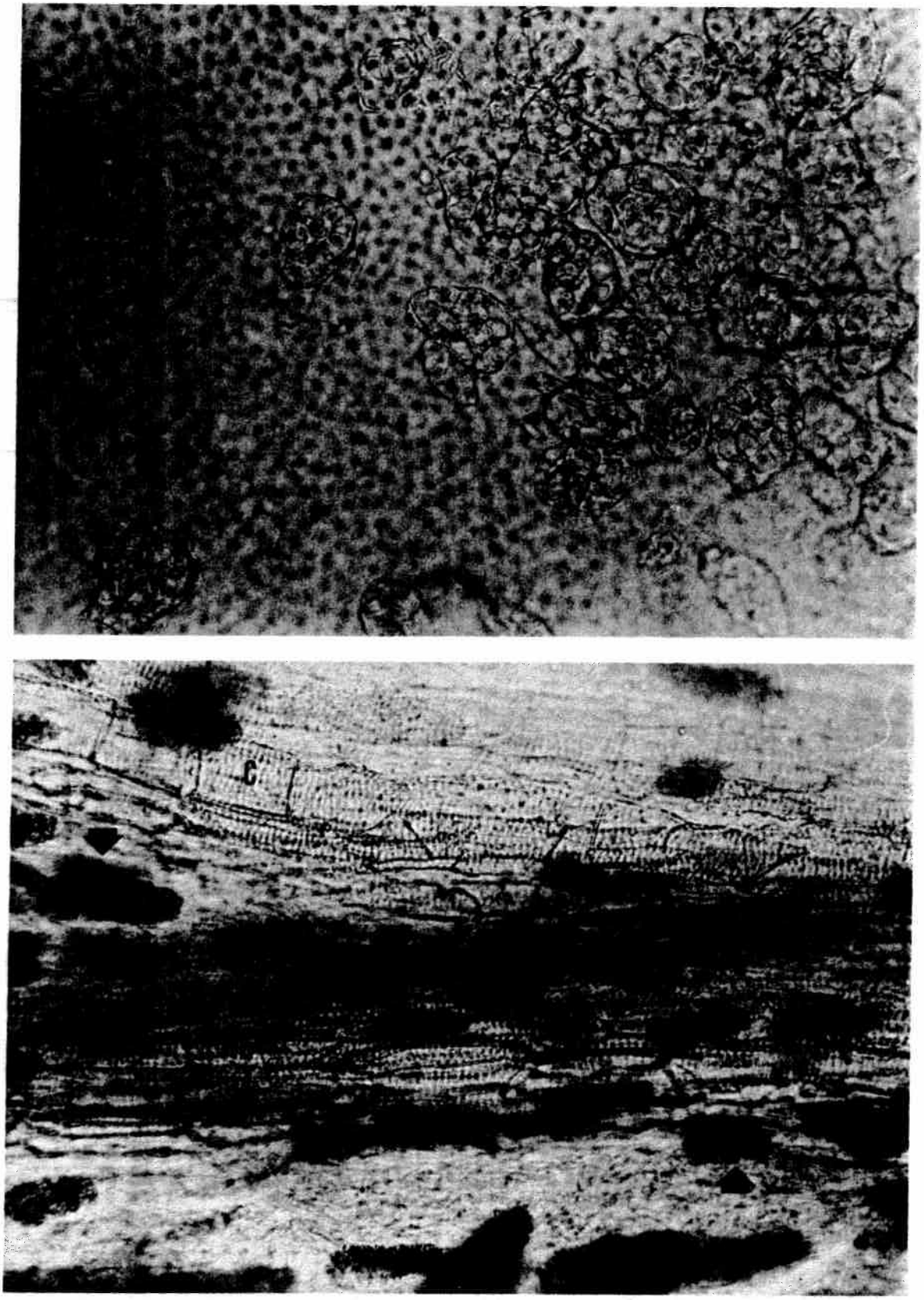


Figure 8.13 Bean. Pulp of pinto bean (X224, taken at 10X) in Mounting Medium A. Note rounded cells containing many starch grains, and seed coat in background. Also see fig. 9.7.

Figure 8.14 Beet pulp (X246, taken at 10X) in Mounting Medium B. Note the elongate druse crystals (arrow) and short, broad conducting cells. Also see fig. 9.9.

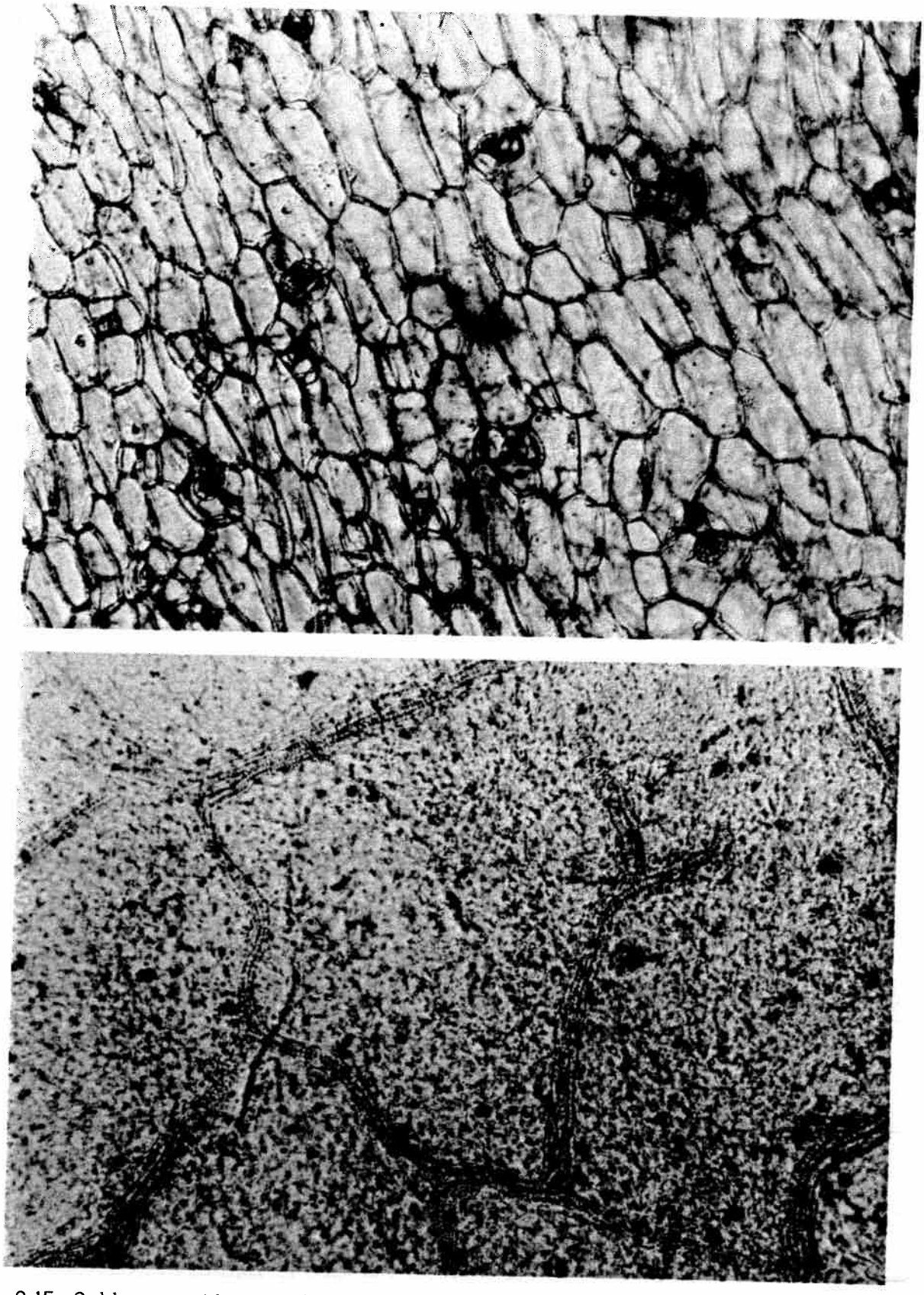


Figure 8.15 Cabbage epidermis (X224, taken at 10X) in Mounting Medium B. Note elongate, smooth-walled epidermal cells. Compare to lettuce (Figure 8.39). Also see fig. 9.10.

Figure 8.16 Cabbage (interior of leaf) (X224, taken at 10X) in Mounting Medium A. Note the pattern formed by the conducting tissues. Also see fig. 9.10.

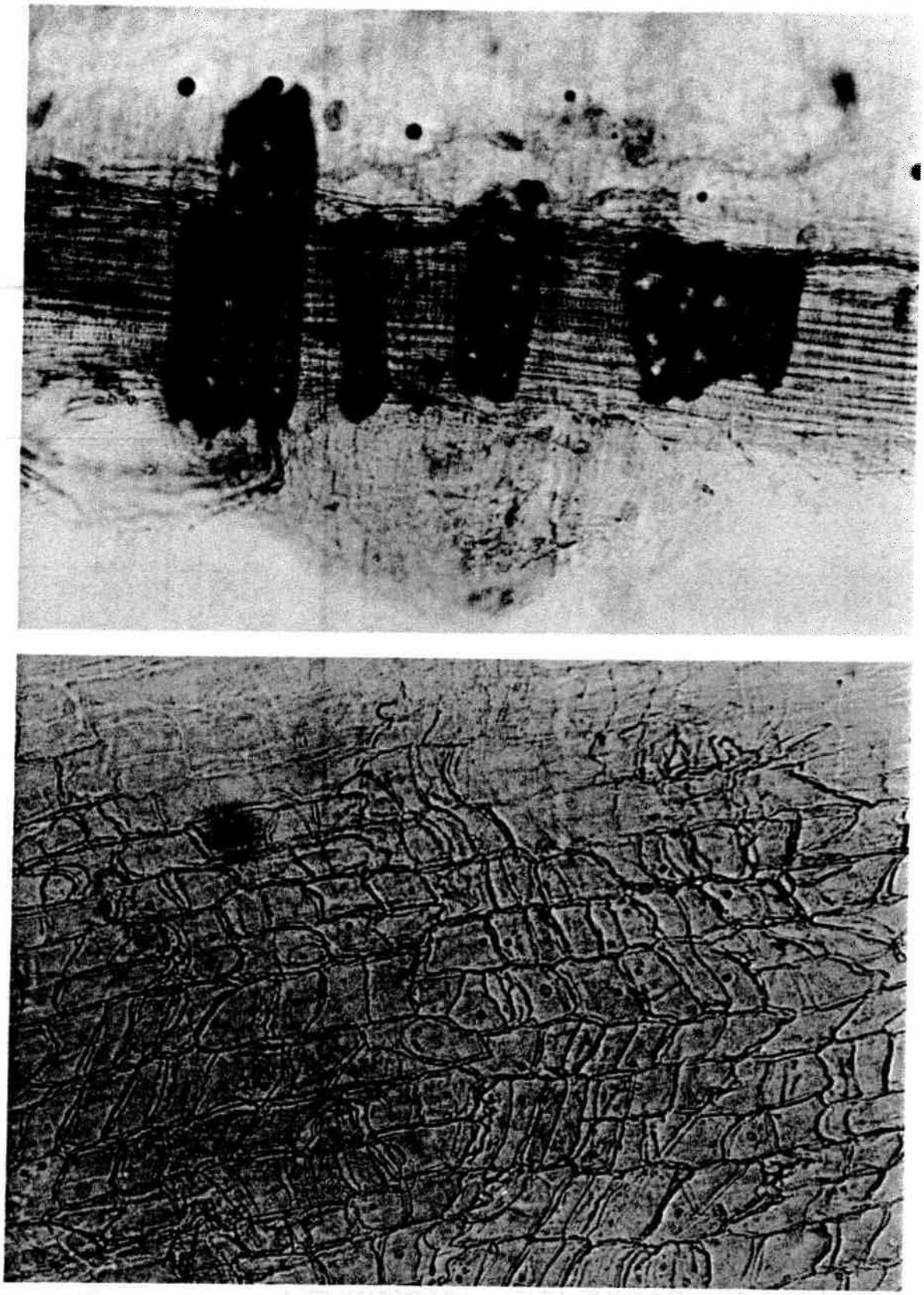


Figure 8.17 Caraway (X276, taken at 10X) in Mounting Medium B. Note very large, thick-walled cells (arrow) oriented at right angles to conducting tissues. Also see fig. 9.11.

Figure 8.18 Carrot pulp (X258, taken at 10X) in Mounting Medium B. Cells compact, brick-like. Also see fig. 9.12.

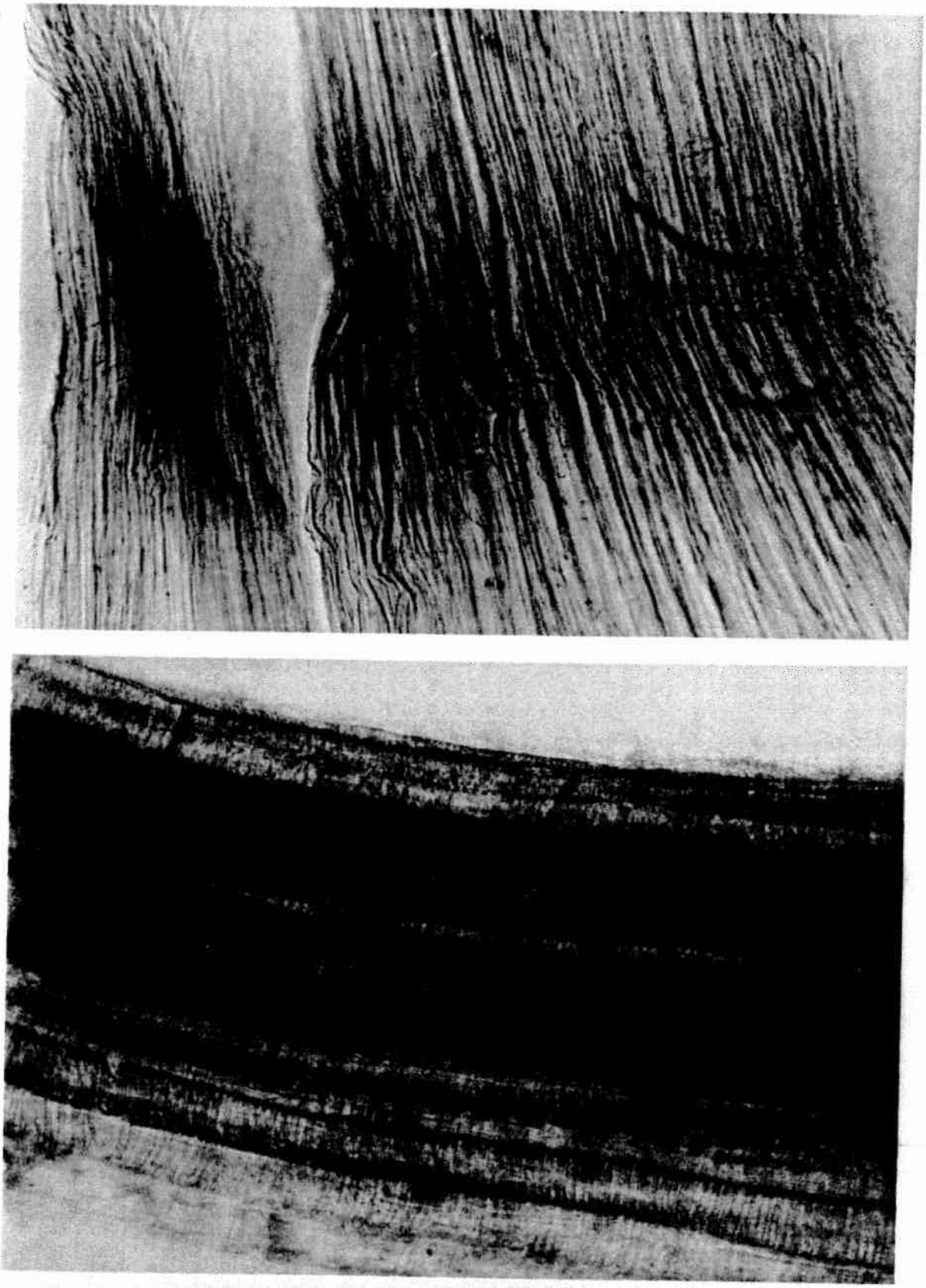
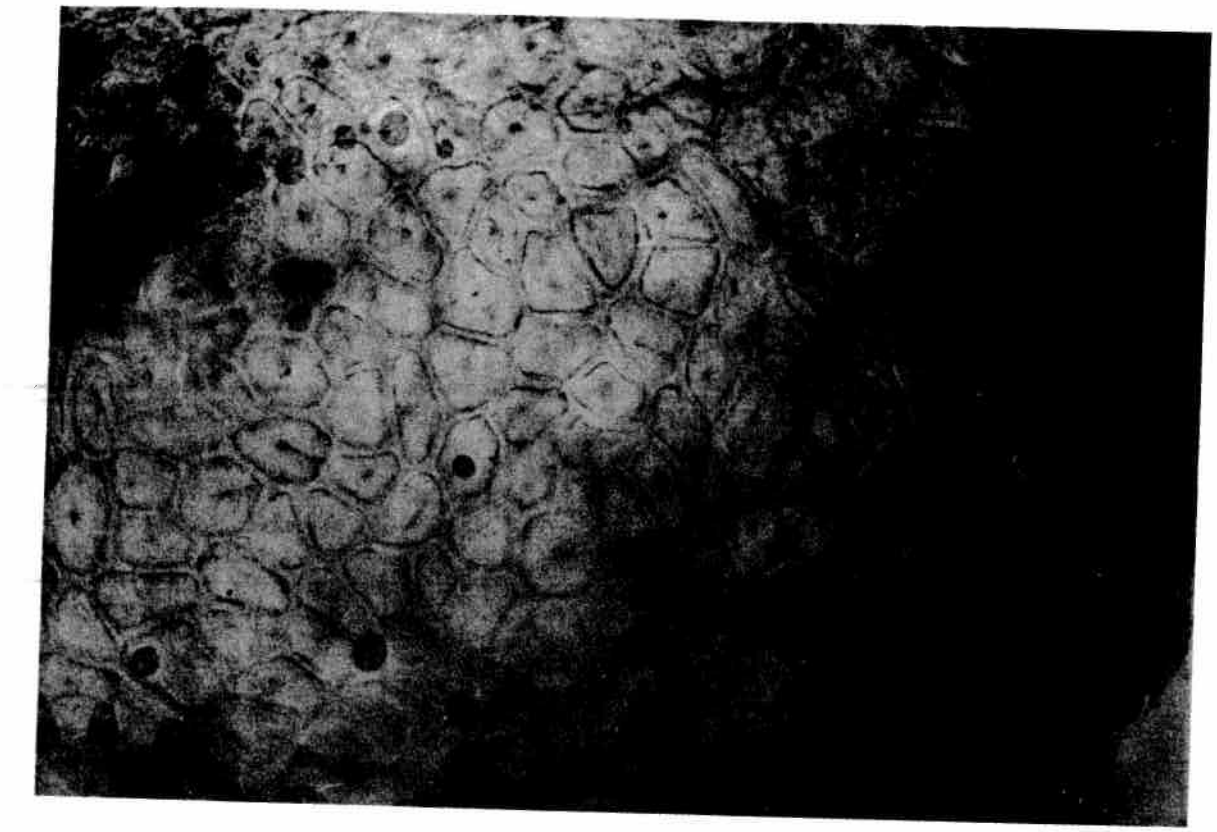


Figure 8.19 Celery skin (X224, taken at 10X) in Mounting Medium B. Cells thin-walled, very elongate. Also see fig. 9.13.

Figure 8.20 Celery pulp (X246, taken at 10X) in Mounting Medium A. Note the very prominent conducting cells (arrow) -- the celery "string." Also see fig. 9.13.



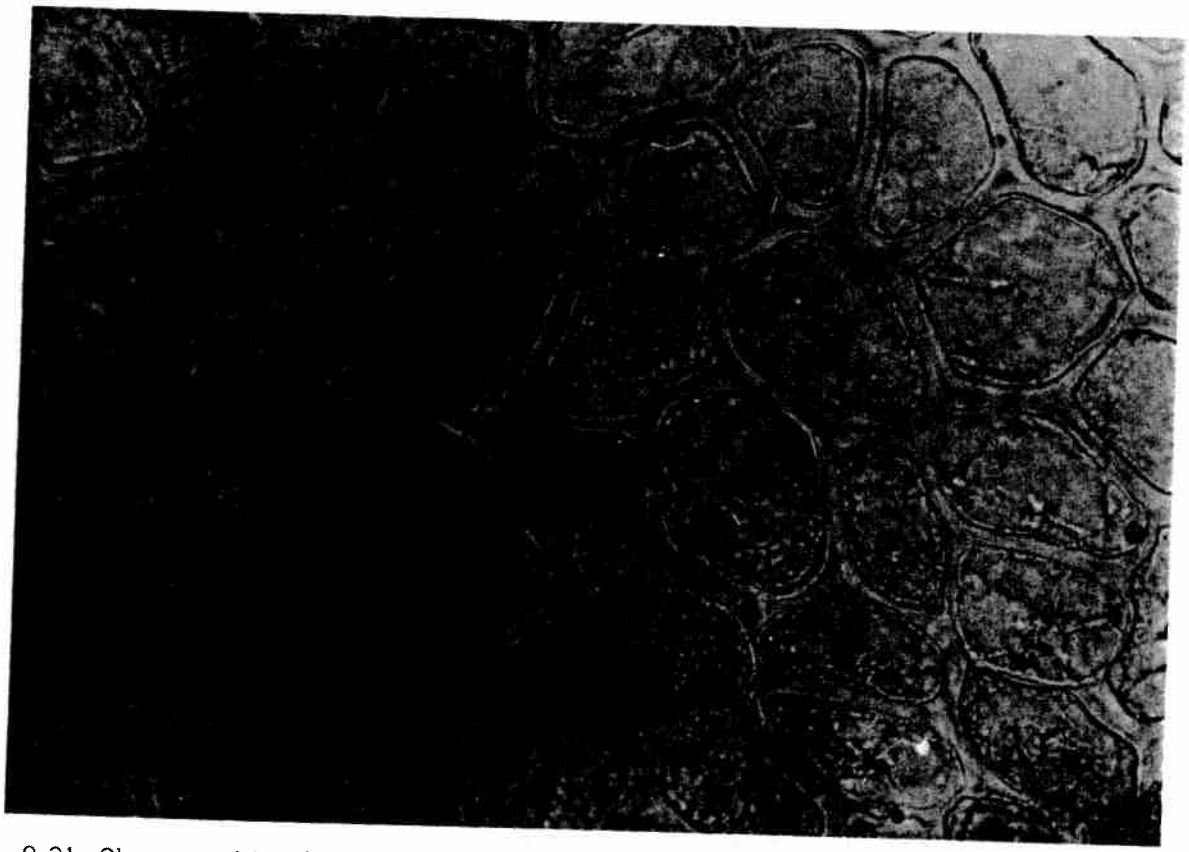
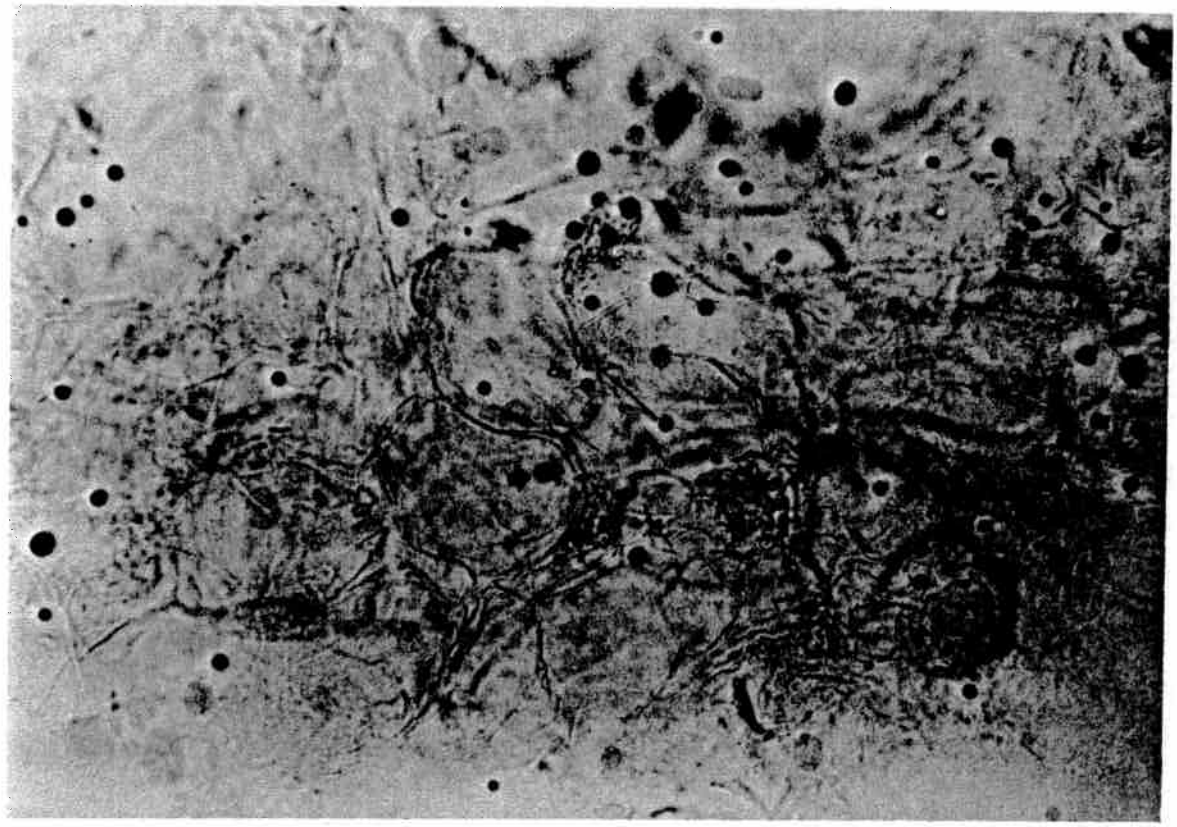


Figure 8.21 Cherry skin (X240, taken at 10X) in Mounting Medium B. Cells moderately thick-walled, blocky, cell walls clearly defined. Compare to apple, plum or prune, and tomato. Also see fig. 9.14.

Figure 8.22 Cherry skin (X600, taken at 25X) in Mounting Medium B. Cells moderately thick-walled, blocky, cell walls clearly defined. Also see fig. 9.14.



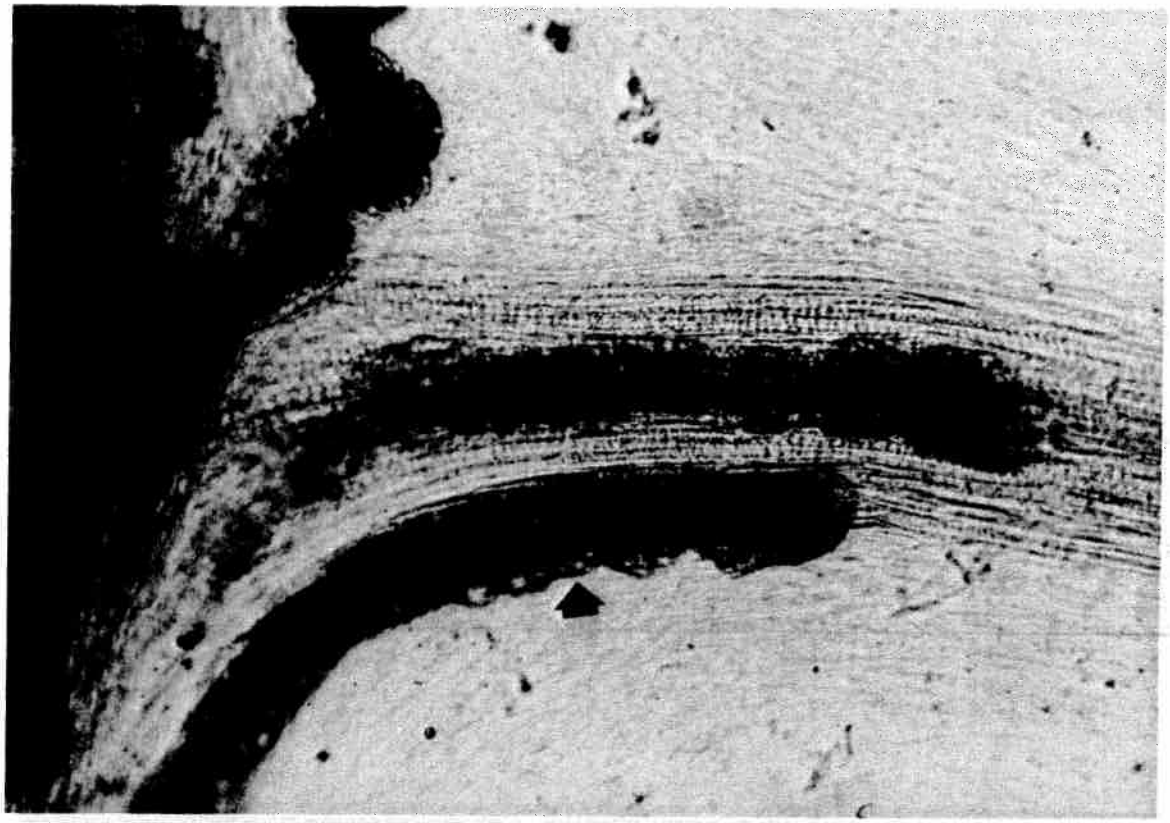
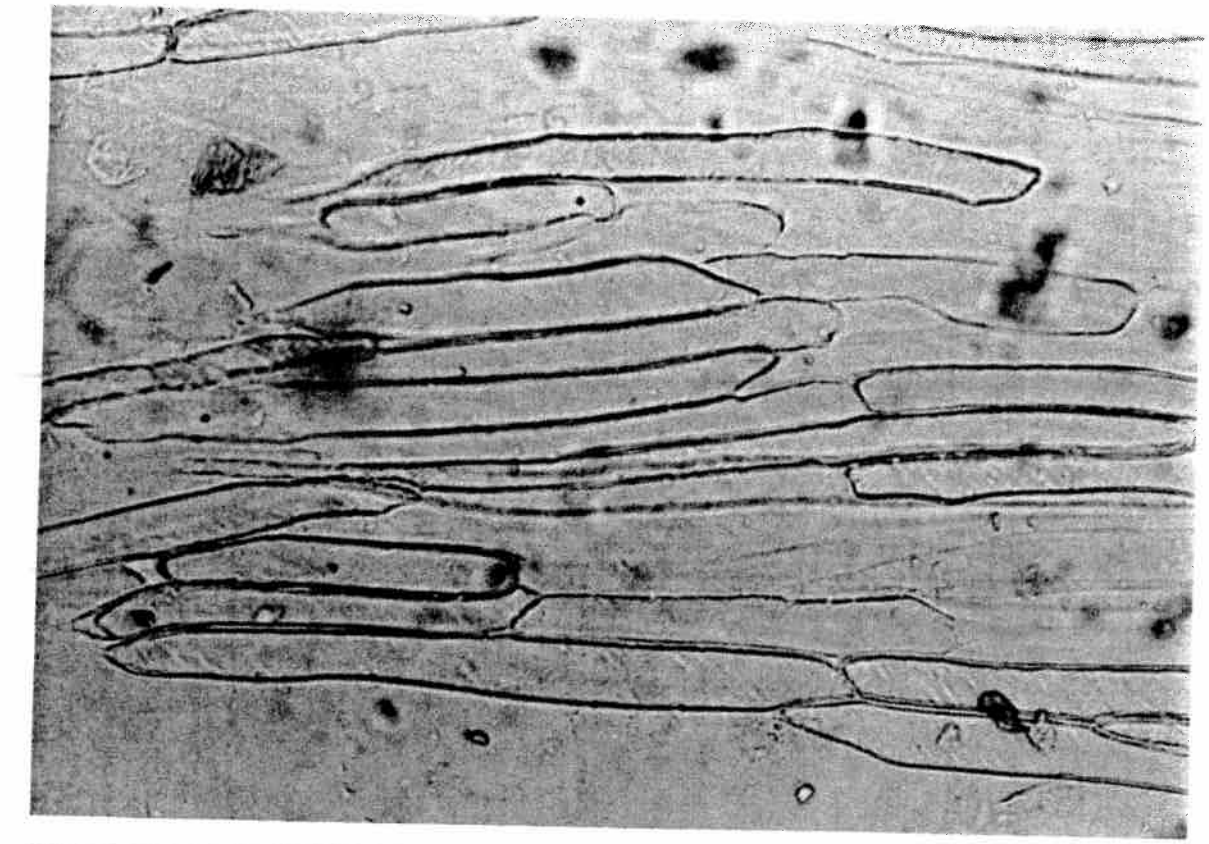


Figure 8.23 Cherry pulp (X224, taken at 10X) in Mounting Medium B. Cells large, thin-walled, with scattered dark droplets of pigment (arrow). Compare to apple, fig, grape or raisin, okra, pear, plum or prune, radish, strawberry, tomato, turnip, and water chestnut. Also see fig. 9.14.

Figure 8.24 Citrus (lemon) pulp (X224, taken at 10X) in Mounting Medium A. Note strings of druse crystals (arrow) adhering to conducting tissues. See also figs. 9.15, 9.17.



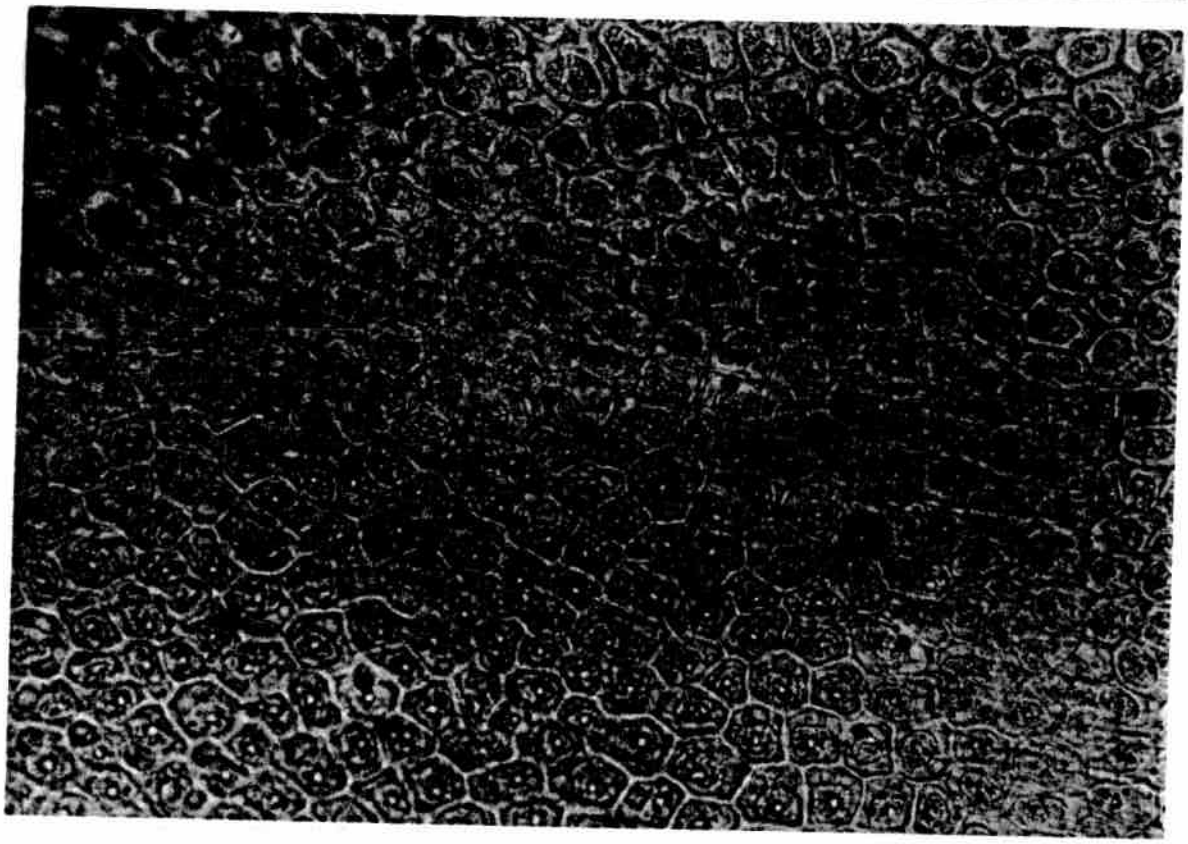


Figure 8.25 Citrus (lemon) section membrane (X224, taken at 10X) in Mounting Medium A. Cells very elongate, tightly packed. See also figs. 9.15, 9.17.

Figure 8.26 Corn seed coat (inside) (X224, taken at 10X) in Mounting Medium A. Cells small, compact, angular. Also see fig. 9.19.

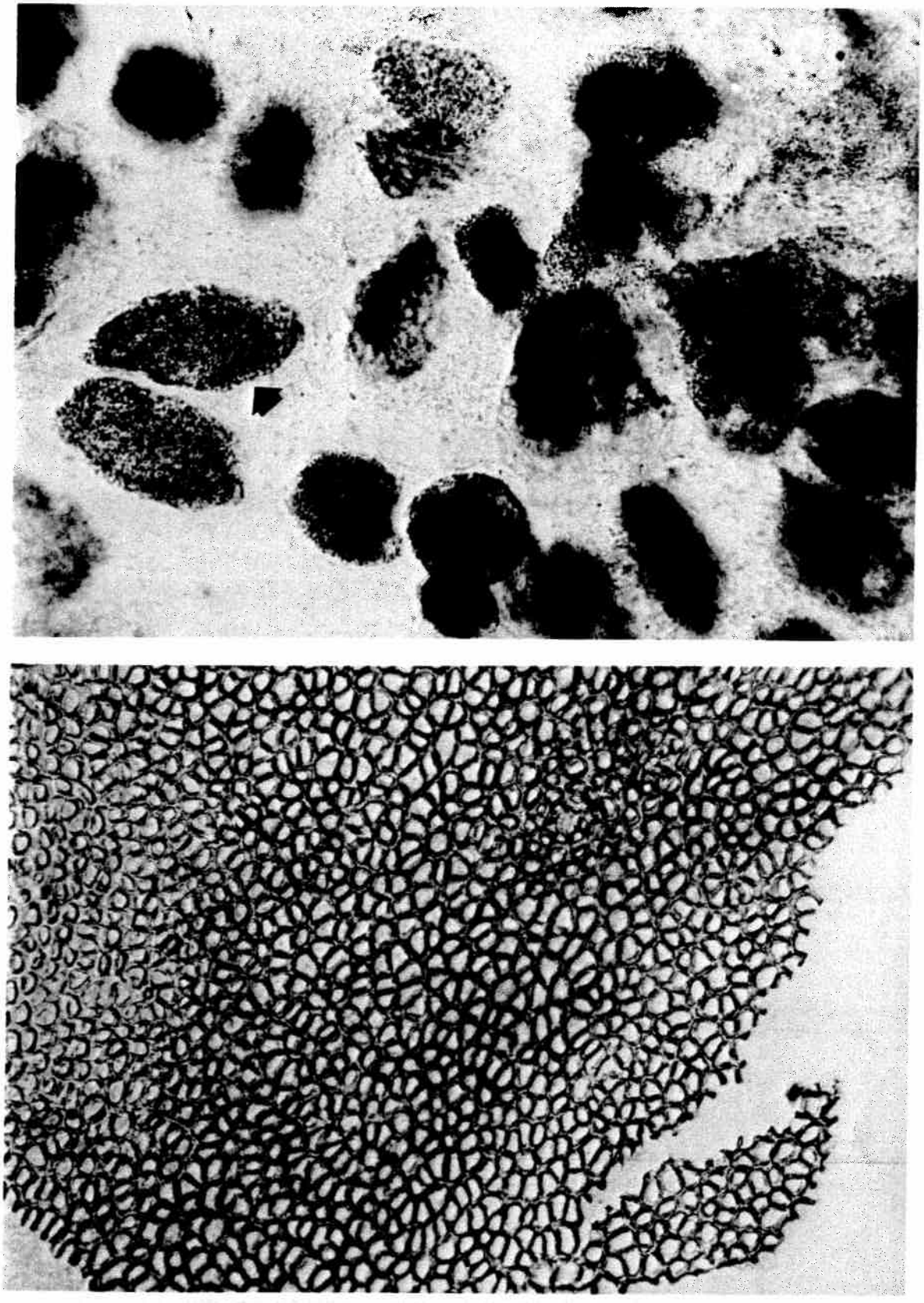


Figure 8.27 Corn pulp (X234, taken at 10X) in Mounting Medium A. Cells (arrow) elongate, packed with starch grains.

Figure 8.28 Cucumber epidermis (X224, taken at 10X) in Mounting Medium B. Cells small, rounded, thick-walled. Compare to bean seed coats.

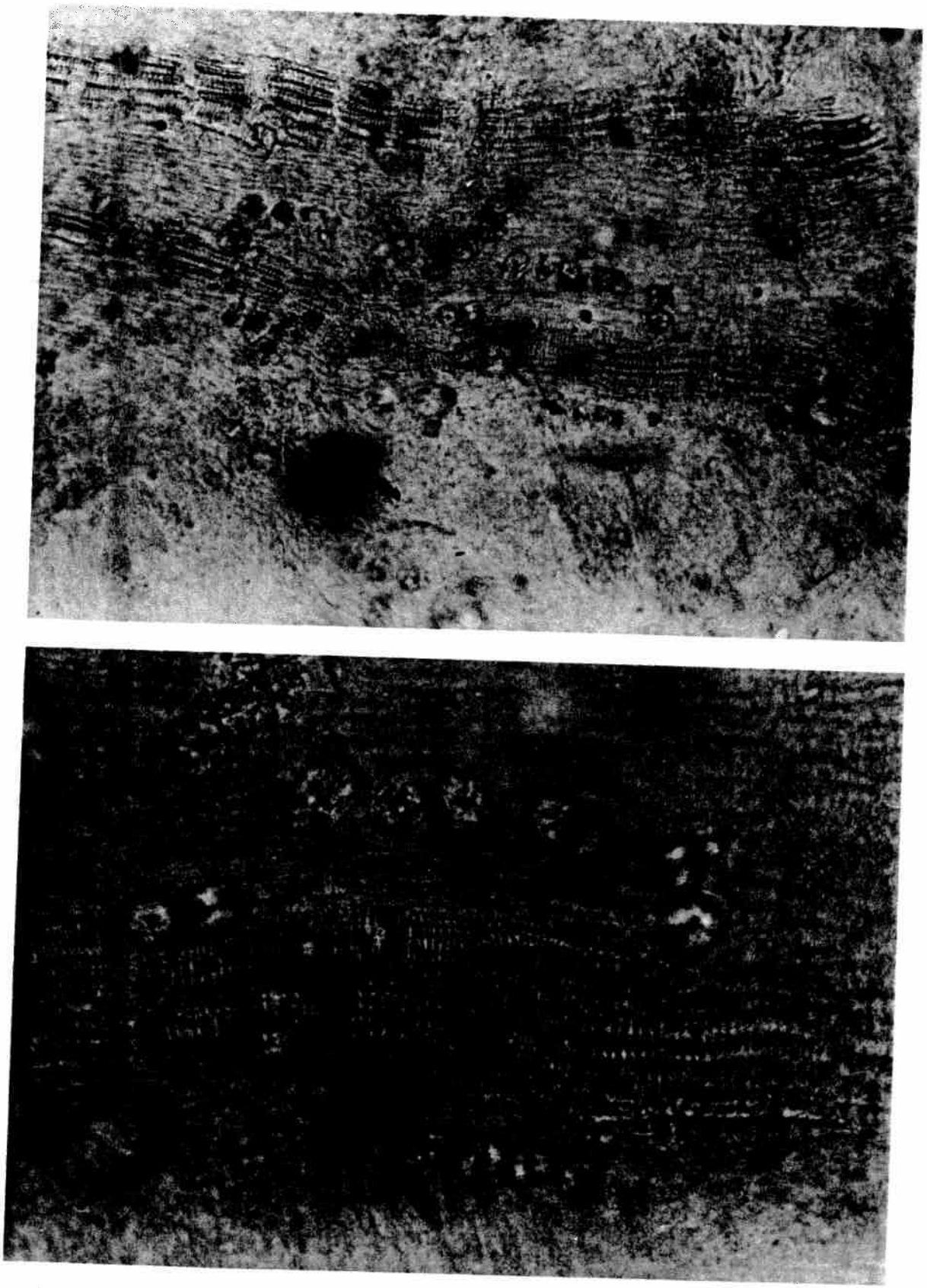


Figure 8.29 Fig pulp (X224, taken at 10X) in Mounting Medium B. Note rounded druses (arrow) along the prominent conducting cells (arrow). Compare to apricot (fig. 8.4). Also see fig. 9.22.

Figure 8.30 Fig pulp (X600, taken at 25X) in Mounting Medium B. Note rounded druses (arrow) along the prominent conducting cells (arrow). Compare to apricot (fig. 8.4). Also see fig. 9.22.

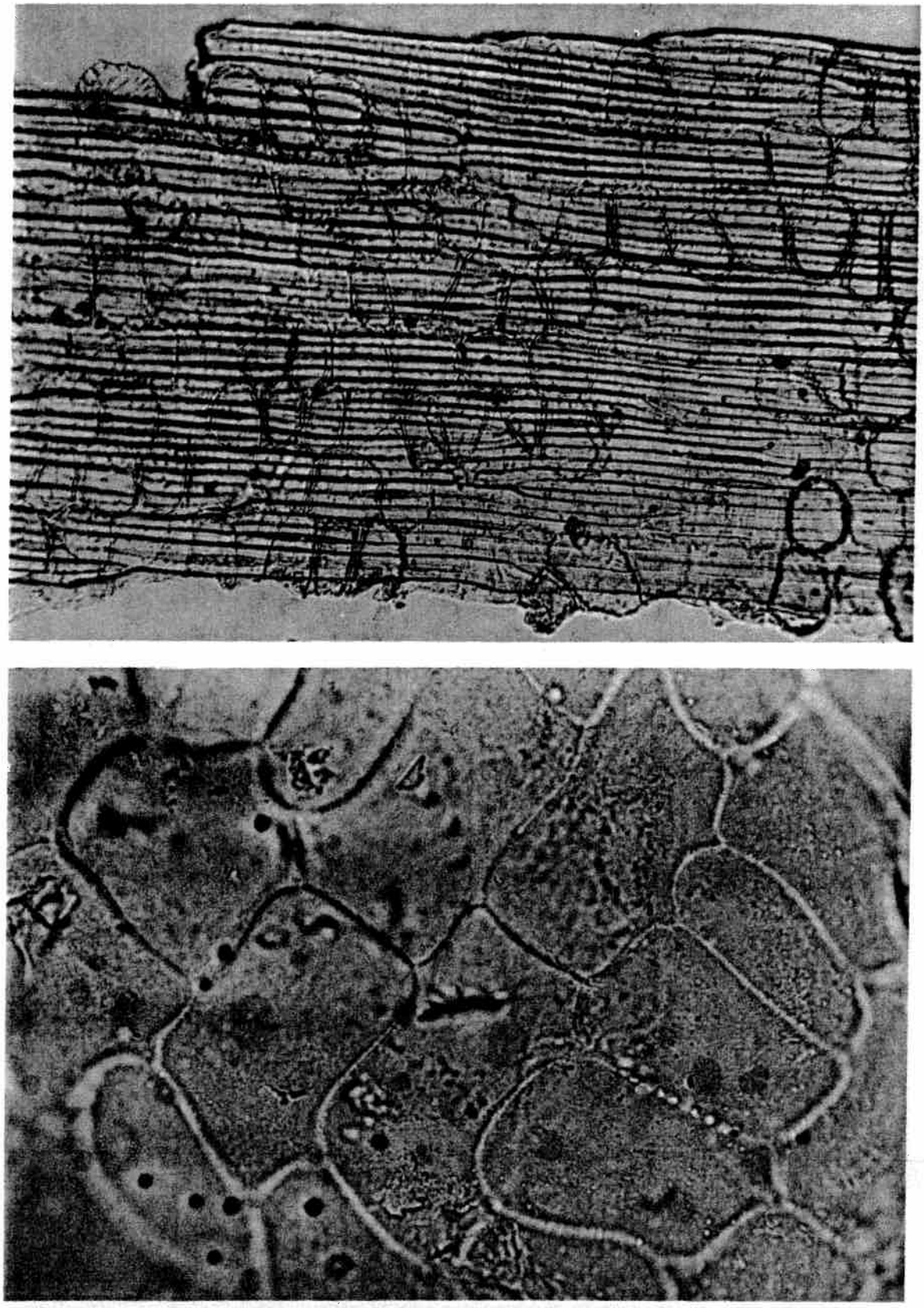
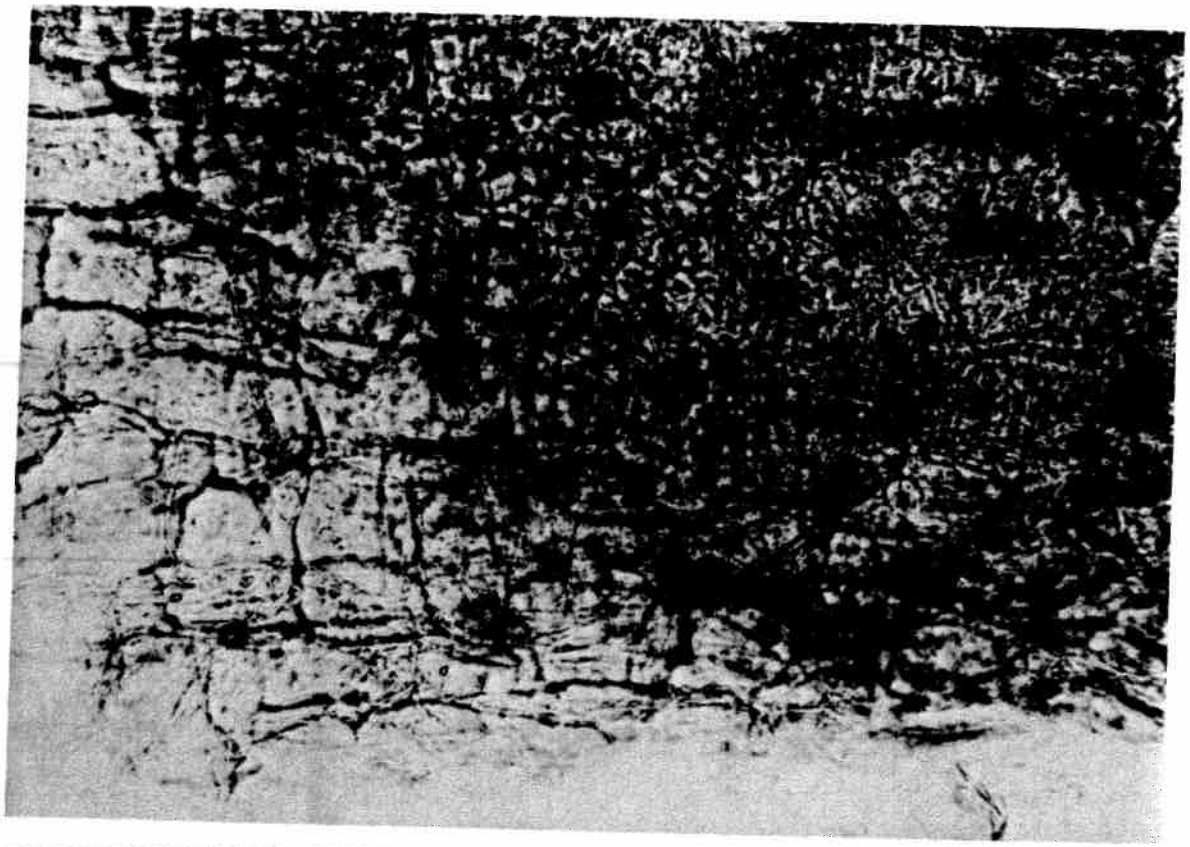


Figure 8.31 Garlic epidermis (X226, taken at 10X) in Mounting Medium A. Cells plate-like. Compare to onion (figs. 8.47, 8.48, 8.49). Also see fig. 9.24.

Figure 8.32 Garlic epidermis (X685, taken at 25X) in Mounting Medium A. Cells plate-like. Compare to onion (figs. 8.47, 8.48, 8.49) Also see fig. 9.24.



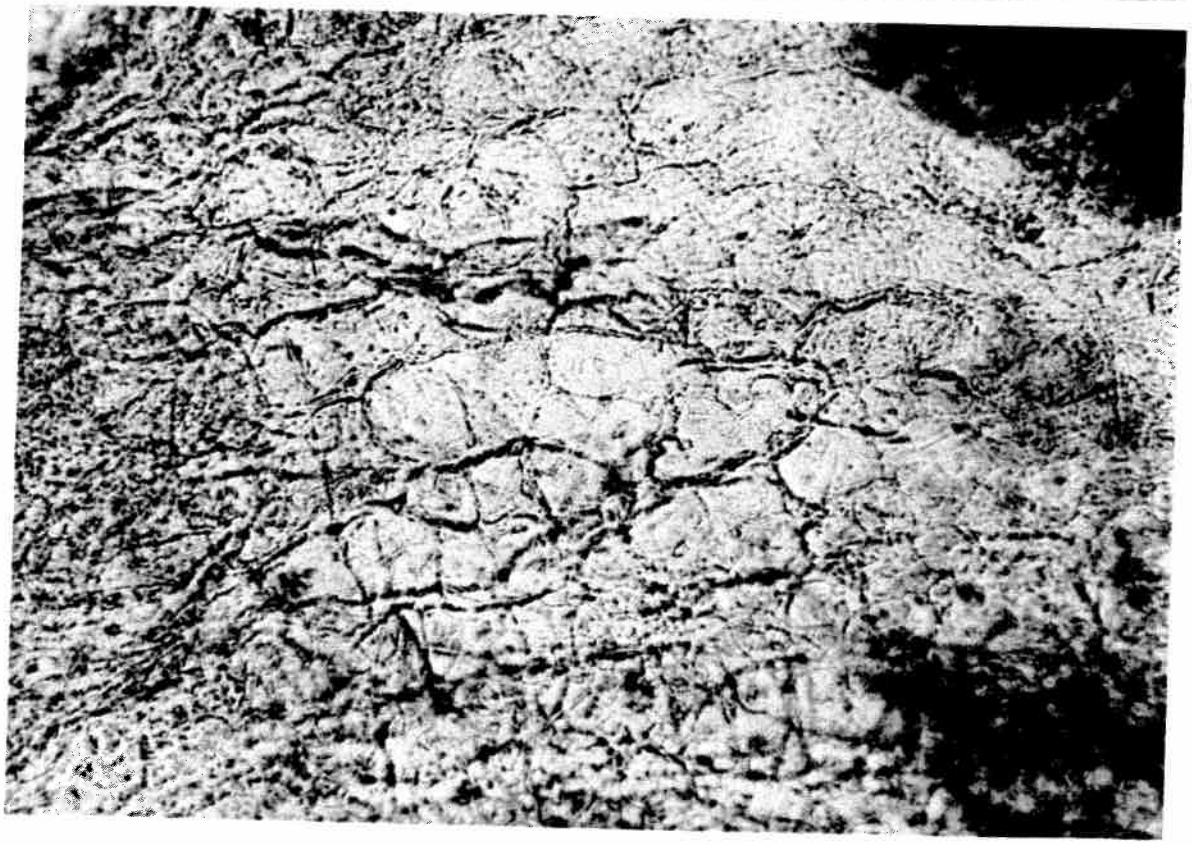
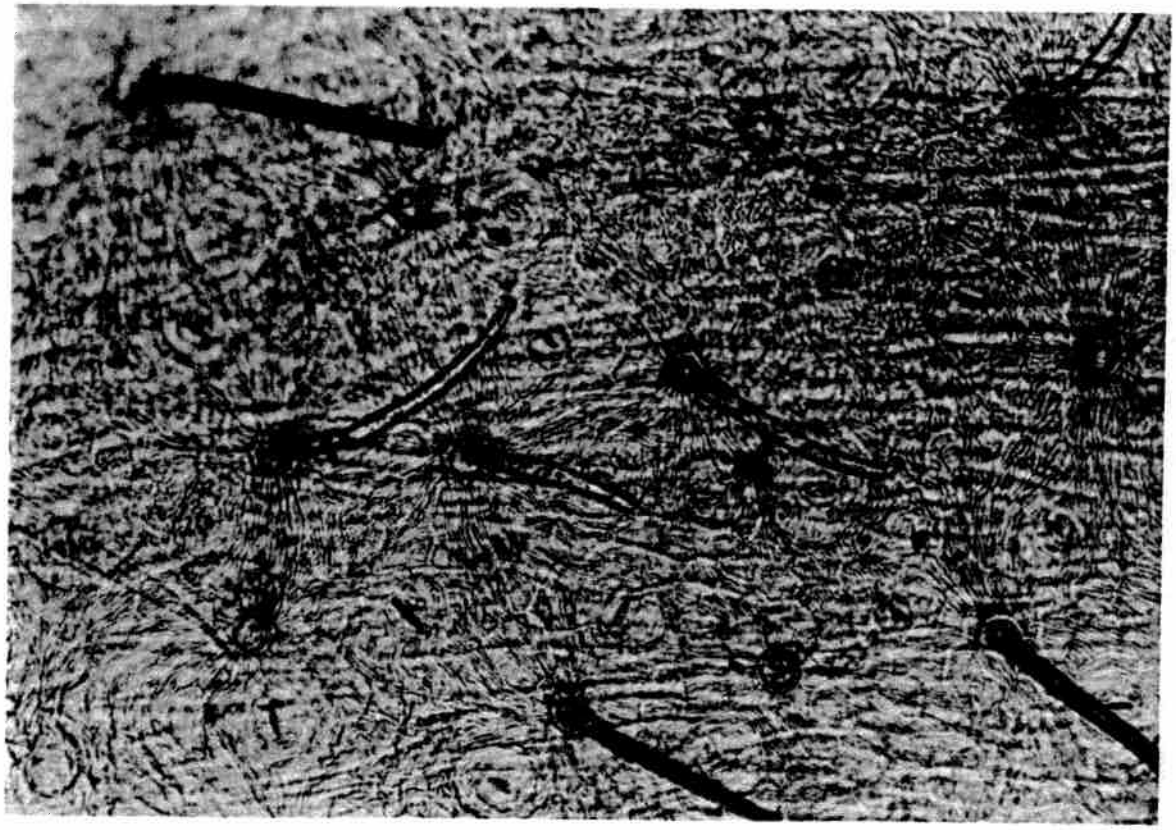


Figure 8.33 Grape or raisin epidermis (X224, taken at 10X) in Mounting Medium A. Cells small, blocky, 0.02--0.03 mm wide. Also see fig. 9.25.

Figure 8.34 Grape or raisin pulp (X224, taken at 10X) in Mounting Medium B. Cells small, somewhat regular with rounded corners, 0.03 mm wide by 0.06 to 0.1 mm long. Compare to apple pulp or applesauce, cherry, fig, okra, pear, plum or prune, radish, strawberry, tomato, turnip, and water chestnut. Also see fig. 9.26.



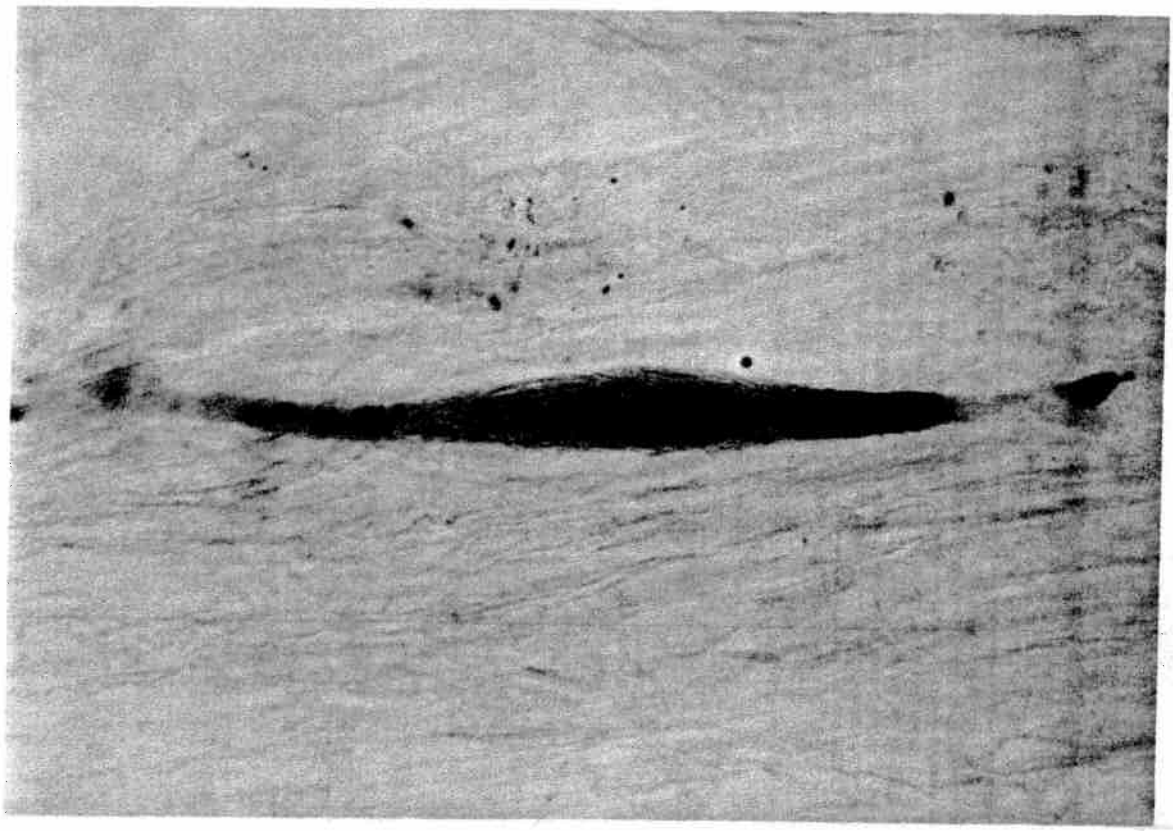
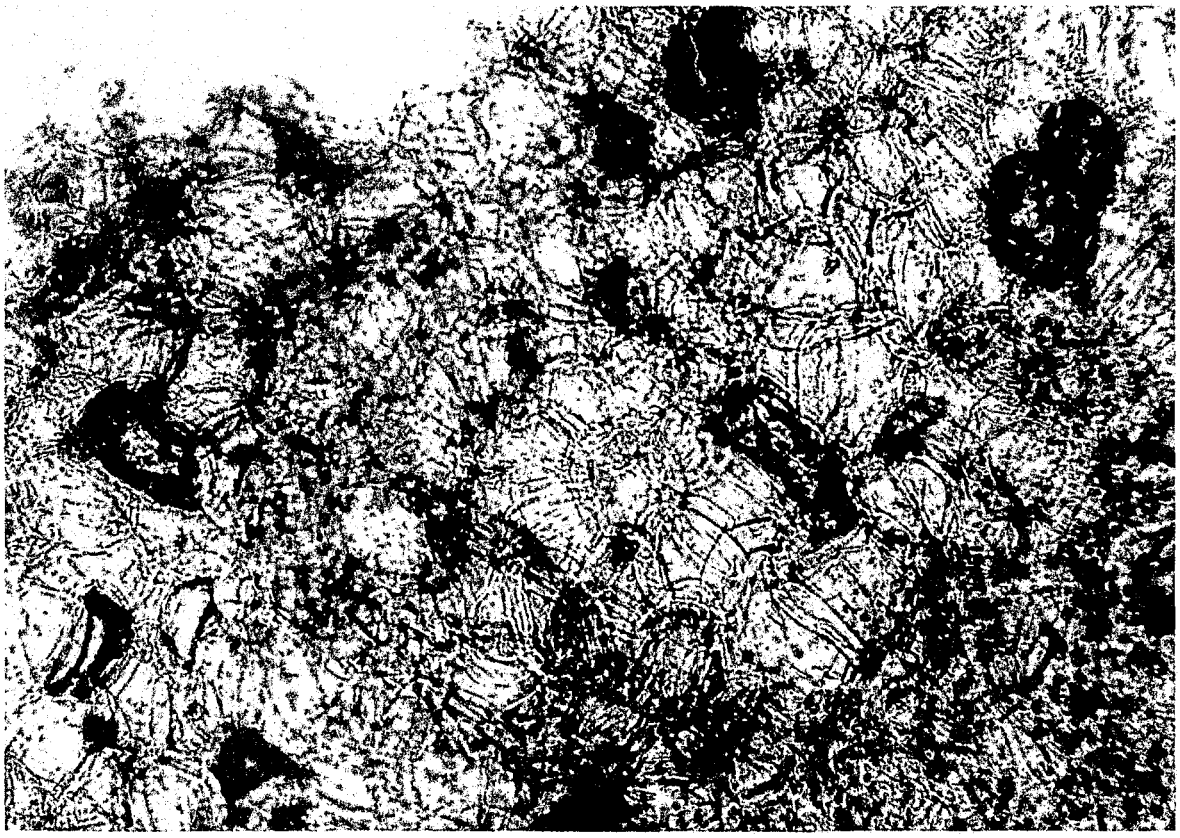


Figure 8.35 Green bean pod.(X226, taken at 10X) in Mounting Medium A. Note widely spaced, unbranched hairs (arrow). Hairs have basal diameter up to 0.03 mm, and hair length to 0.4 mm. Compare to apricot, okra, peach, and oregano. For seed characteristics, see "Beans." Also see fig. 9.27.

Figure 8.36 Kiwi fruit seed (X234, taken at 10X) in Mounting Medium B. Elongate, spindle shaped seed covered with raphides. Cells 0.4 mm wide, and greater than 8 mm long.



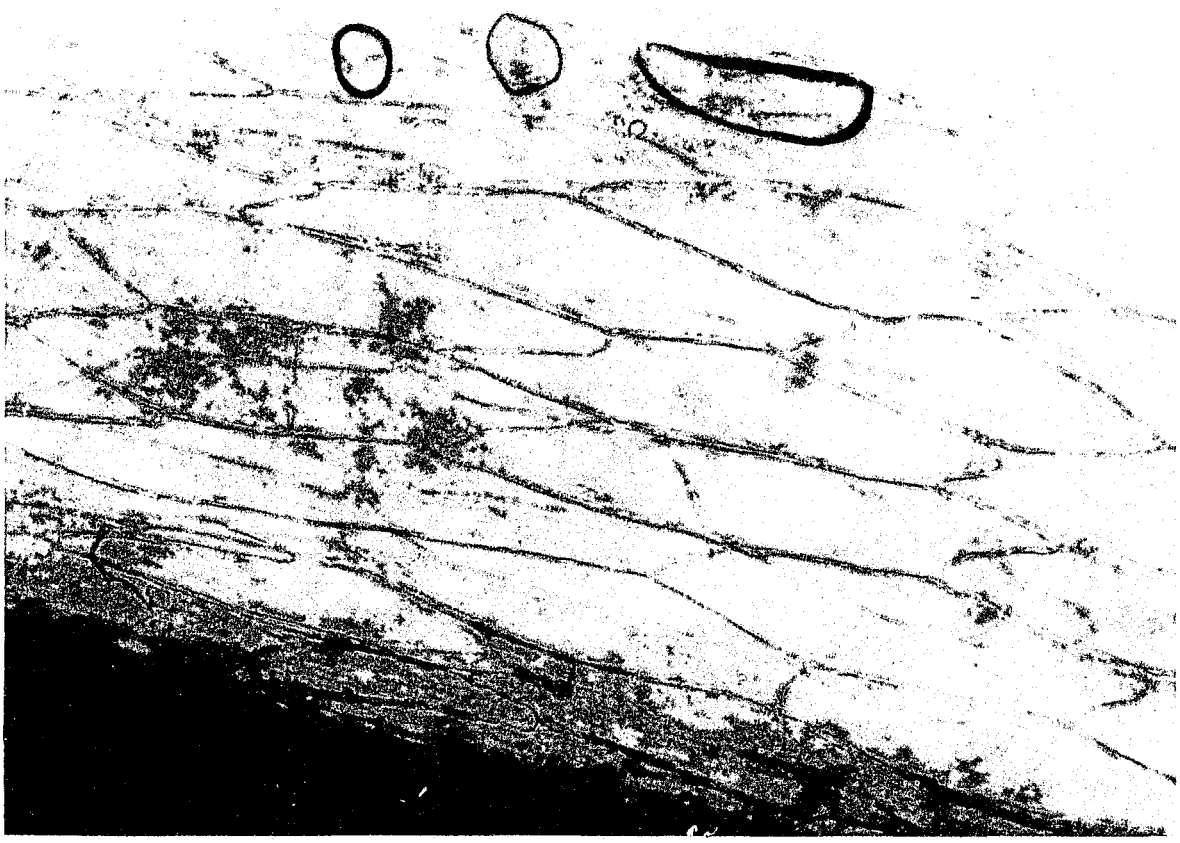
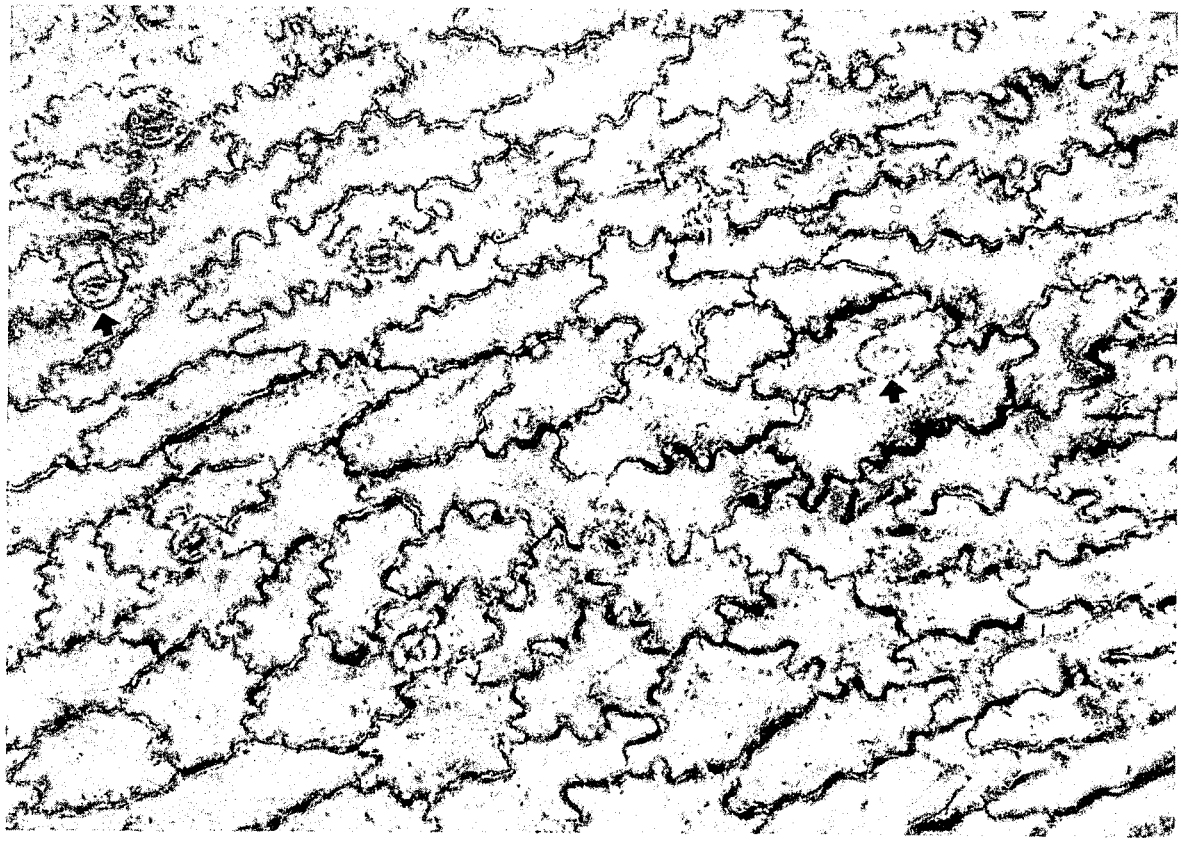


Figure 8.37 Kiwi fruit pulp (X224, taken at 10X) in Mounting Medium A. Pulp cells have a crocheted appearance. Cells range from 0.06 X0.06 mm to 0.09 X0.15 mm. Also see fig. 9.28.

Figure 8.38 Lettuce (X224, taken at 10X) in Mounting Medium B. Elongate cells with pointed ends, 0.04 to 0.05 mm wide and 0.12 to 0.20 in length. Compare to cabbage (fig. 8.15).



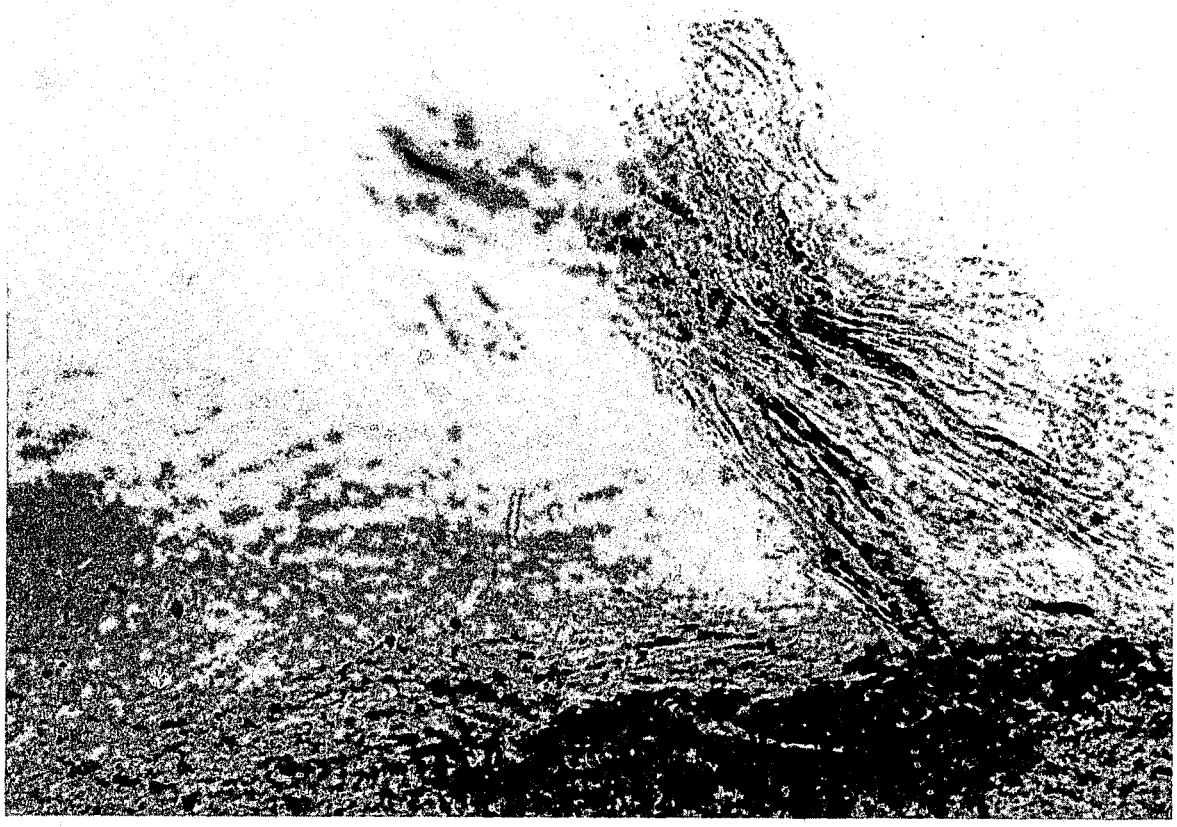


Figure 8.39 Lettuce (X224, taken at 10X) in Mounting Medium B. Lower epidermis cells. Note wavy edges on cells and numerous stomates (arrow). Also see fig. 9.29.

Figure 8.40 Mushroom (X224, taken at 10X) in Mounting Medium B. Fibrous appearing structure without internal cell walls. Very difficult material to distinguish. Also see fig. 9.30.

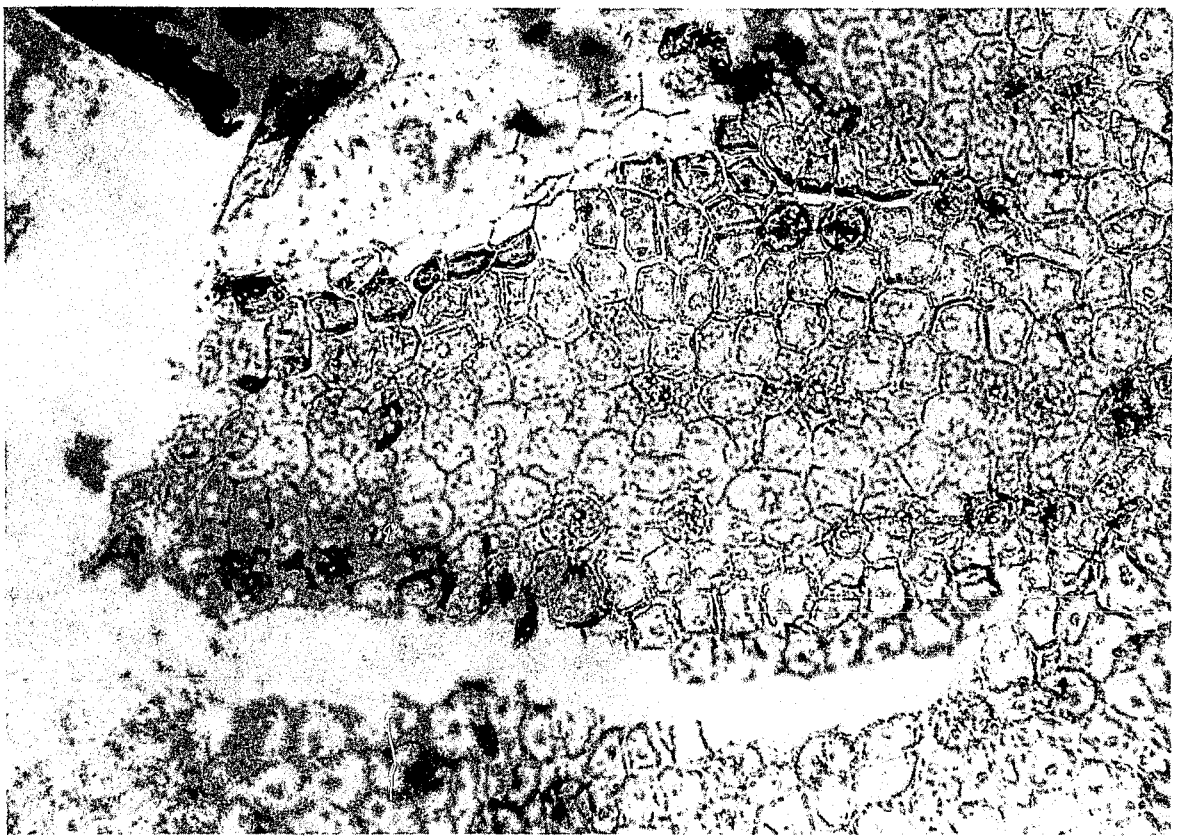
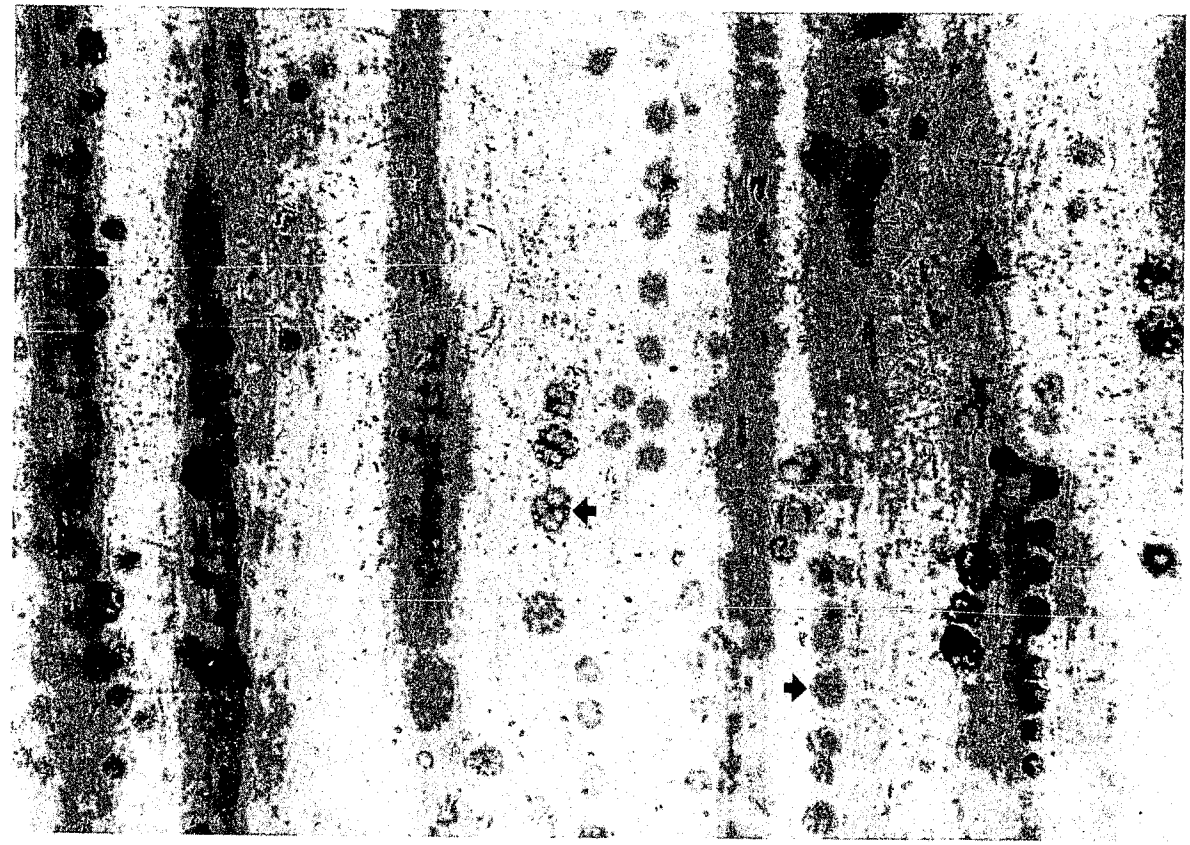


Figure 8.41 Okra epidermis (X224, taken at 10X) in Mounting Medium A. Epidermal cells multi-faceted, 0.03 to 0.04 mm in diameter.

Figure 8.42 Okra hairs (X226, taken at 10X) in Mounting Medium A. Hairs large, 0.07 wide and 0.7 mm in length. Compare to apricot skin, green bean pod, oregano, peach, rosemary, strawberry, and sweet basil.



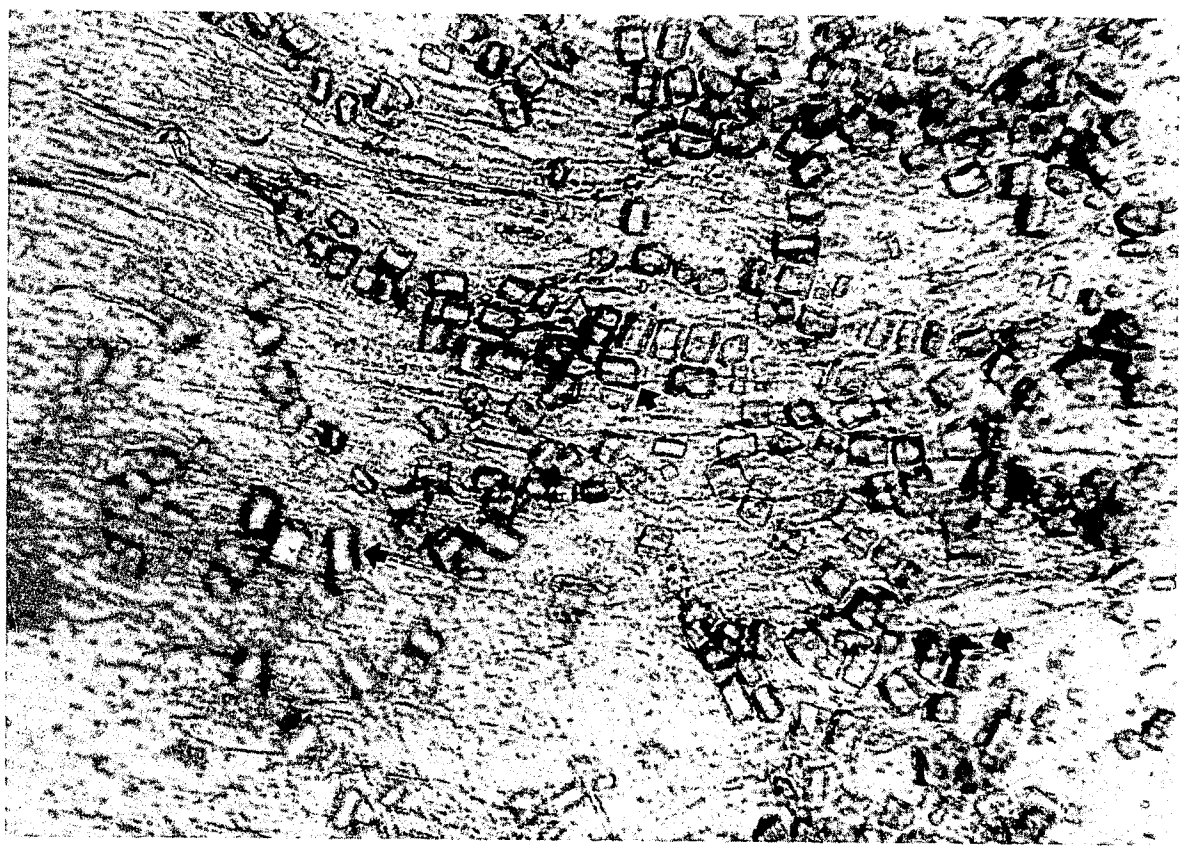
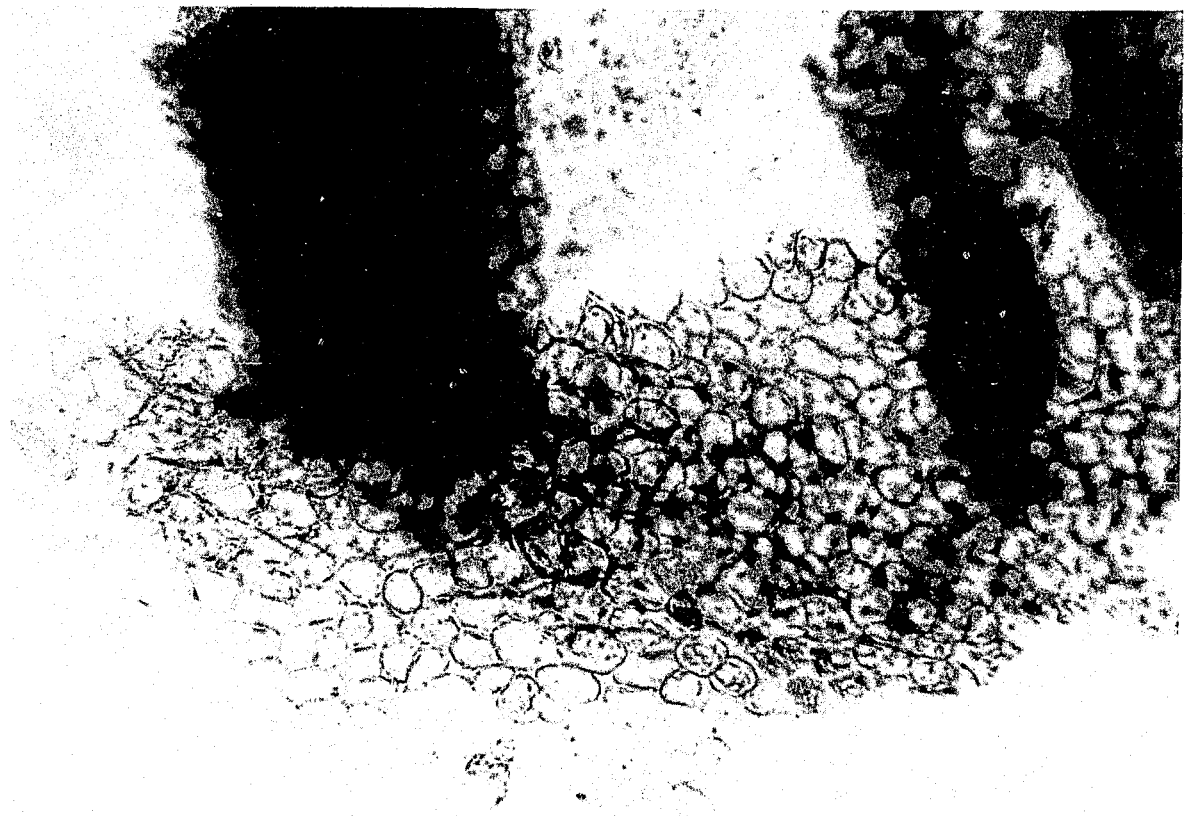


Figure 8.43 Okra pulp (X224, taken at 10X) in Mounting Medium A. Note rounded druses (arrow) in conducting tissue. Compare to apple pulp or applesauce, cherry, fig, grape or raisin, pear, plum or prune, radish, strawberry, tomato, turnip, and water chestnut. Also see fig. 9.31.

Figure 8.44 Okra pulp (X224, taken at 10X) in Mounting Medium A. Note prismatic crystals (arrow).



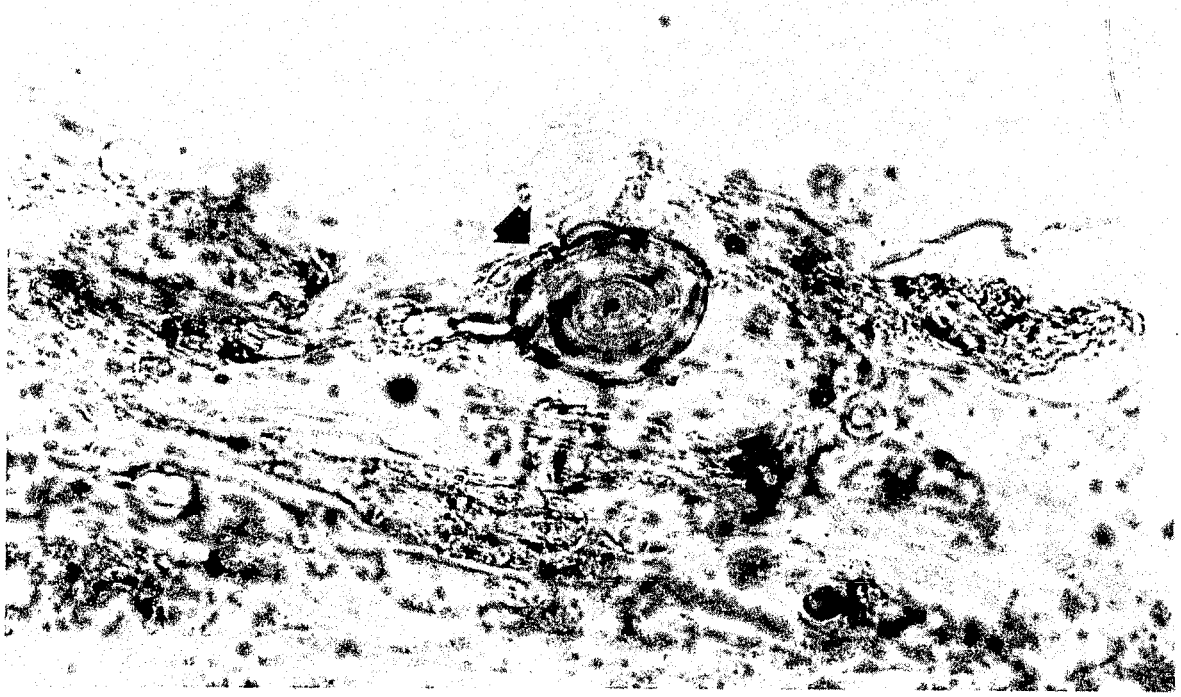
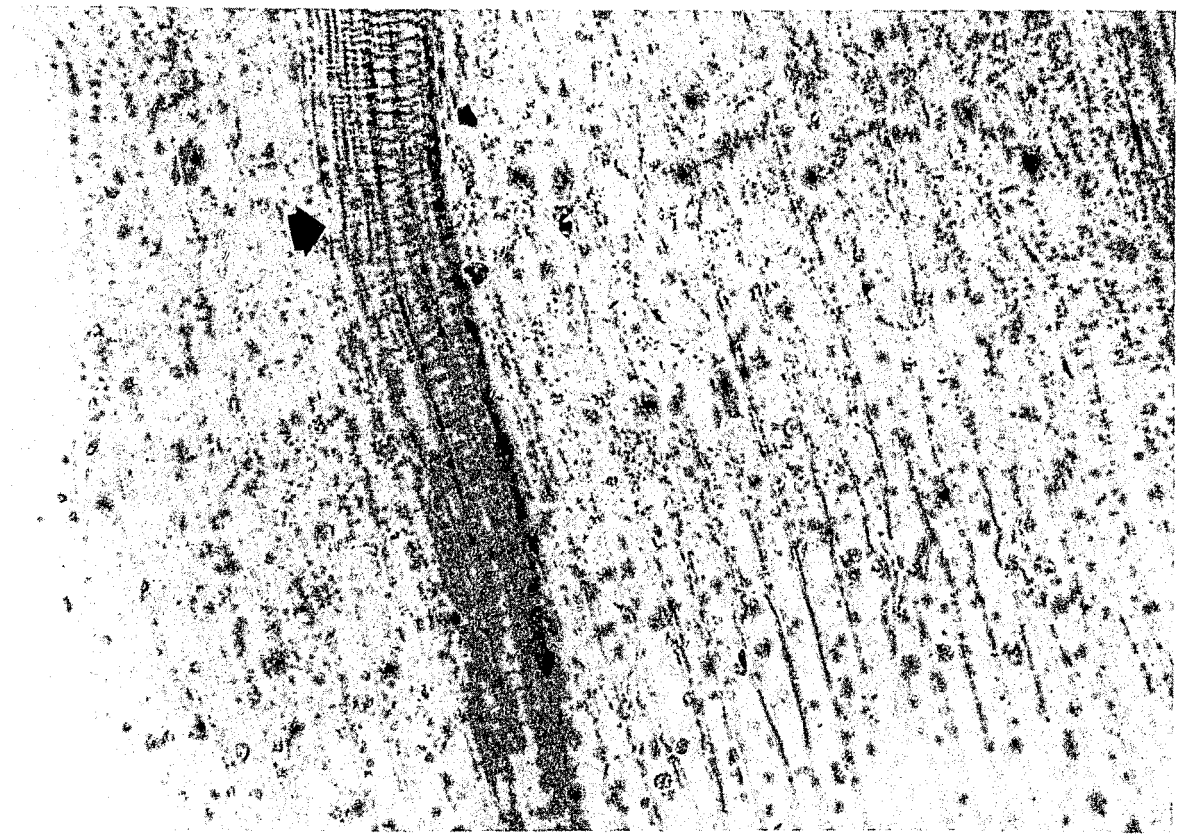


Figure 8.45 Olive skin (X224, taken at 10X) in Mounting Medium B. Note tightly packed, blocky cells with dimensions of 0.02 to 0.04 mm diameter. Compare with okra (fig. 8.38).

Figure 8.46 Olive pulp (X560, taken at 25X) in Mounting Medium A. Note large inclusion (arrow) which is characteristic. Also see fig. 9.33.



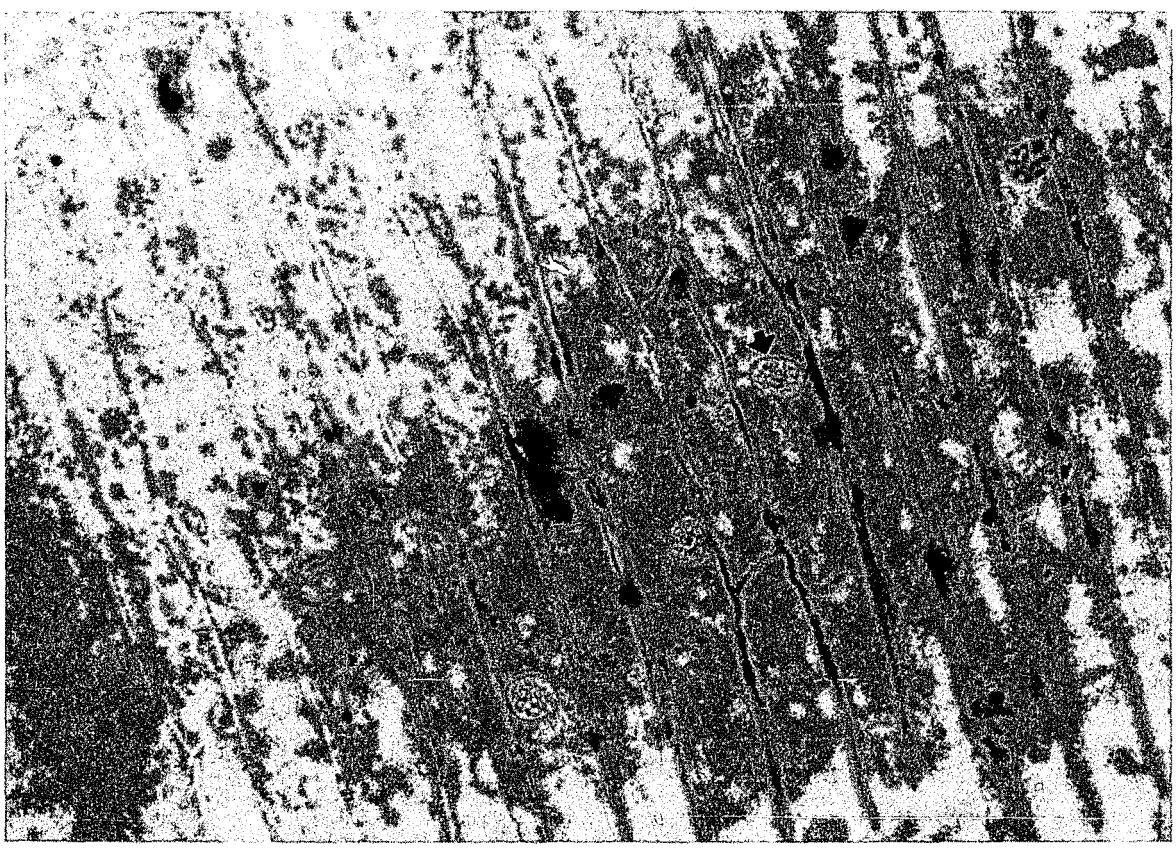
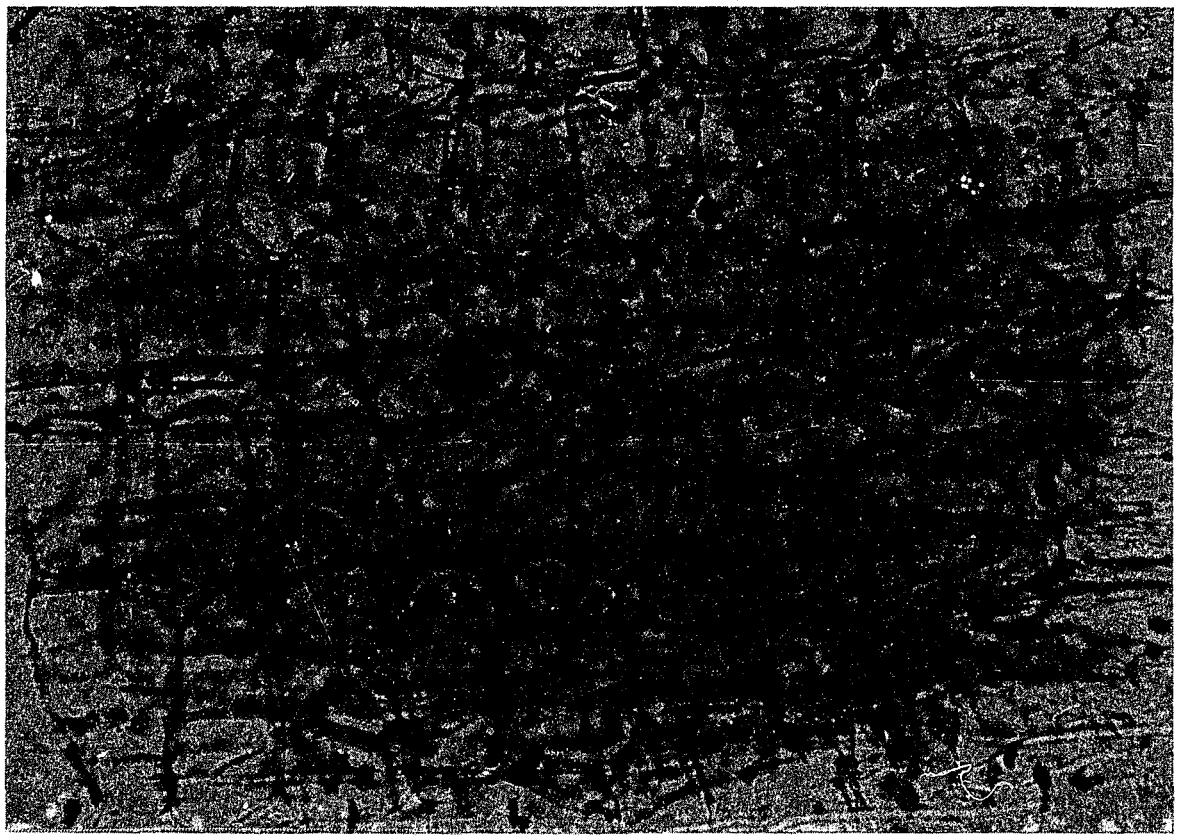


Figure 8.47 Onion (X246, taken at 10X) in Mounting Medium B. Note elongate rectangular cells and conducting cells (arrow). Also see fig. 9.34.

Figure 8.48 Onion (X575, taken at 25X) in Mounting Medium B. High magnification of rectangular epidermal cells, width is 0.02 to 0.03 mm and length is 0.09 to 0.34 mm. Note prominant nucleus (arrow). Compare to garlic (figs. 8.31, 8.32). Also see fig. 9.34.



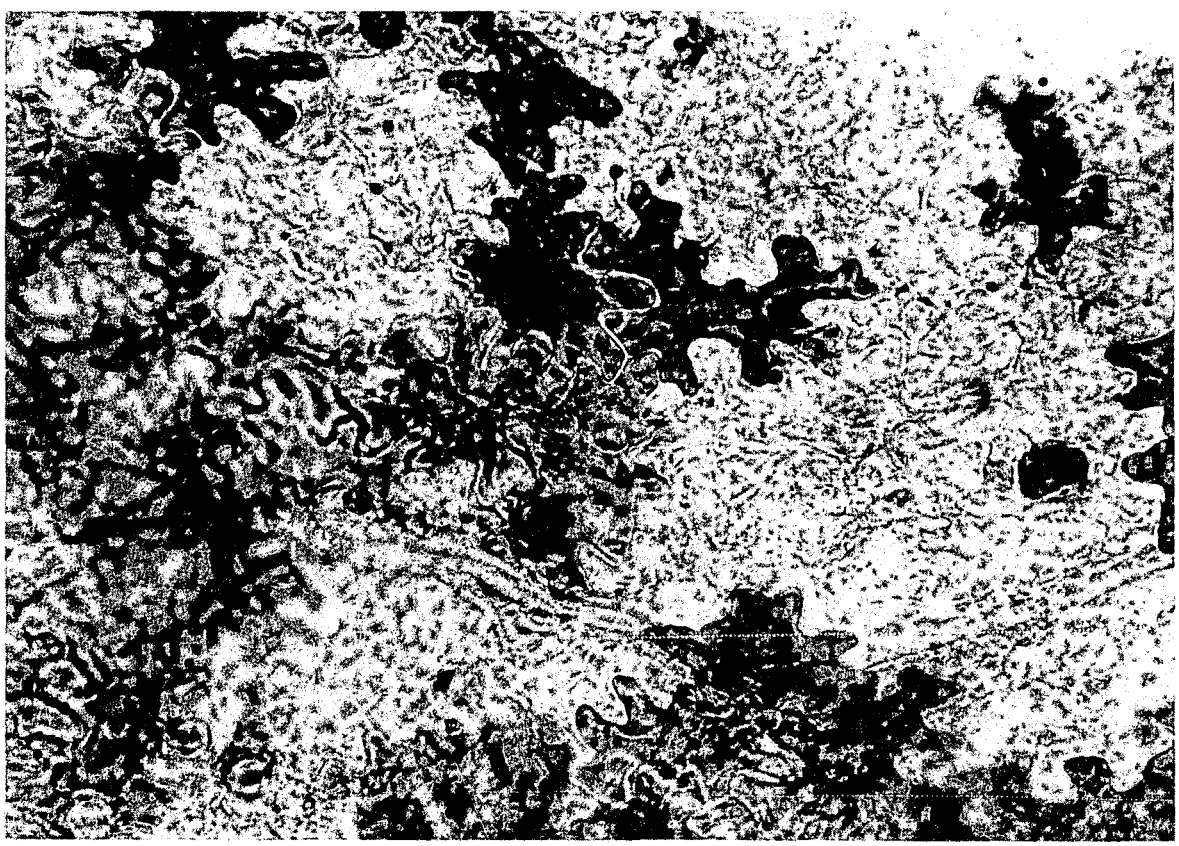


Figure 8.49 Onion (X560, taken at 25X) in Mounting Medium B. High magnification of square epidermal cells ( 0.05 X0.15 mm) next to layer of elongate cells. Compare to garlic (figs. 8.31, 8.32). Also see fig. 9.34.

Figure 8.50 Oregano (X240, taken at 25X) in Mounting Medium A. Epidermal cells look like puzzle pieces. Compare to lettuce (fig. 8.39) and peppers (figs. 8.58, 8.59), be sure to note magnification differences between photos. See also figs. 9.36. 9.37.

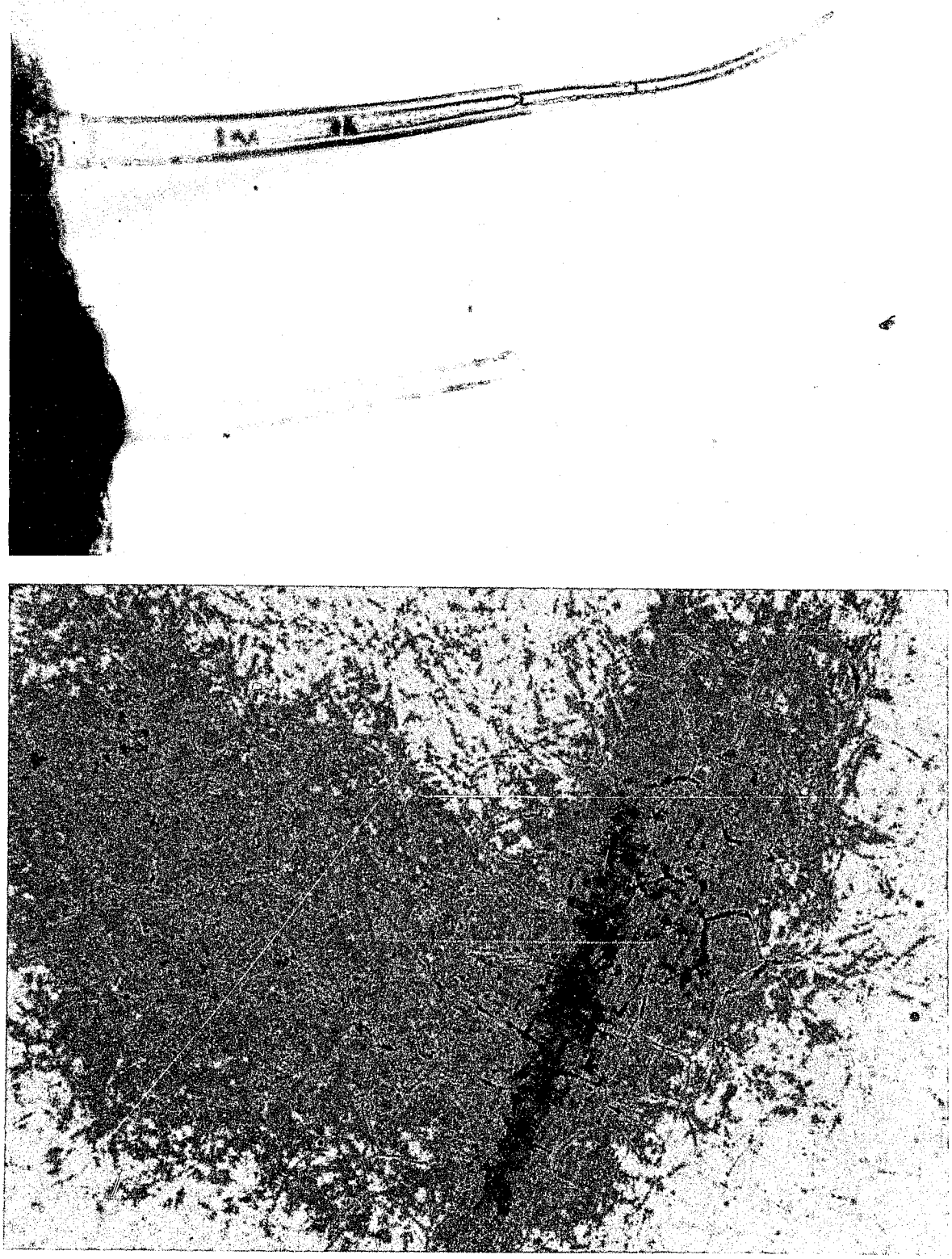


Figure 8.51 Oregano (X224, taken at 10X) in Mounting Medium A. Unbranched epidermal hair with base diameter 0.03 to 0.05 mm. Hairs may be short (0.1 mm) to long (5.0 mm). Compare to apricot skin, green bean pod, okra, peach, rosemary, strawberry, and sweet basil. See also figs. 9.36, 9.37.

Figure 8.52 Papaya (X244, taken at 10X) in Mounting Medium B. Rounded, thin-walled parenchyma, 0.04 to 0.07 mm in diameter.

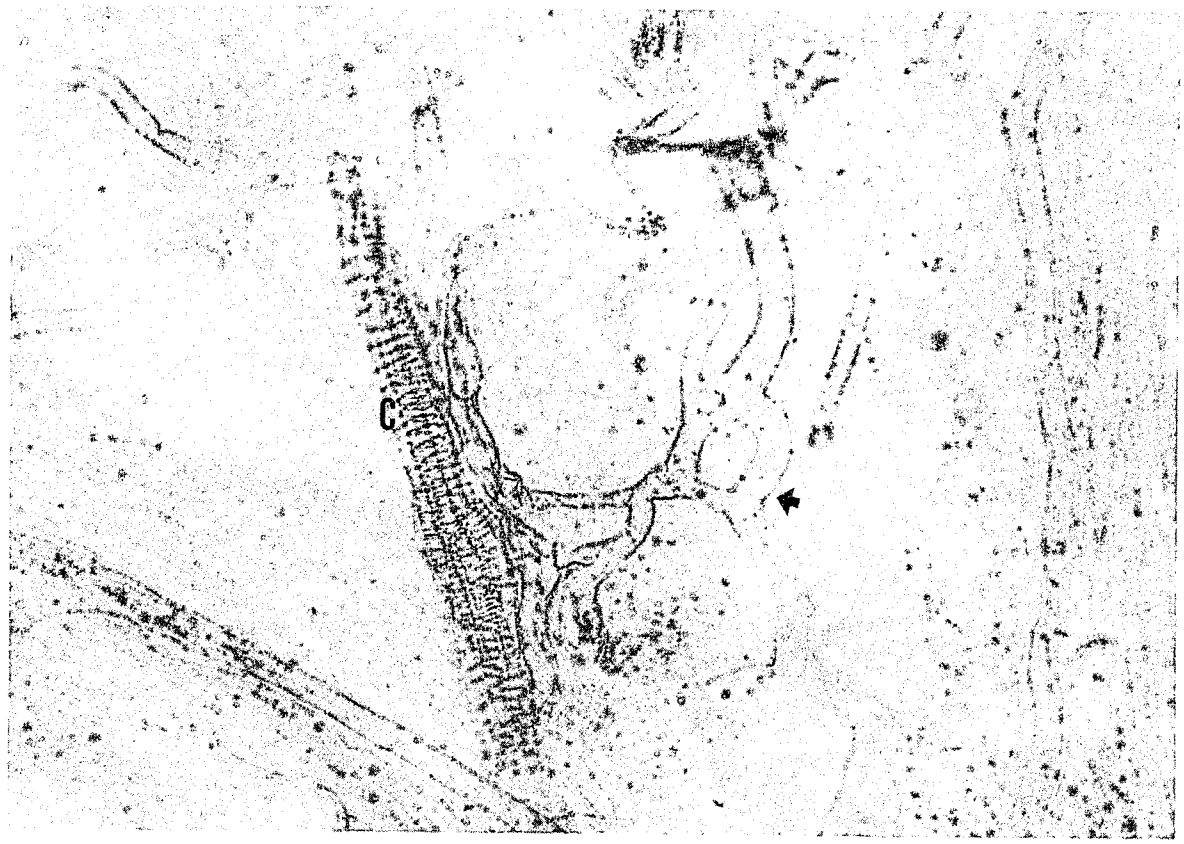
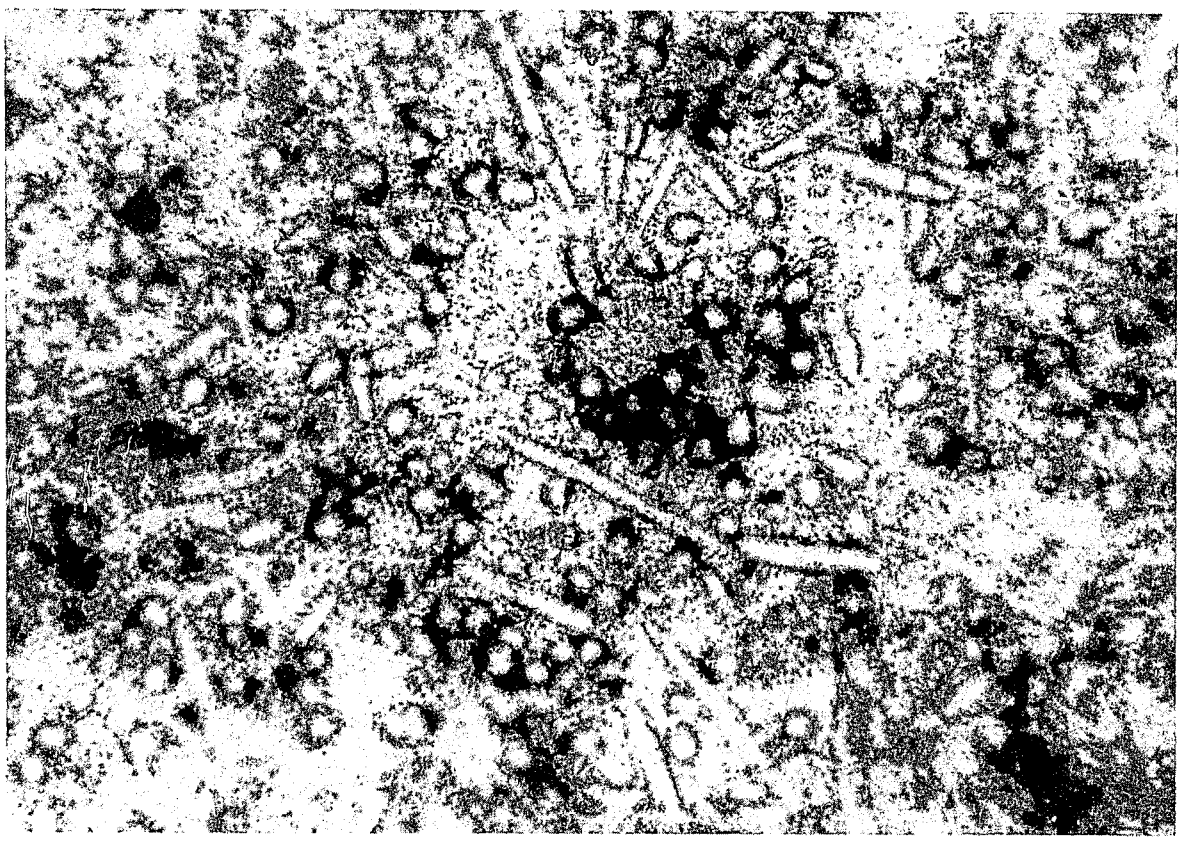



Figure 8.53 Papaya (X244, taken at 10X) in Mounting Medium B. Note fungal hyphae (arrow) lacking cross walls associated with the conducting cells (C).

Figure 8.54 Peach (X226, taken at 10X) in Mounting Medium A. Epidermis shown on edge; note short, dense, unbranched hairs. Compare to apricot skin, green bean pod, okra, oregano, rosemary, strawberry, and sweet basil.



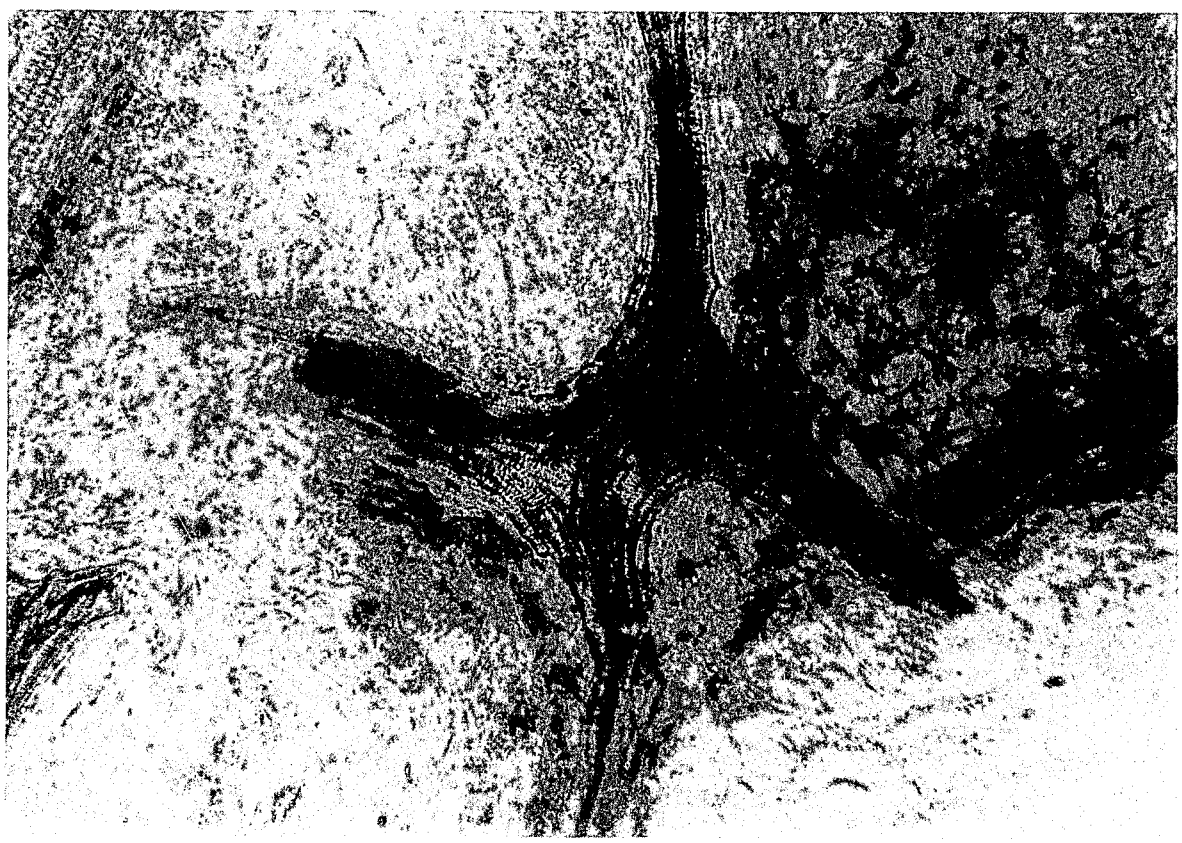
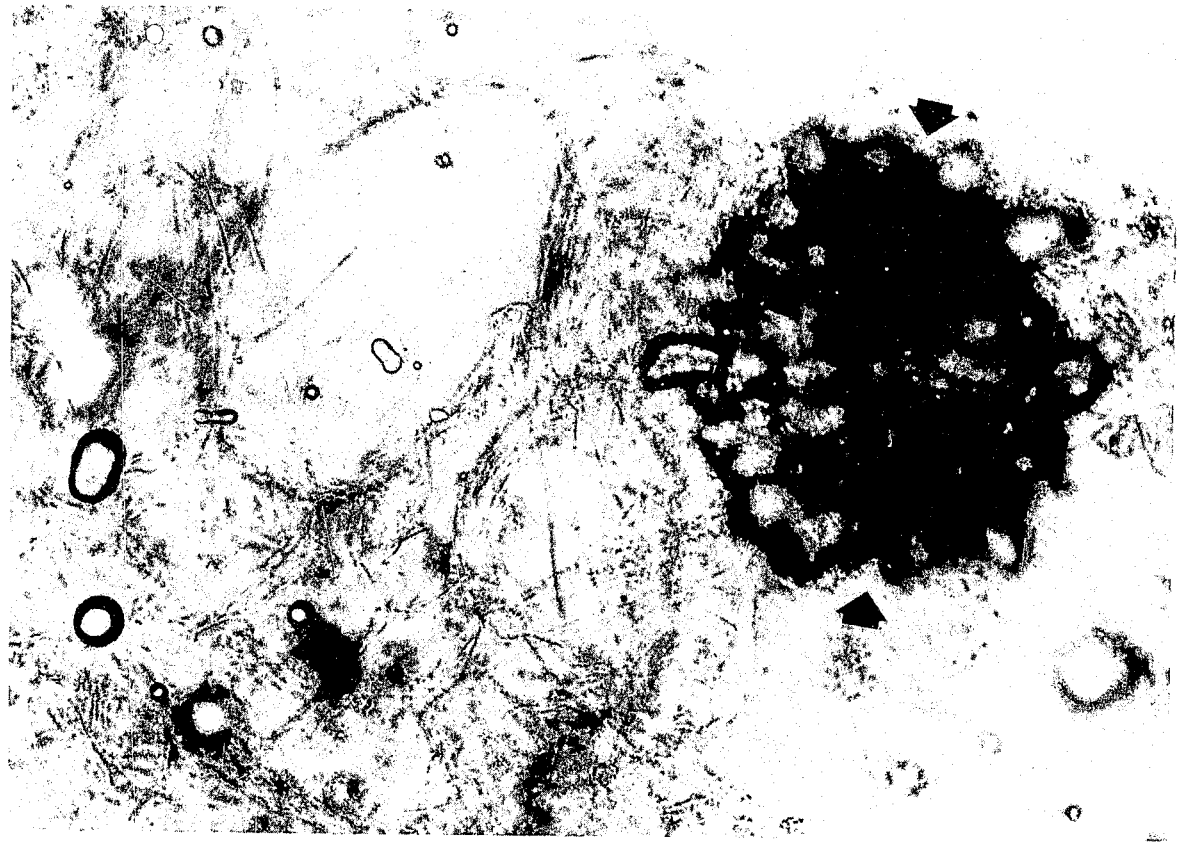


Figure 8.55 Peach (X244, taken at 10X) in Mounting Medium A. Epidermis shown from above; note short, dense, unbranched hairs. Compare to apricot skin, green bean pod, okra, oregano, rosemary, strawberry, and sweet basil.

Figure 8.56 Peach (X226, taken at 10X) in Mounting Medium A. Pulp with prominent conducting tissue.



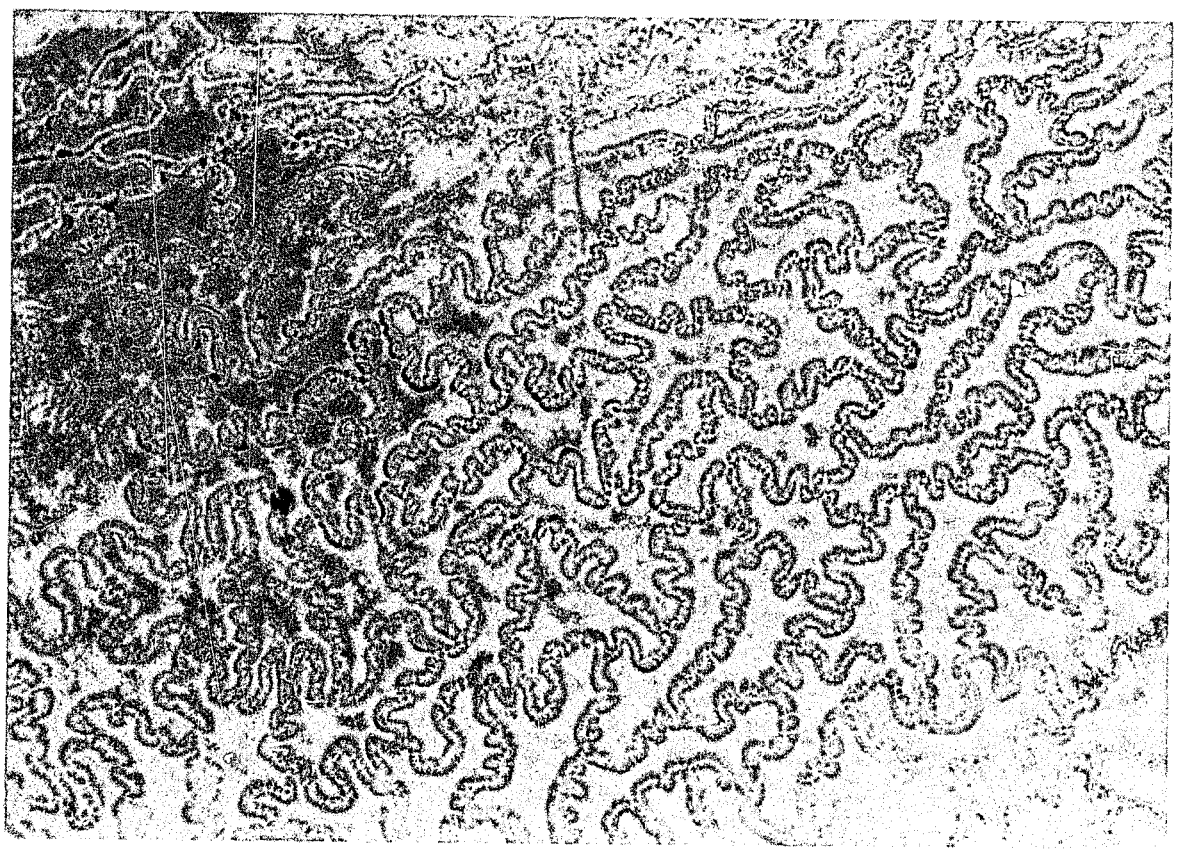


Figure 8.57 Pear pulp (X224, taken at 10X) in Mounting Medium B. Note large, thin-walled parenchyma cells and large inclusions (stone cells, arrow). Compare to apple, fig, okra, radish, tomato, turnip, or water chestnut.

Figure 8.58 Pepper (green, bell, chili) epidermis (X224, taken at 10X) in Mounting Medium A. Note thick-walled, wavy cell margins. Compare to lettuce and oregano (figs. 8.39 and 8.51). See also figs. 9.40, 9.41.

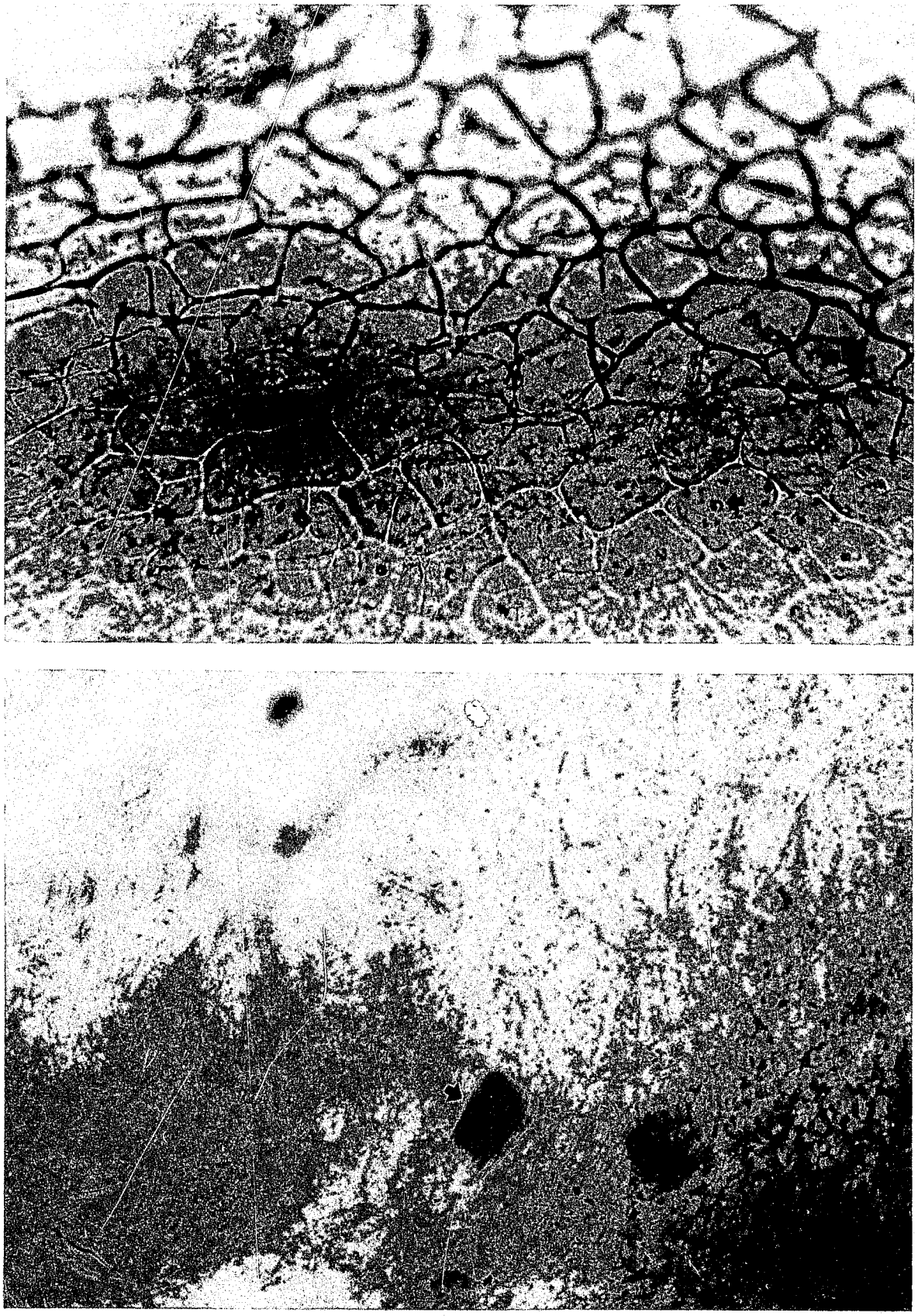
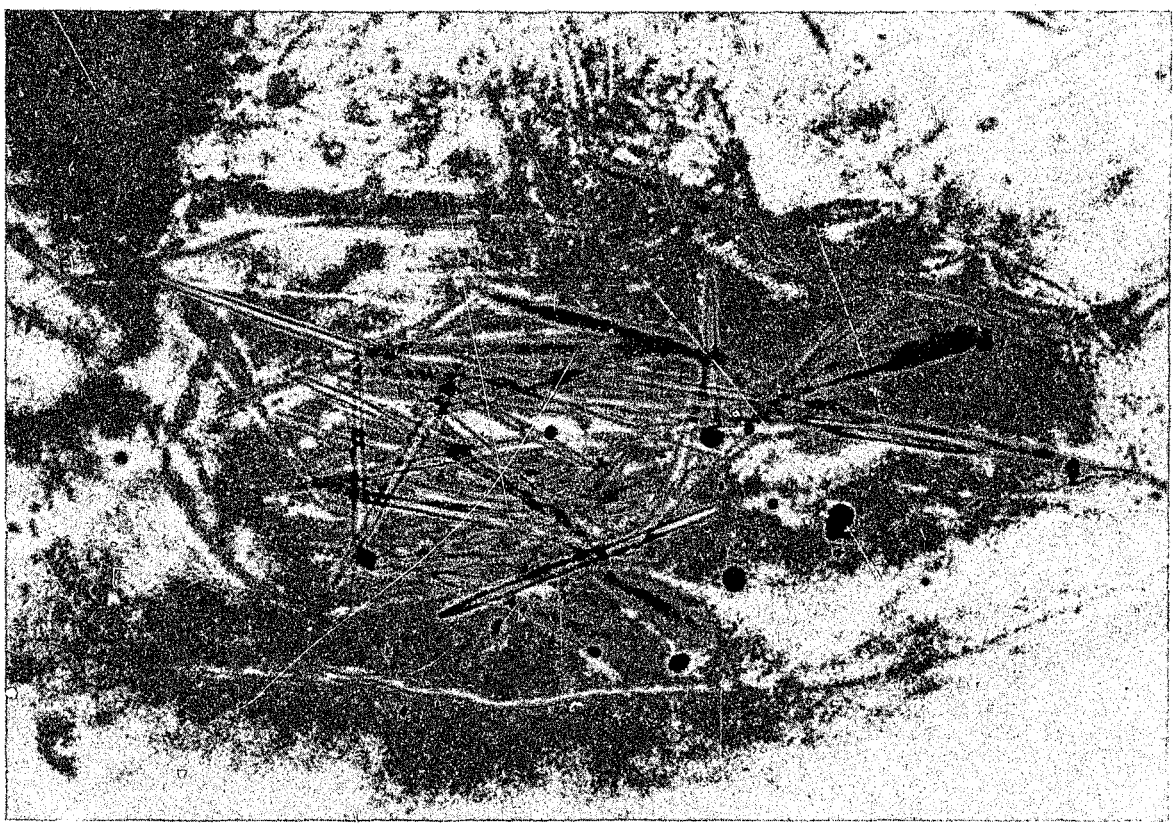


Figure 8.59 Pepper (green, bell, chili) pulp (X224, taken at 10X) in Mounting Medium B. Note packed, blocky cells with clearly defined nuclei (arrow). Also see fig. 9.40.

Figure 8.60 Pineapple pulp (X224, taken at 10X) in Mounting Medium B. Note stacked raphides (arrow). Also see fig. 9.42.



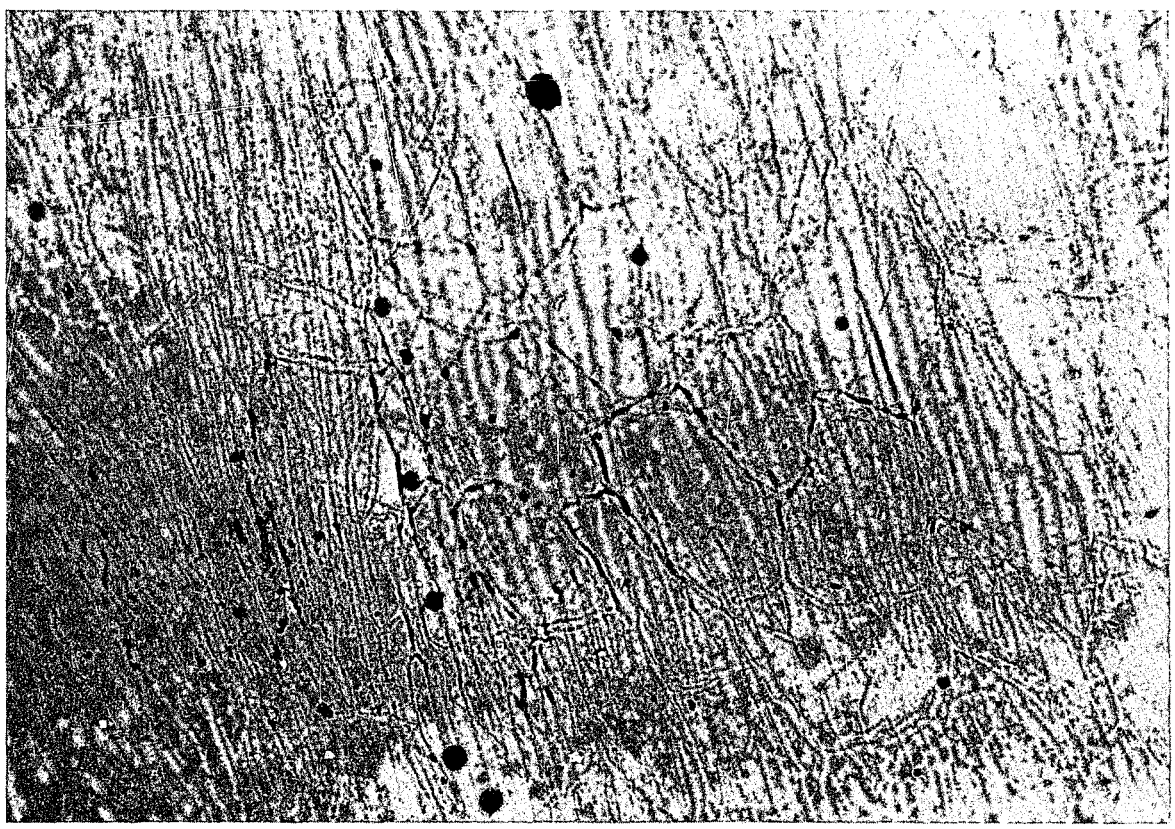
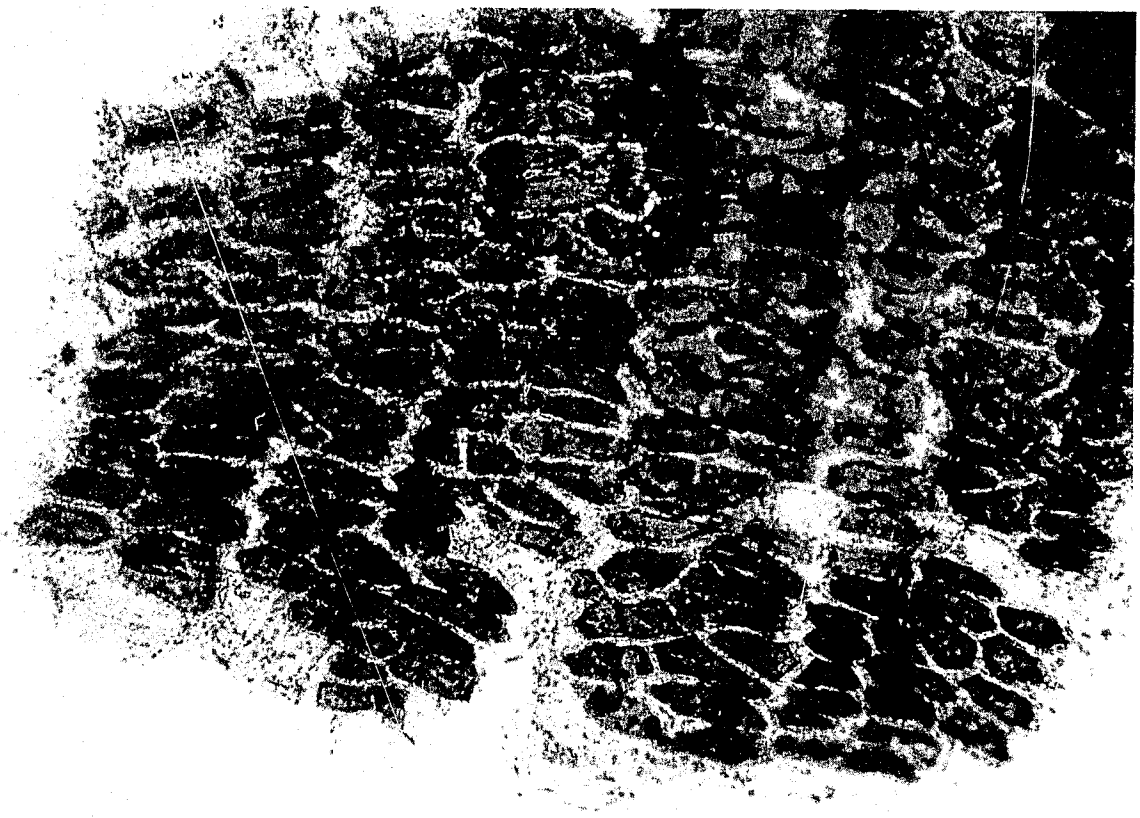


Figure 8.61 Pineapple pulp (X224, taken at 25X) in Mounting Medium A. Note individual raphides (needle-shaped crystals). Also see fig. 9.42.

Figure 8.62 Pineapple pulp (X280, taken at 10X) in Mounting Medium A. Cells very thin-walled, irregular-shaped, loosely packed. Also see fig. 9.42.



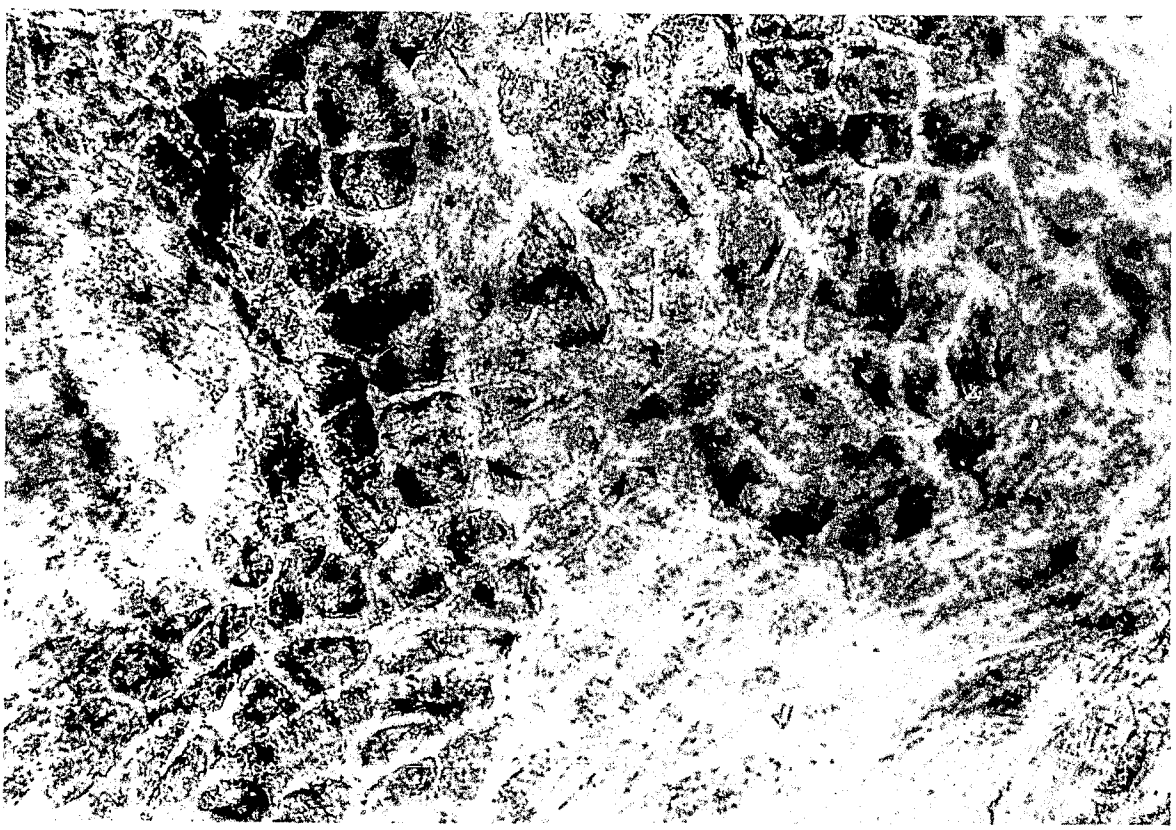


Figure 8.63 Poppy seed coat (X240, taken at 10X) in Mounting Medium A. Cells very thick-walled, angularly-shaped, tightly packed. Also see fig. 9.44.

Figure 8.64 Plum or prune epidermis (X224, taken at 10X) in Mounting Medium A. Cells thick-walled. Compare to cherry (fig. 8.21), blocky, square-cornered.

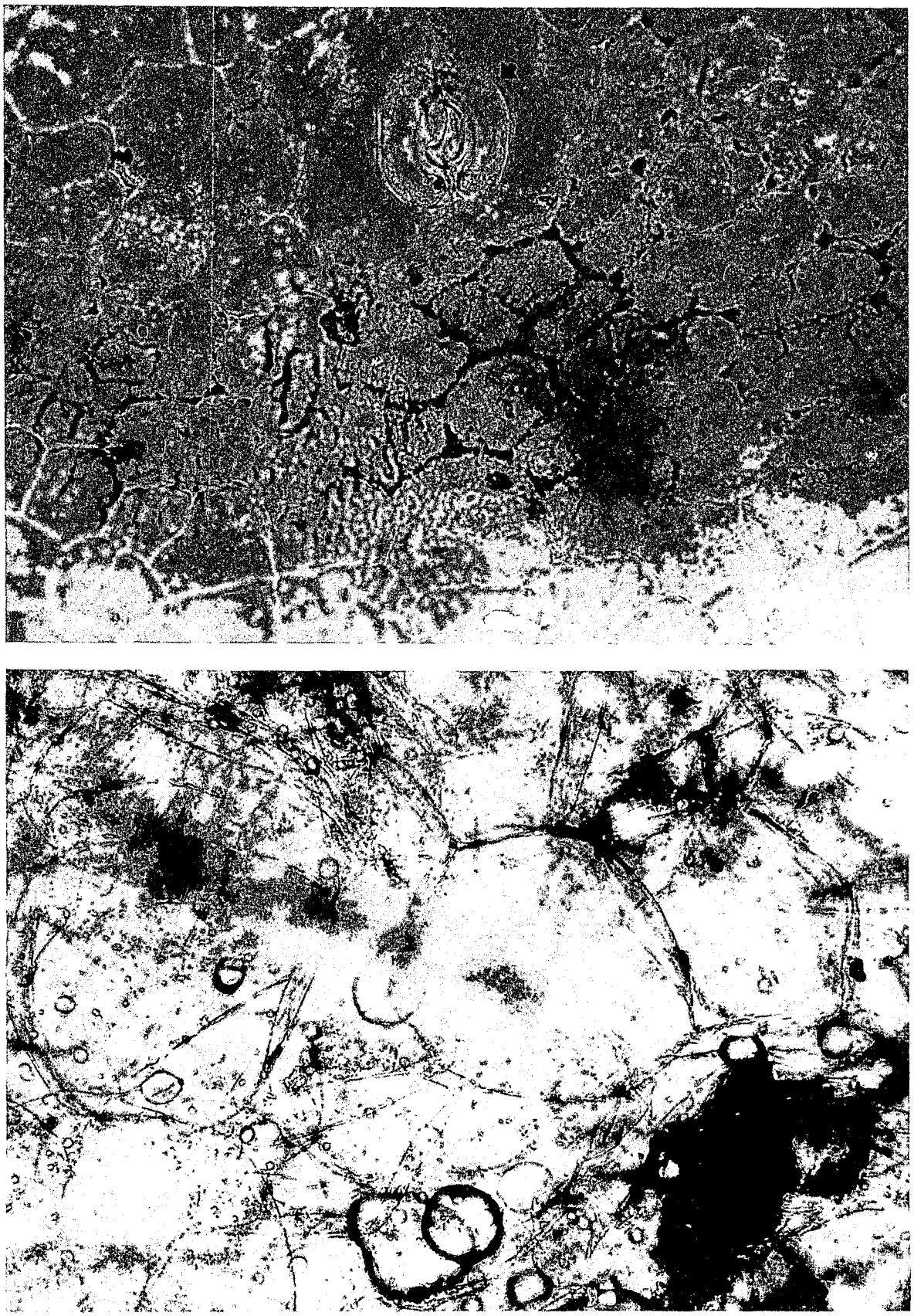
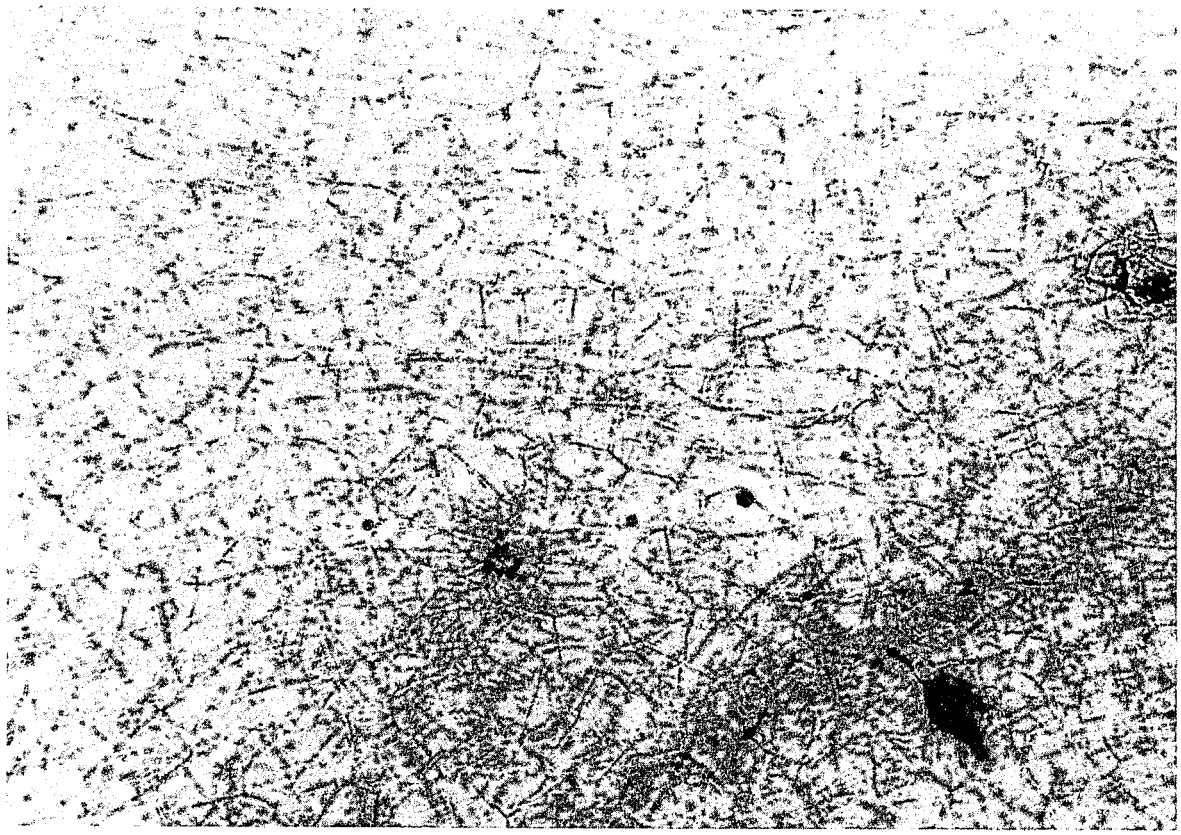


Figure 8.65 Plum or prune epidermis (X615, taken at 25X) in Mounting Medium A. Cells thick-walled, blocky, square-cornered. Note distinctive stomate (arrow). Compare to apple, cherry, and tomato.

Figure 8.66 Plum or prune pulp (X224, taken at 10X) in Mounting Medium B. Cells very thin-walled, rounded. Note druses (arrow). Compare to apple, cherry, fig, okra, pear, radish, strawberry, tomato, turnip, and water chestnut.



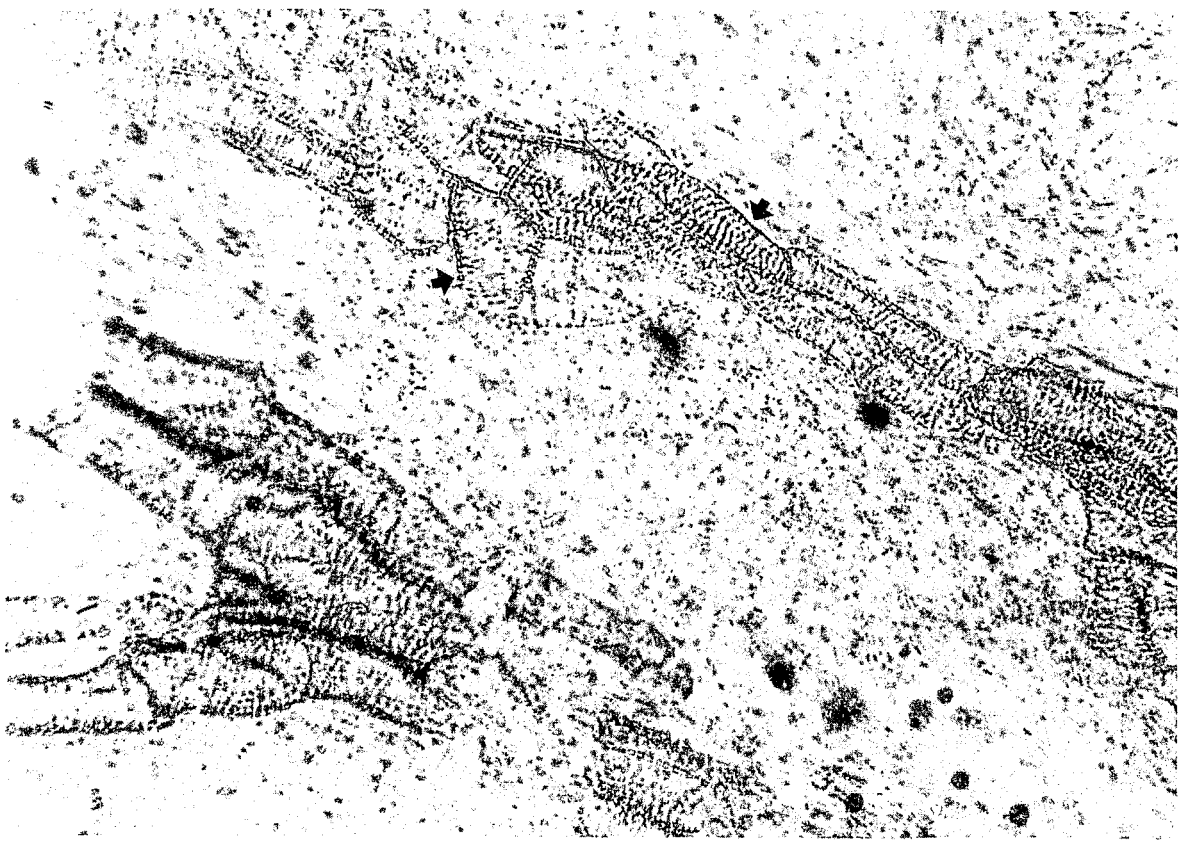


Figure 8.67 Radish pulp (X234, taken at 10X) in Mounting Medium B. Tightly packed, thin-walled, angular cells 0.04--0.09 mm in diameter. Compare to apple, cherry, fig, okra, pear, strawberry, or turnip. Also see fig. 9.48.

Figure 8.68 Radish pulp (X234, taken at 10X) in Mounting Medium A. Note short, broad conducting cells (arrow) arranged in branched patterns. Compare to beet (fig. 8.14) and turnip (fig. 8.81). Also see fig. 9.45.

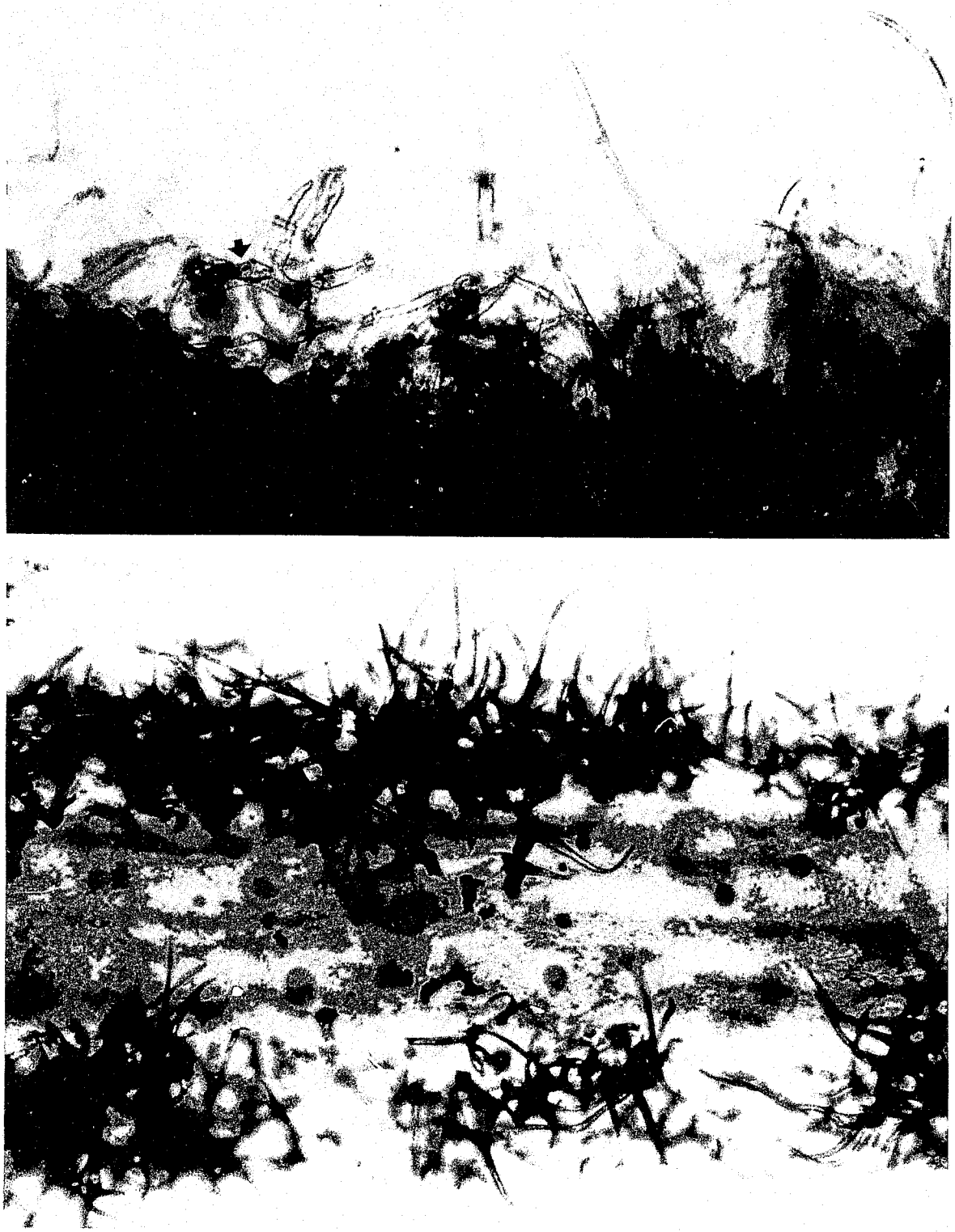


Figure 8.69 Rosemary (X250, taken at 10X) in Mounting Medium A. Note tangled, branched hairs (arrow) on leaf surface. Compare to apricot skin, green bean pod, okra, oregano, peach, strawberry, and sweet basil. Also see fig. 9.46.

Figure 8.70 Rosemary (X224, taken at 10X) in Mounting Medium B. Note branched hairs on stem. Also see fig. 9.46.

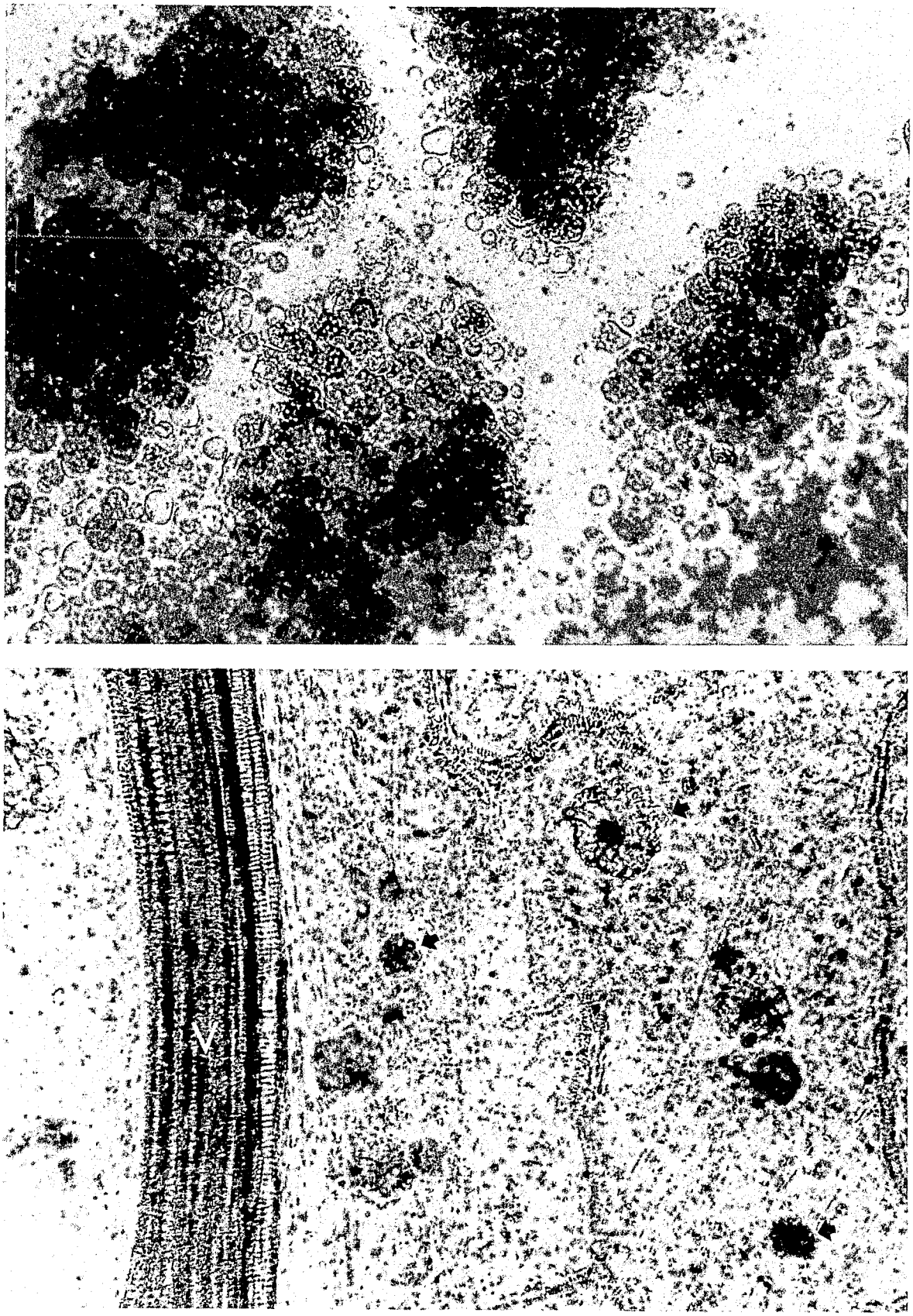
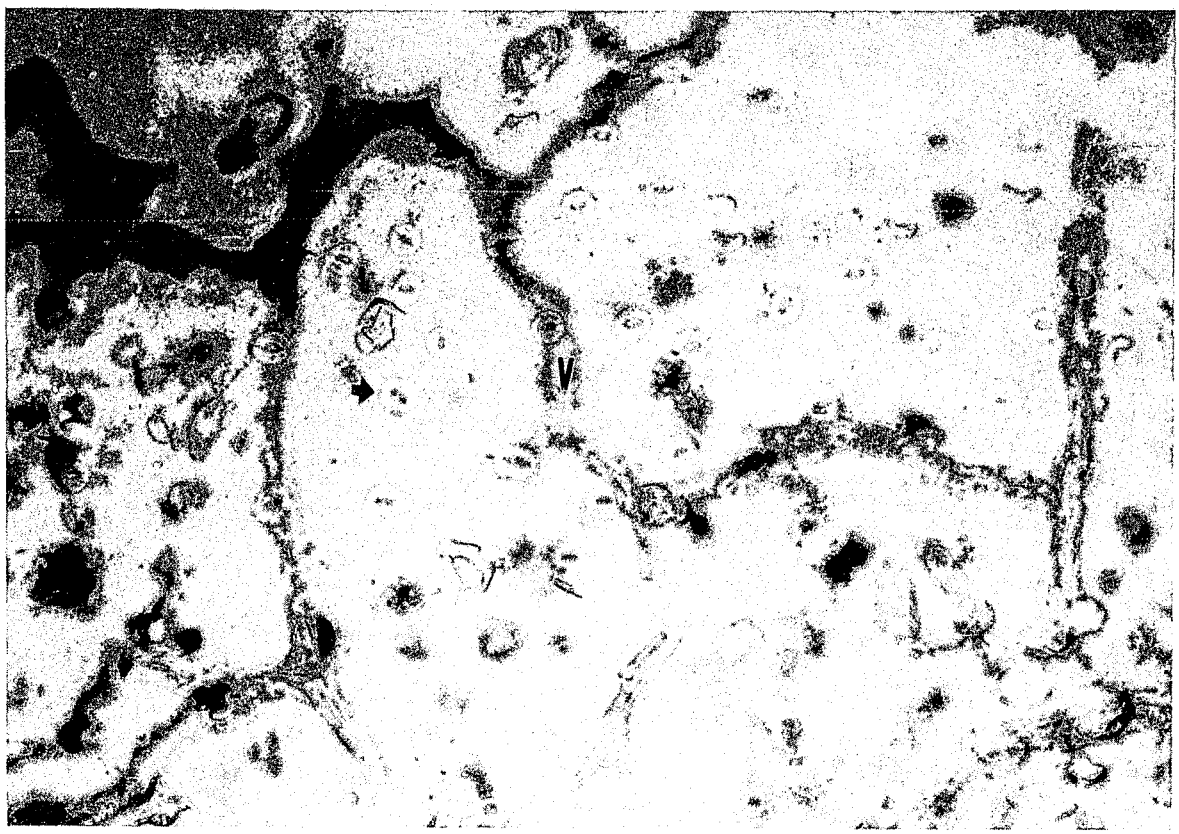


Figure 8.71 Sesame (X246, taken at 10X) in Mounting Medium A. Epidermal cells small, 0.03 mm in diameter, rounded, thick-walled. See also figs. 9.47, 9.48.

Figure 8.72 Spinach (X224, taken at 10X) in Mounting Medium A. Note variously-sized druses (arrows). Also see fig. 9.49.



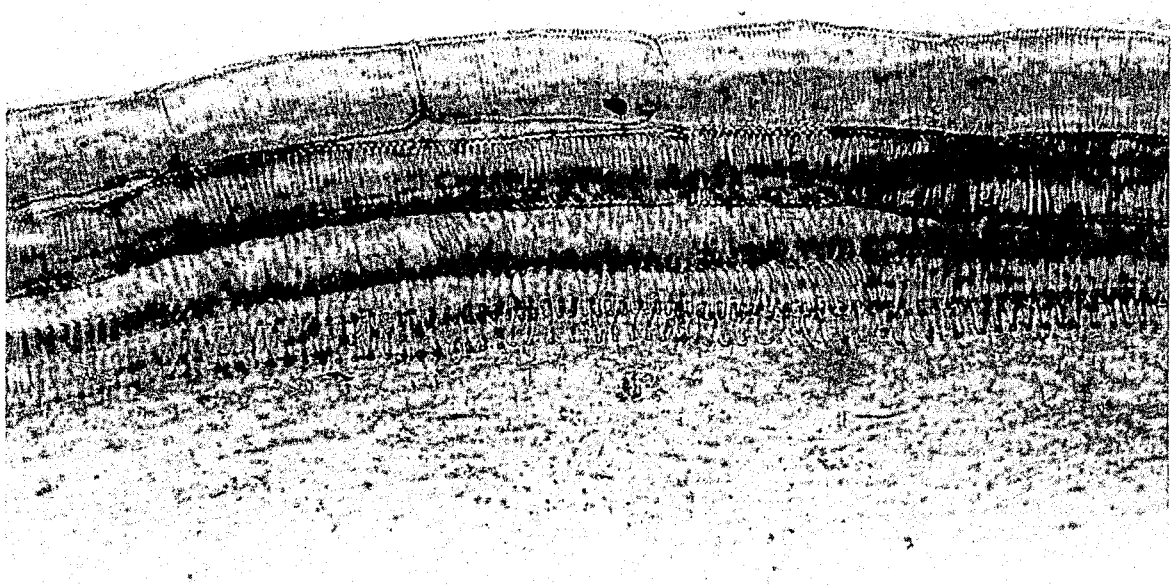
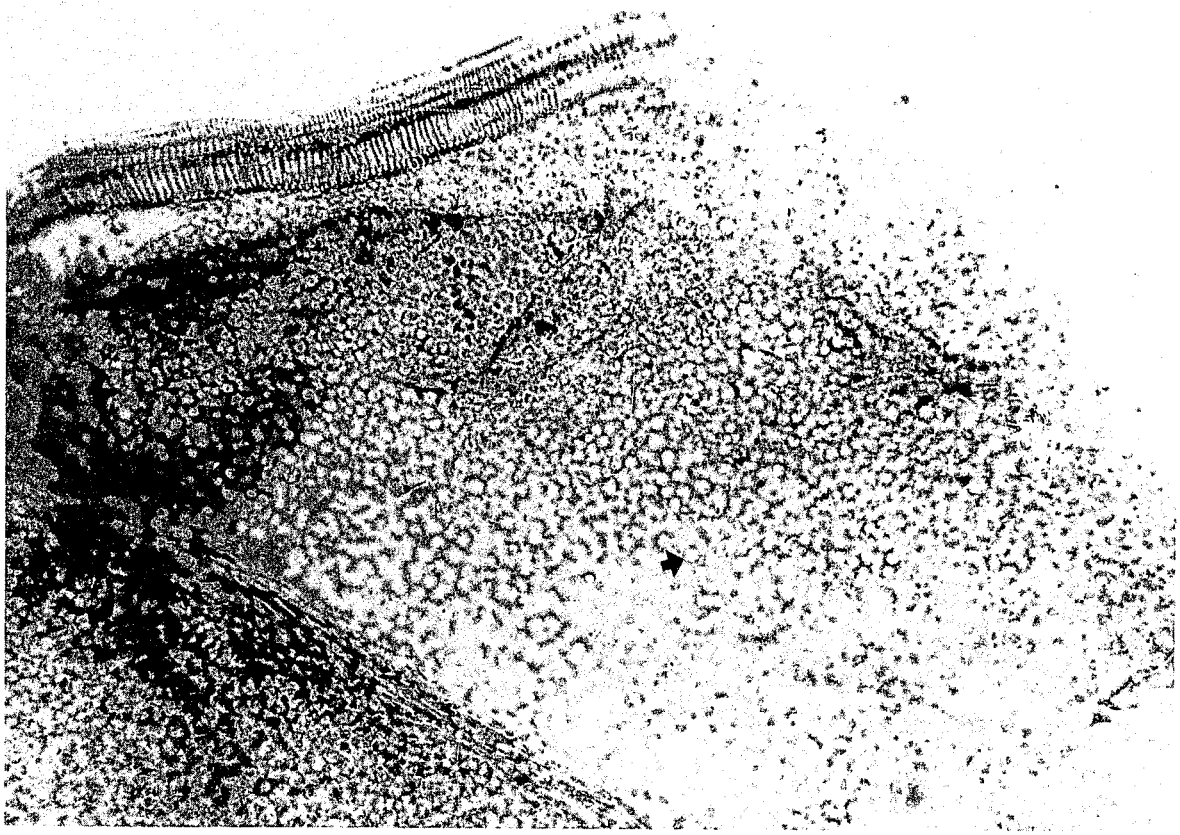



Figure 8.73 Spinach leaf undersurface (X224, taken at 10X) in Mounting Medium A. Note numerous stomates (arrow) and distinctive veins (V)

Figure 8.74 Squash (zucchini, crookneck, pumpkin, etc.) pulp (X224, taken at 10X) in Mounting Medium A. Conducting cells of similar widths but different lengths prominent among indistinct parenchyma cells. Also see fig. 9.50.



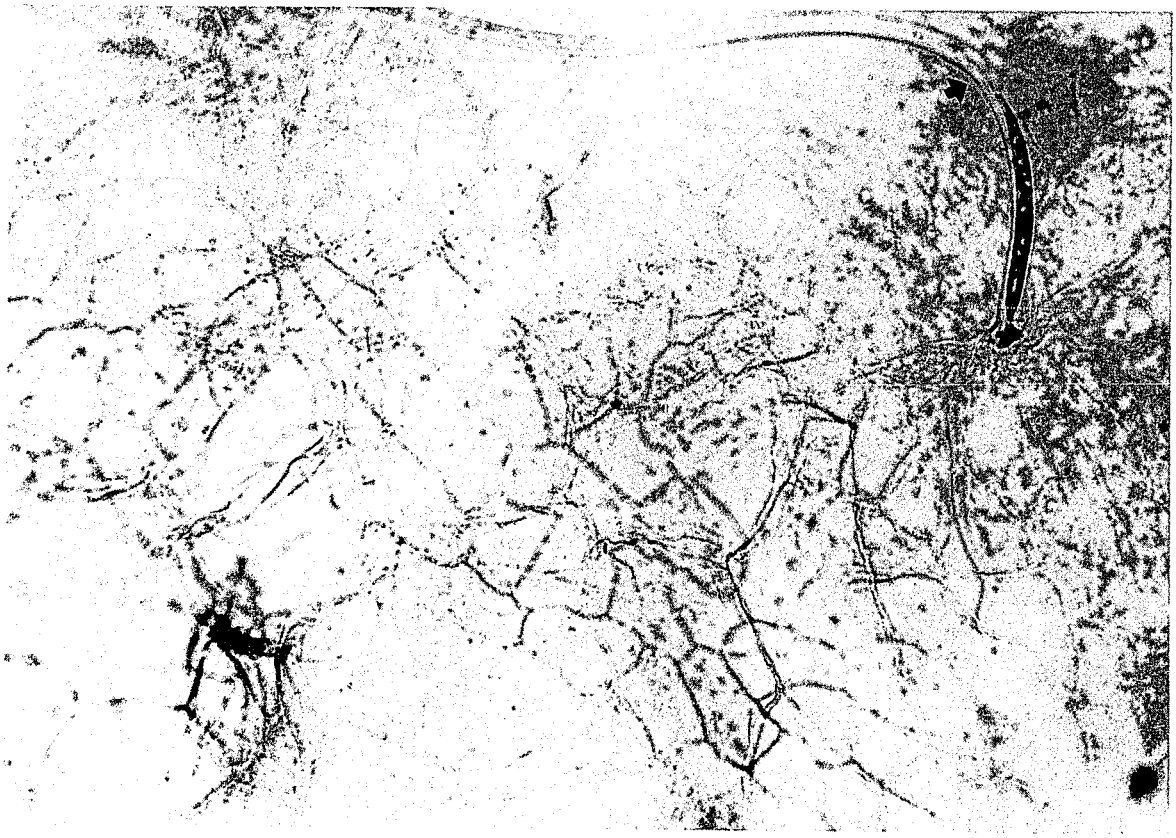


Figure 8.75 Squash (zucchini, crookneck, pumpkin, etc.) pulp (X224, taken at 10X) in Mounting Medium A. Cells indistinct, but with conspicuous starch grains present (arrow). Also see fig. 9.50.

Figure 8.76 Strawberry (X224, taken at 10X) in Mounting Medium B. Note very long (0.5 mm or more), fine hairs on surface (arrow). Compare to apricot, green bean pod, okra, oregano, peach, rosemary, and sweet basil.

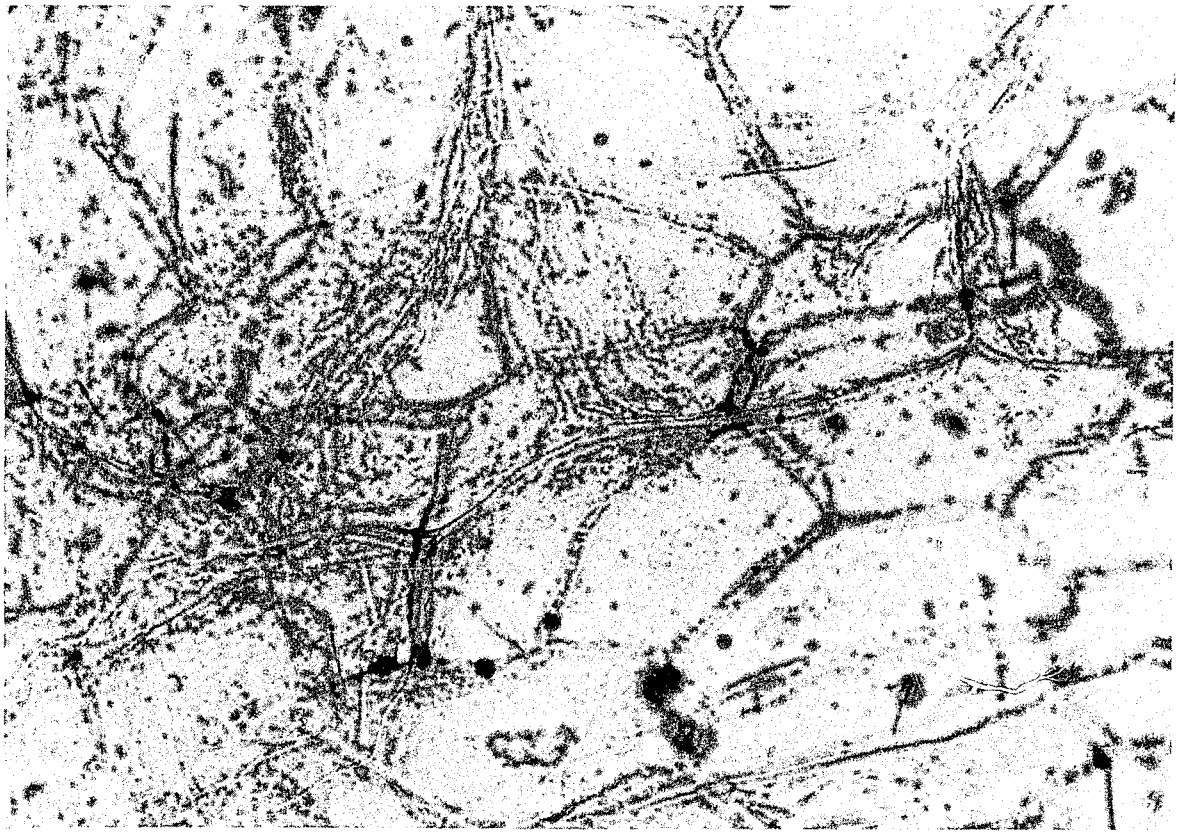
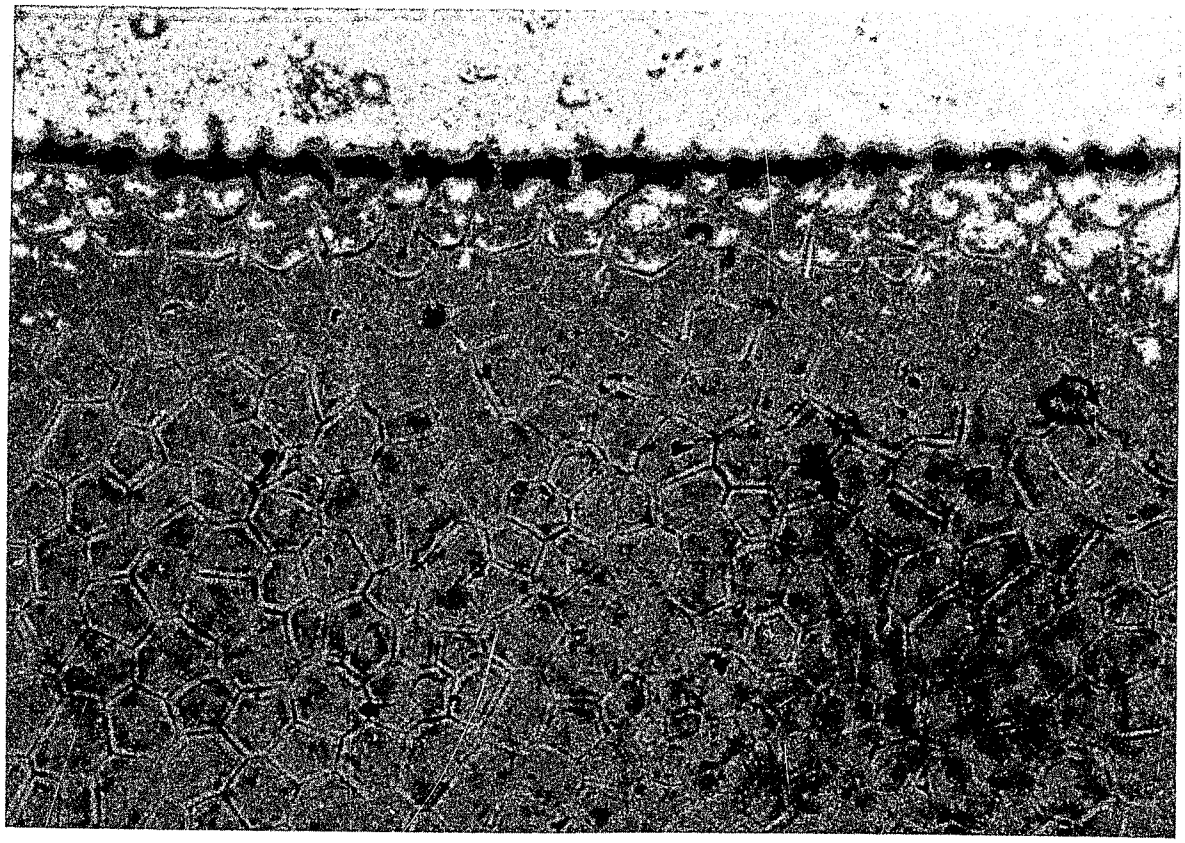


Figure 8.77 Strawberry pulp (X600, taken at 25X) in Mounting Medium A. Note relatively thick-walled, angular cells. Compare to apple pulp or applesauce, cherry, fig, okra, pear, radish, tomato, and water chestnut. Also see fig. 9.51.

Figure 8.78 Sweet basil epidermis (X224, taken at 10X) in Mounting Medium A. Note unbranched hairs (arrow). Compare to apricot skin, green bean pod, okra, oregano, peach, rosemary, and strawberry. Also see fig. 9.53.



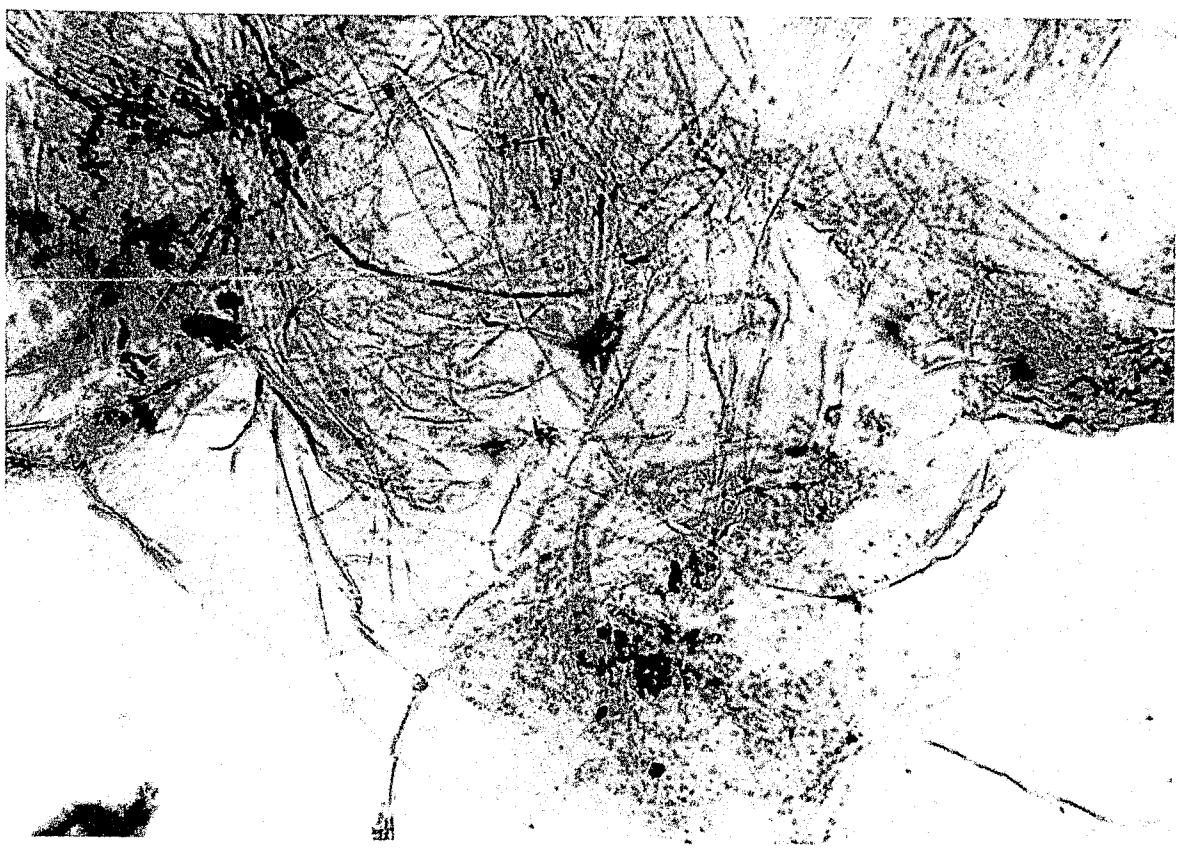
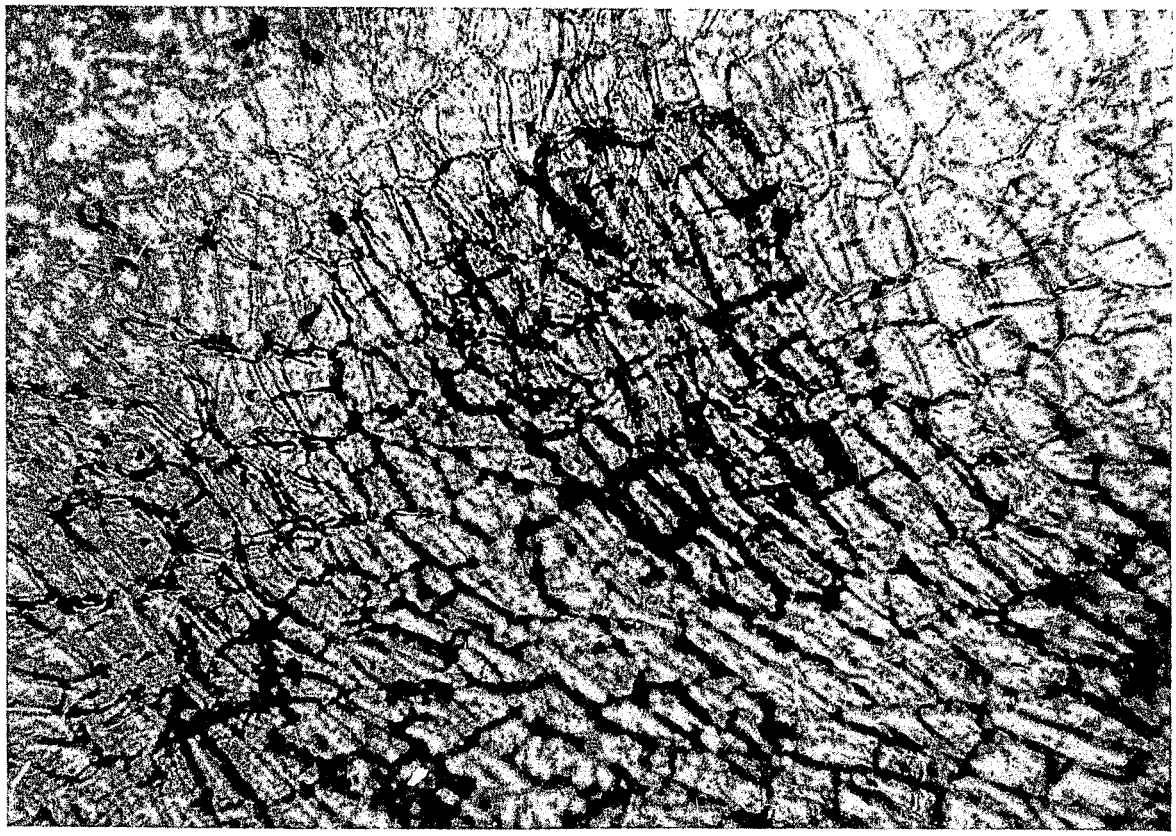


Figure 8.79 Tomato skin (X292, taken at 10X) in Mounting Medium A. Note tightly packed, sharply angular, relatively thin-walled cells. Compare to apple, cherry, and plum. Also see fig. 9.54.

Figure 8.80 Tomato pulp (X224, taken at 10X) in Mounting Medium B. Note very large, thin-walled, easily deformed cells. Compare to apple pulp or applesauce, cherry, fig, grape or raisin, okra, pear, radish, turnip, and water chestnut.



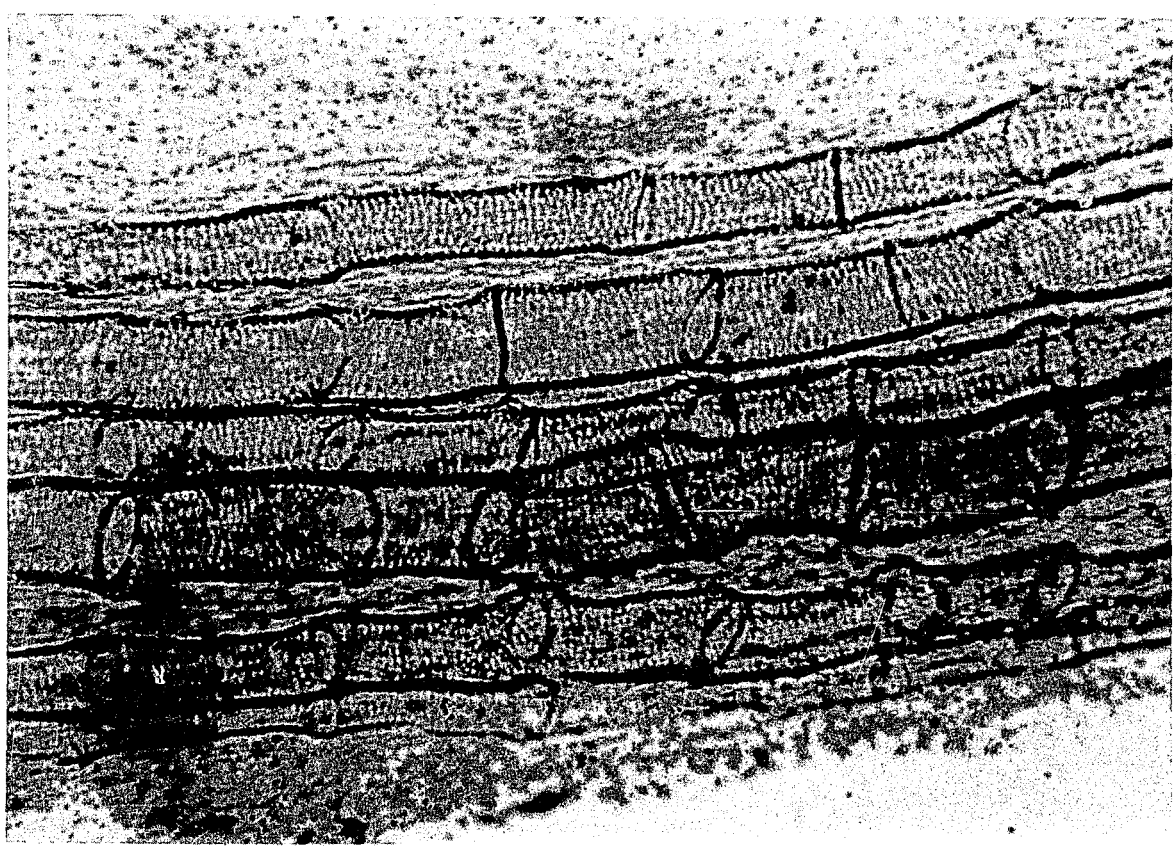


Figure 8.81 Turnip pulp (X220, taken at 10X) in Mounting Medium A. Thin-walled cells relatively small, 0.04 to 0.06 by 0.12 to 0.20 mm. Compare to apple, okra, pear, plum or prune, radish, strawberry, or tomato. Also see fig. 9.55.

Figure 8.82 Turnip conducting cells (X224, taken at 10X) in Mounting Medium B. All cells similar in diameter and length, occurring in unbranched strands. Compare to radish (fig. 8.66).

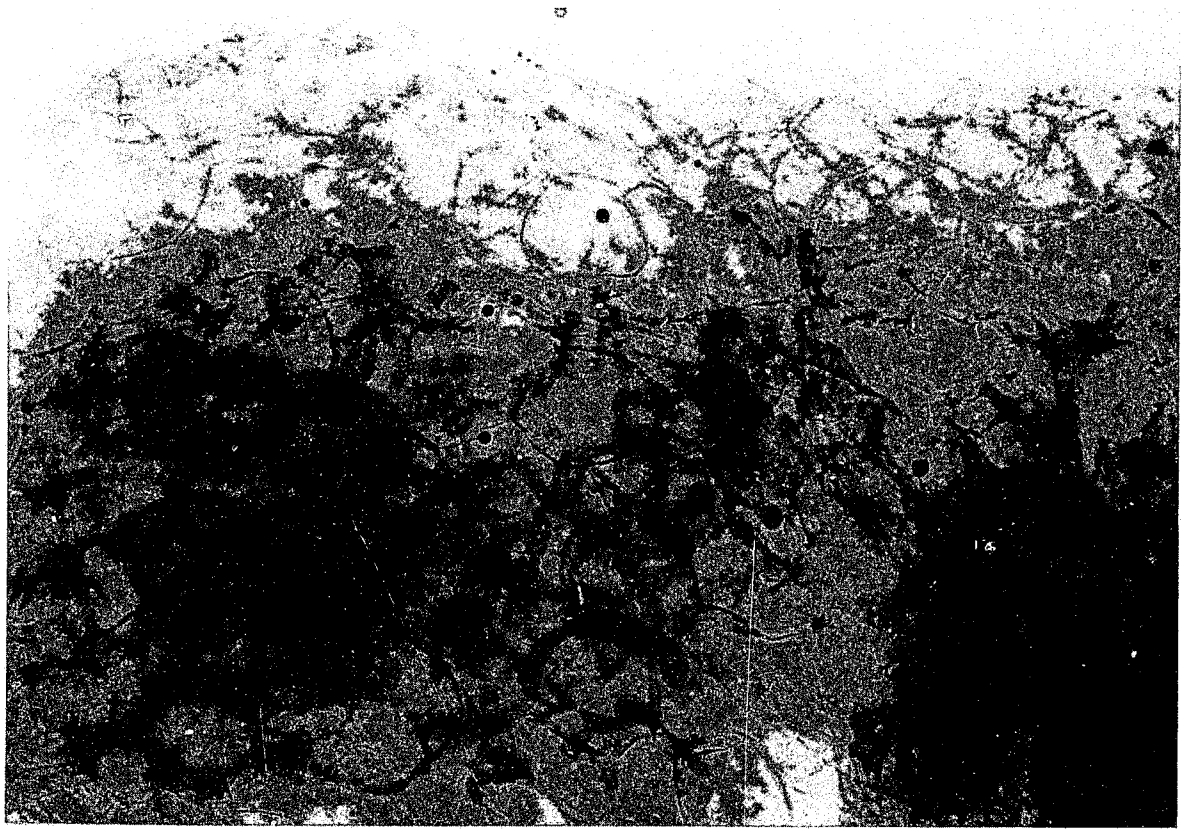


Figure 8.83 Water Chestnut pulp (X224, taken at 10X) in Mounting Medium B. Cells rounded and regular in shape, walls not particularly thin. A few conducting cells present. Compare to apple pulp or applesauce, cherry, fig, grape or raisin, okra, pear, plum or prune, radish, strawberry, tomato, and turnip. Also see fig. 9.56.

# CHAPTER 9

## Scanning Electron Microscopy of Plant Foods Commonly Found in Stomach Contents

### The Scanning Electron Microscope and How it Works

As explained in Chapter 6, the light microscope is a system of lenses that focuses and transmits light energy from a light source, through a specimen, and thence to the eye of the observer. On the way, the image of the specimen is magnified. The same principle is used by the electron microscope, except that the energy used is not light, but a stream of electrons, and the "lenses" are magnets. The image of the specimen is produced on a phosphorescent screen. There are two basic kinds of electron microscopes: the transmission electron microscope (TEM), in which the beam of electrons is transmitted through the specimen, and the scanning electron microscope (SEM), in which the beam of electrons "scans" the outer surface of the specimen but does not pass through it. This is possible because the specimen has been prepared in such a way that it is "electron dense". This usually means that it has been coated with gold, platinum, palladium, or some alloy of these metals. Both kinds of electron microscopes can provide magnifications far beyond any that can be achieved by the light microscope, although for the purposes described in this guide, the high magnifications are not necessary.

The advantage of the SEM over the light microscope (LM) and the TEM is its ability to form a clear image of the outside of an object so that surface features that can barely be seen with the LM practically leap from the image provided by the SEM. For example, compare the LM photograph of green bean pod (fig. 8.35) and the SEM photograph of the same plant part (fig. 9.27). The SEM is also an extremely useful tool for observing objects too opaque to be properly studied with the compound light microscope, such as seeds (see figs. 9.11, 9.23, 9.32, 9.38, 9.44, 9.52).

The disadvantages of the SEM are its relative expense, and the more technical expertise needed to prepare the specimen and examine it with the microscope. This section of the guide is provided should you need to consult with an SEM laboratory in order to be certain of your identification of a plant food beyond what is possible with this guide. The techniques used in the preparation of the photographs for this book are described here, so that the electron microscopist that you may ask to help you will be able to make comparable photographs.

## Preparation of Specimens:

The technique used to prepare specimens for the photographs presented here (figures 9.1 through 9.56) is a modification of the "OTO" technique of Brown, Cohen and Gilmartin (Taxon 36:in press. 1987). The protocol is as follows:

1. Place specimen (taken directly from the sample and not dehydrated) in 2% aqueous solution of osmium tetroxide for 1 hour

2. Rinse 5 times (5--10 minutes each time) with water

3. Place specimen in saturated solution of thiocarbohydrazide for 1 hour

4. Repeat step 2

5. Repeat step 1

6. Repeat step 2

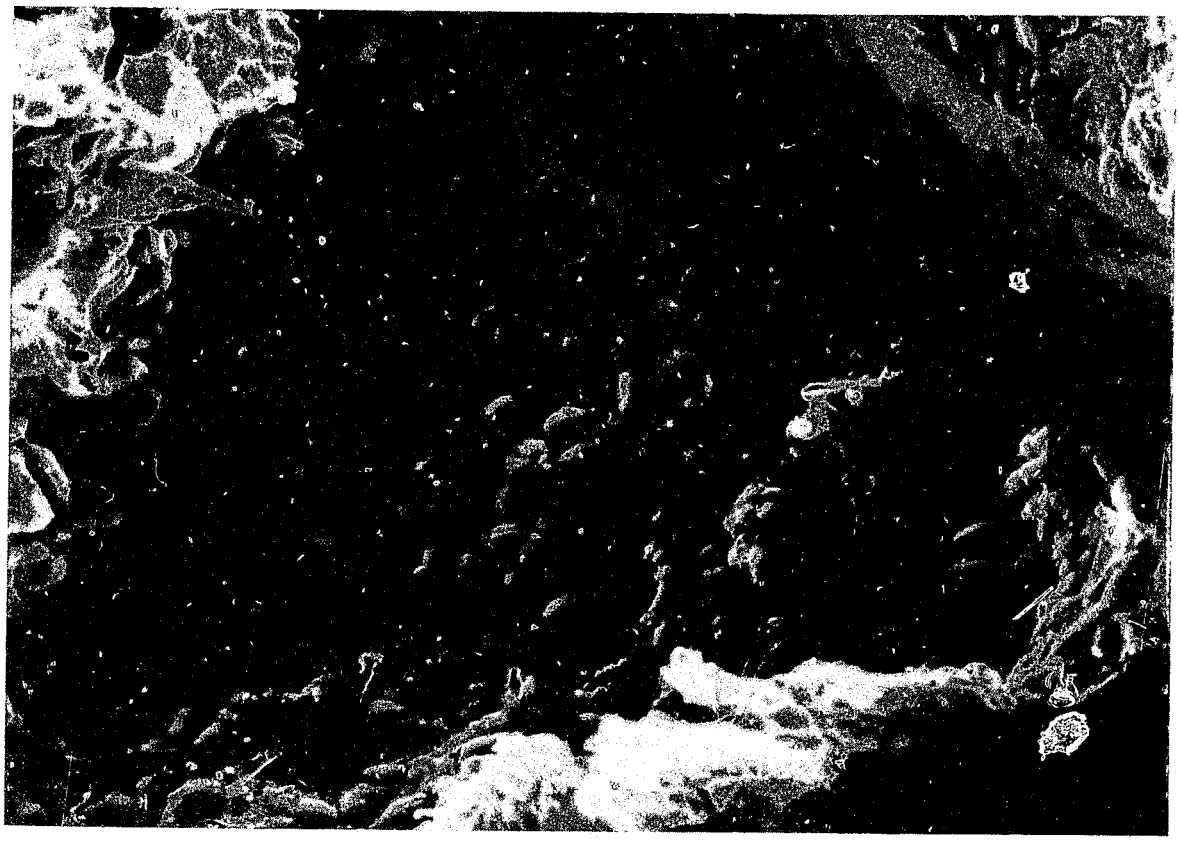
7. Dehydrate (in 10-minute increments) through a series of 30%, 50%, 70%, 85%, 90%, 95%, and three changes of absolute ethanol (it is suggested that one of these last three changes be at least 12 hours long)

8. Critical Point Dry ($CO_2$ method)

Observation and Photography of Specimens:

After critical point drying, the specimens used here were mounted on stubs with double-sided SEM tape (a 3M product). The tape edges and undersides of the specimens not in contact with the tape were then painted with silver paint. Stubs were sputter-coated with approximately 400 nanometers of gold.

Our photographs were made on an AMR 1000 at an accelerating voltage of 20kV, spot size 4, 17° tilt, working distance 23, and magnification 140X, unless otherwise indicated. As reproduced here, the final image is X280.



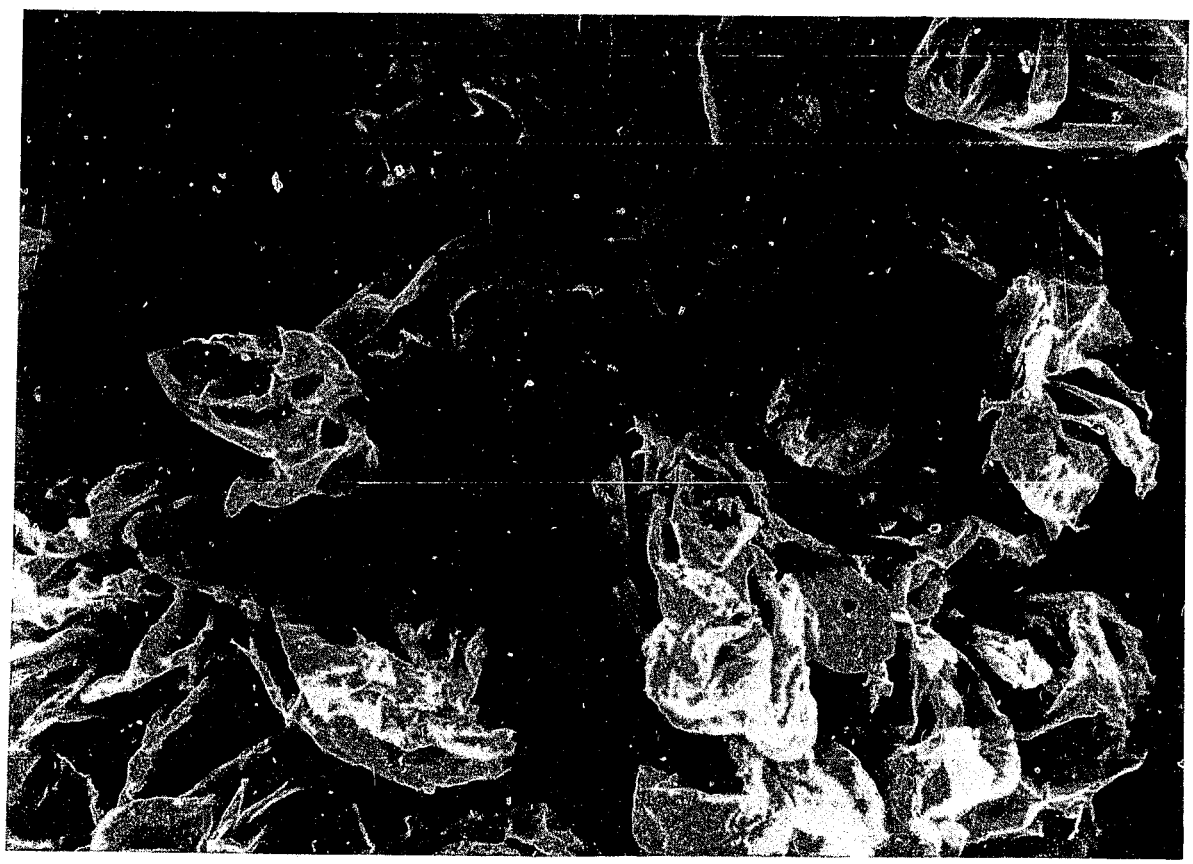
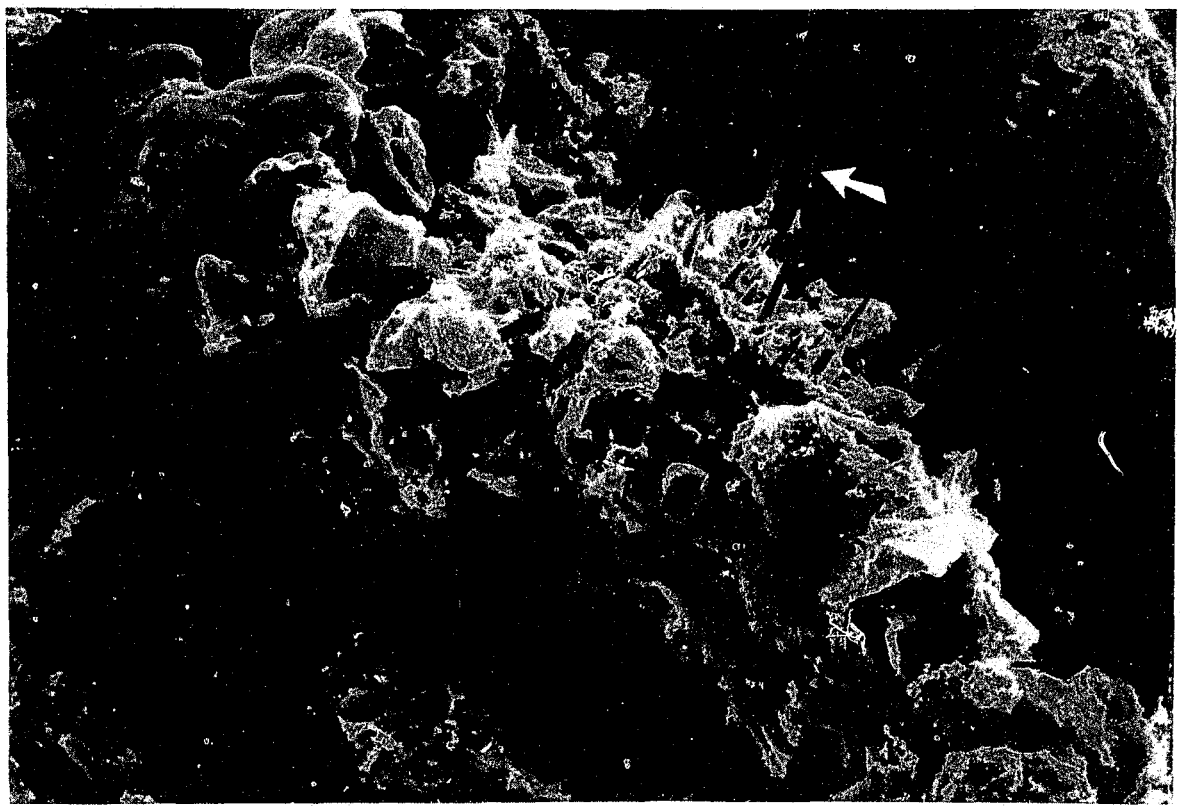


Figure 9.1 Apple peel. Cells blocky, with rounded corners. Also see fig. 8.1.

Figure 9.2 Apple pulp or applesauce. Cells large, thin-walled. Also see fig. 8.2.



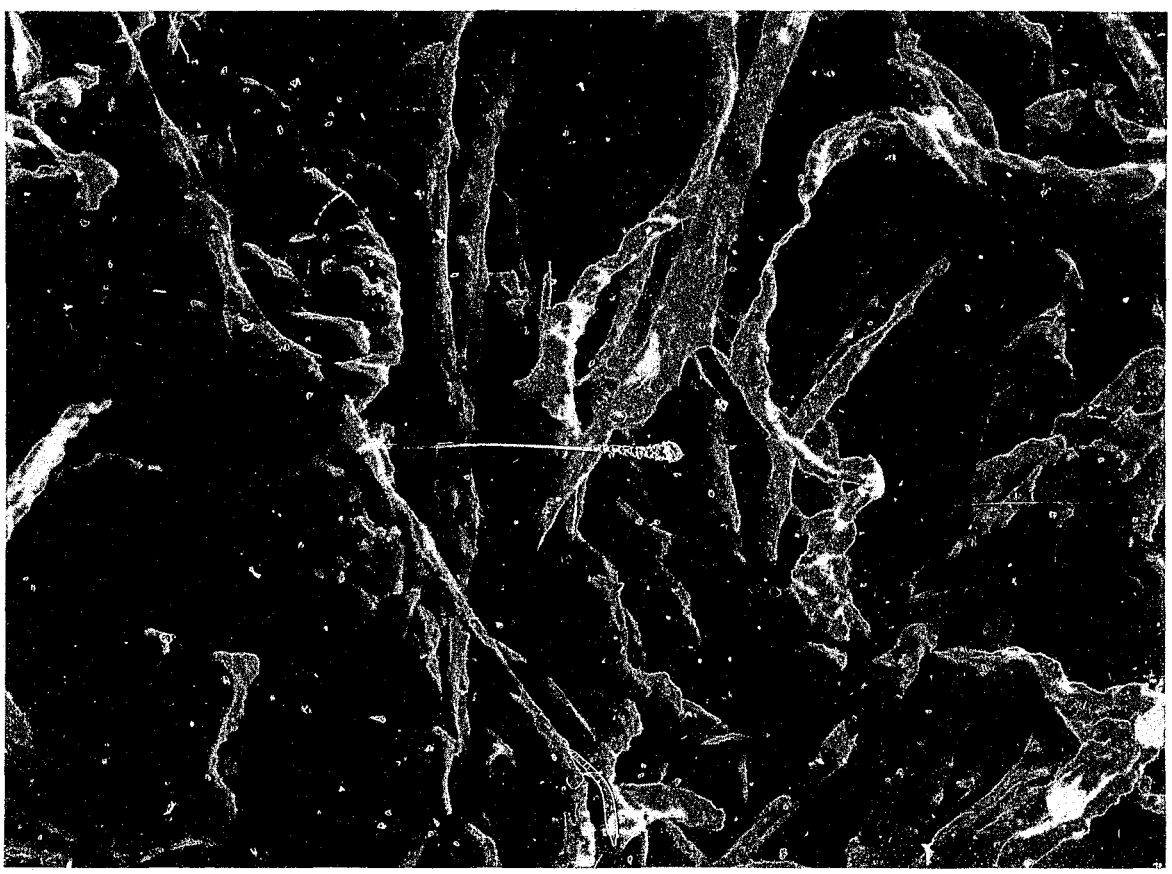


Figure 9.3 Apricot peel and pulp. Note the unbranched hairs (arrow). Also see figs. 8.3 and 8.4.

Figure 9.4 Banana pulp. Large, thick-walled, fibrous conducting cells predominant. Also see figs. 8.5 and 8.6.

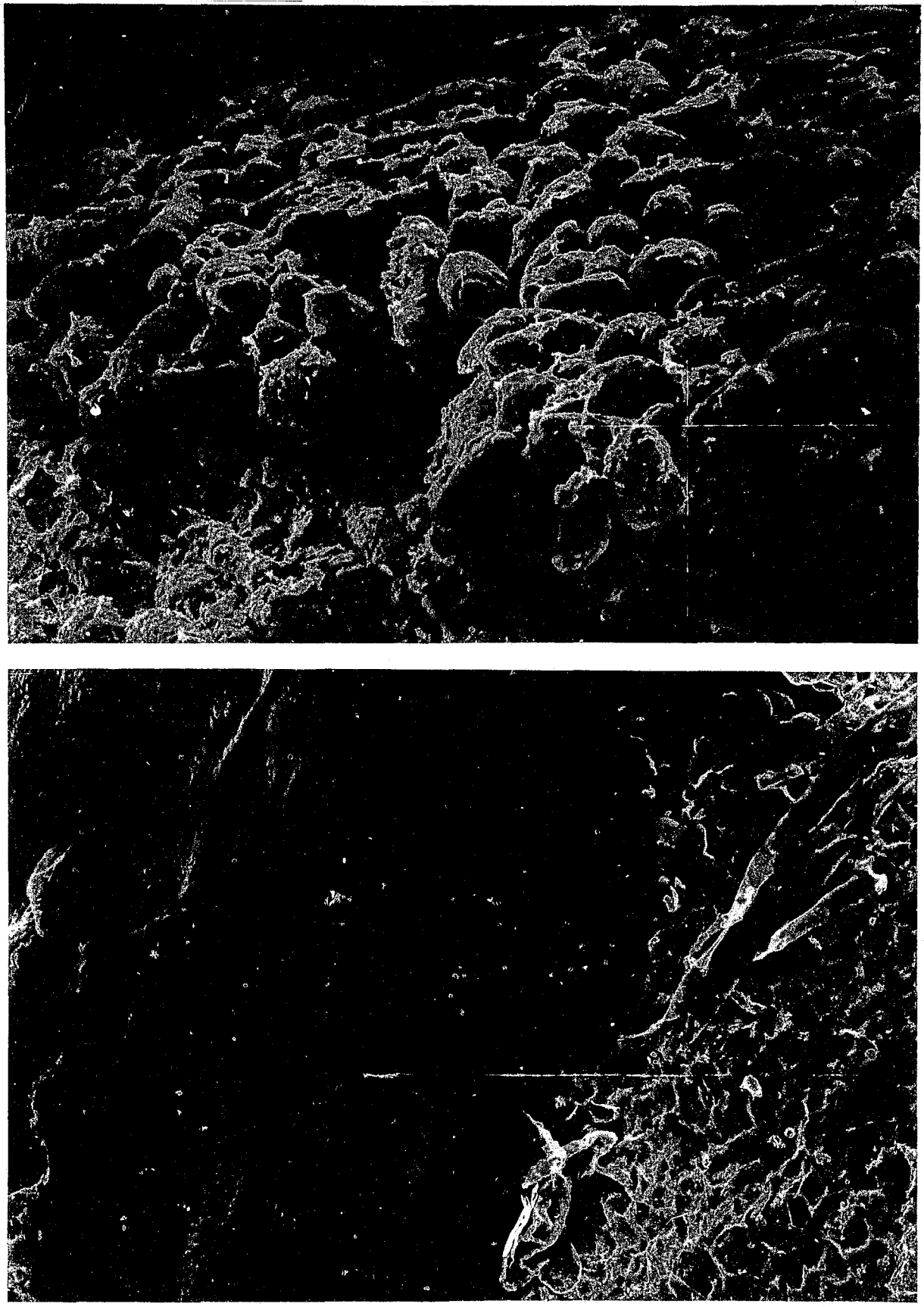


Figure 9.5 Bean. Pulp of garbanzo bean. Note the large, ellipsoid cells filled with starch grains. Also see fig. 8.10.

Figure 9.6 Bean. Seed coat of garbanzo bean. Note the difference between the outside (to the left) and the inside (to the right) of the seed coat. Also see fig. 8.7.

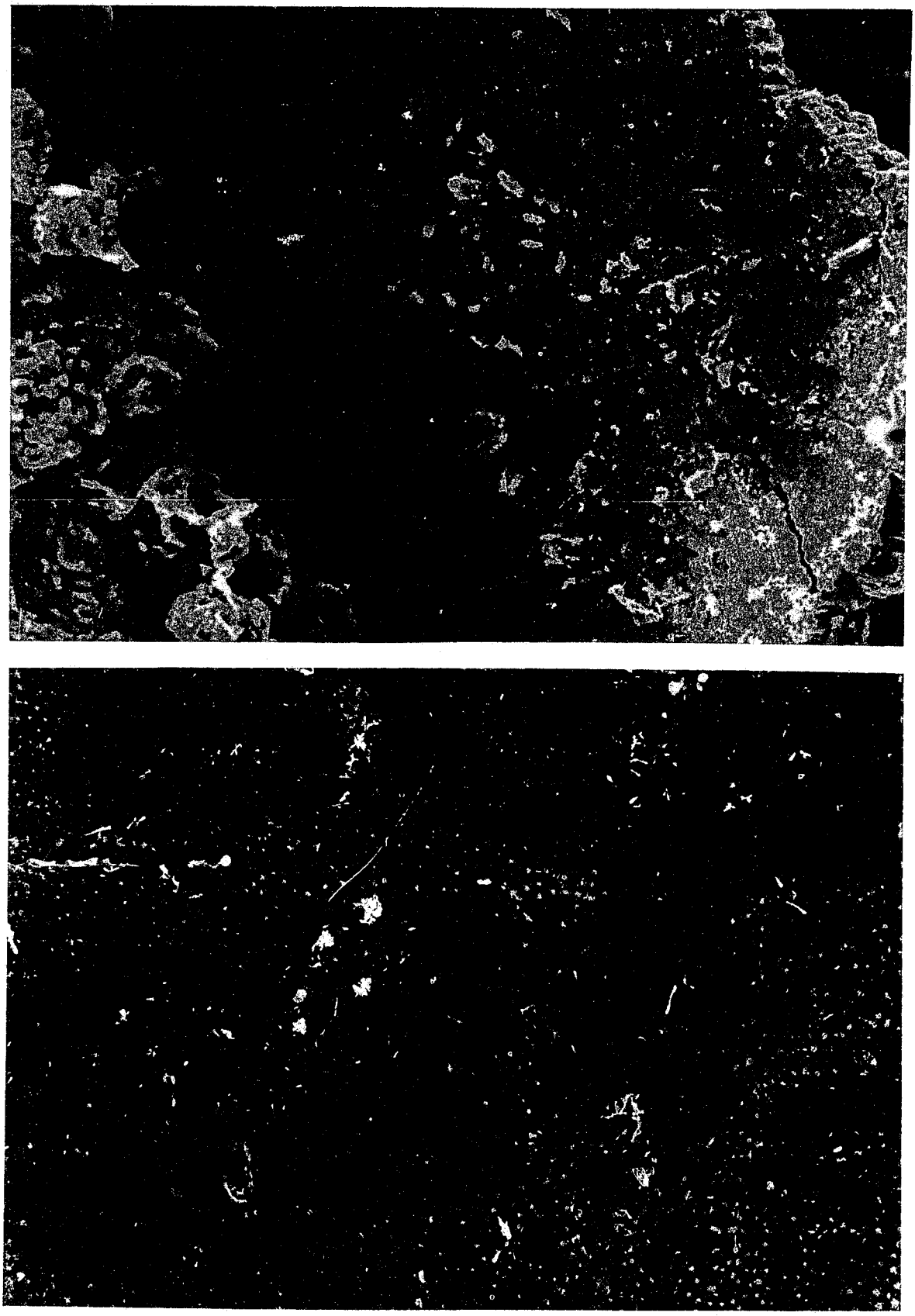


Figure 9.7 Bean. Seed coat (to the right) and pulp (to the left) of pinto bean. 8.13.

Figure 9.8 Bean. Outer seed coat of pea. Also see figs. 8.8 and 8.9.

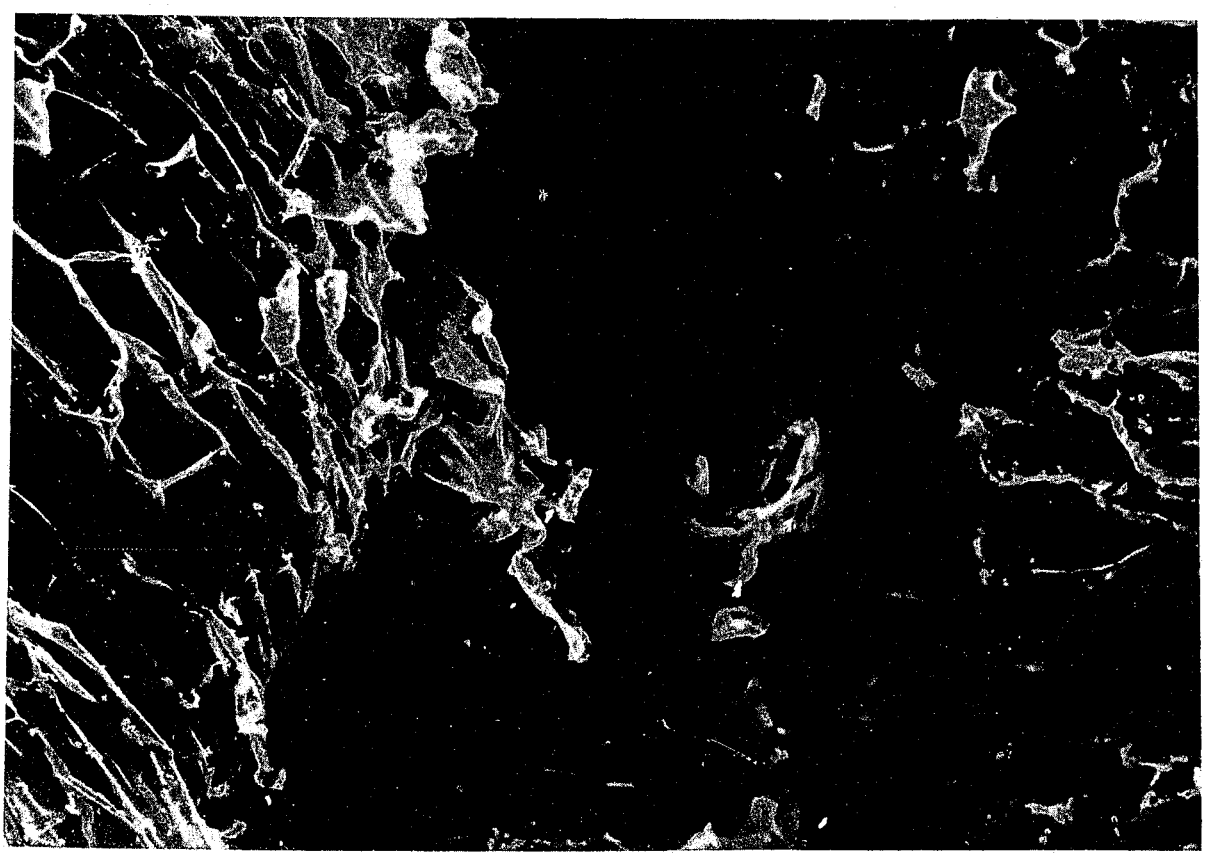
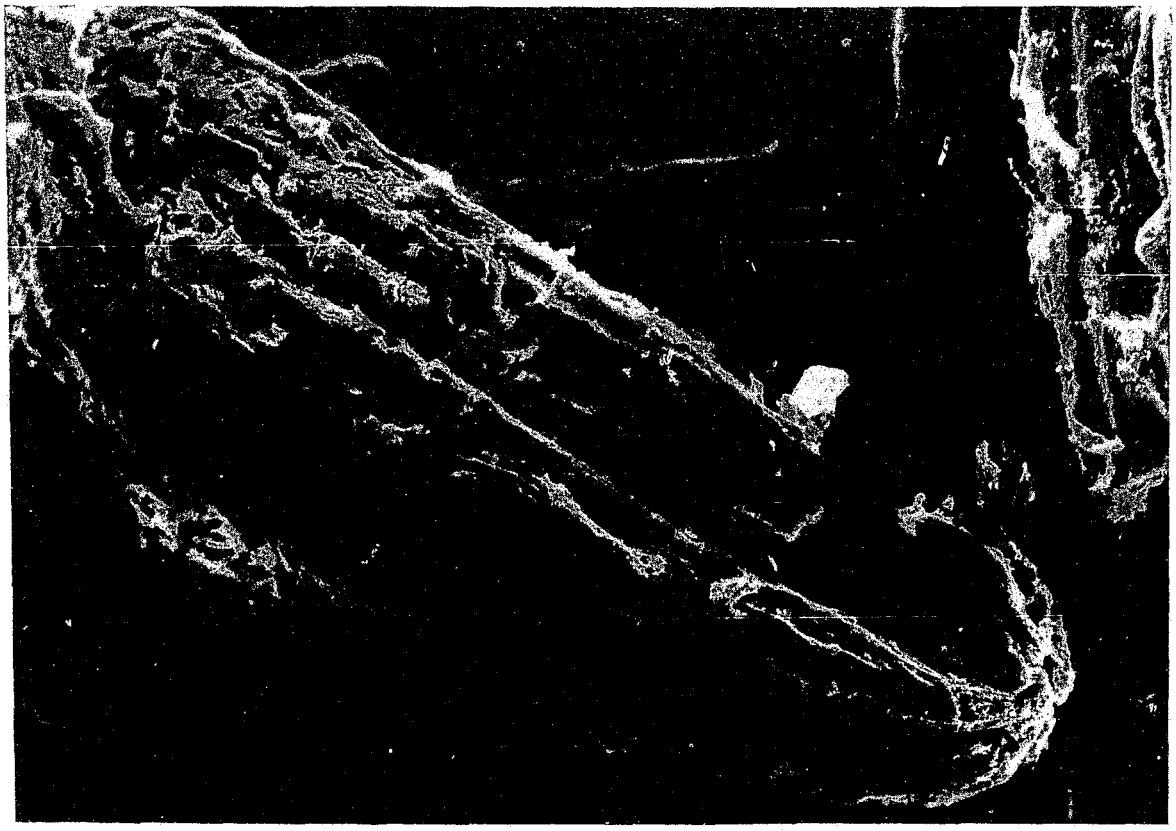


Figure 9.9 Beets. Note the predominant conducting cells. Also see fig. 8.14.

Figure 9.10 Cabbage. Note the elongate, smooth-walled epidermal cells (upper left) and the more rounded interior cells (lower right). Also see figs. 8.15 and 8.16.



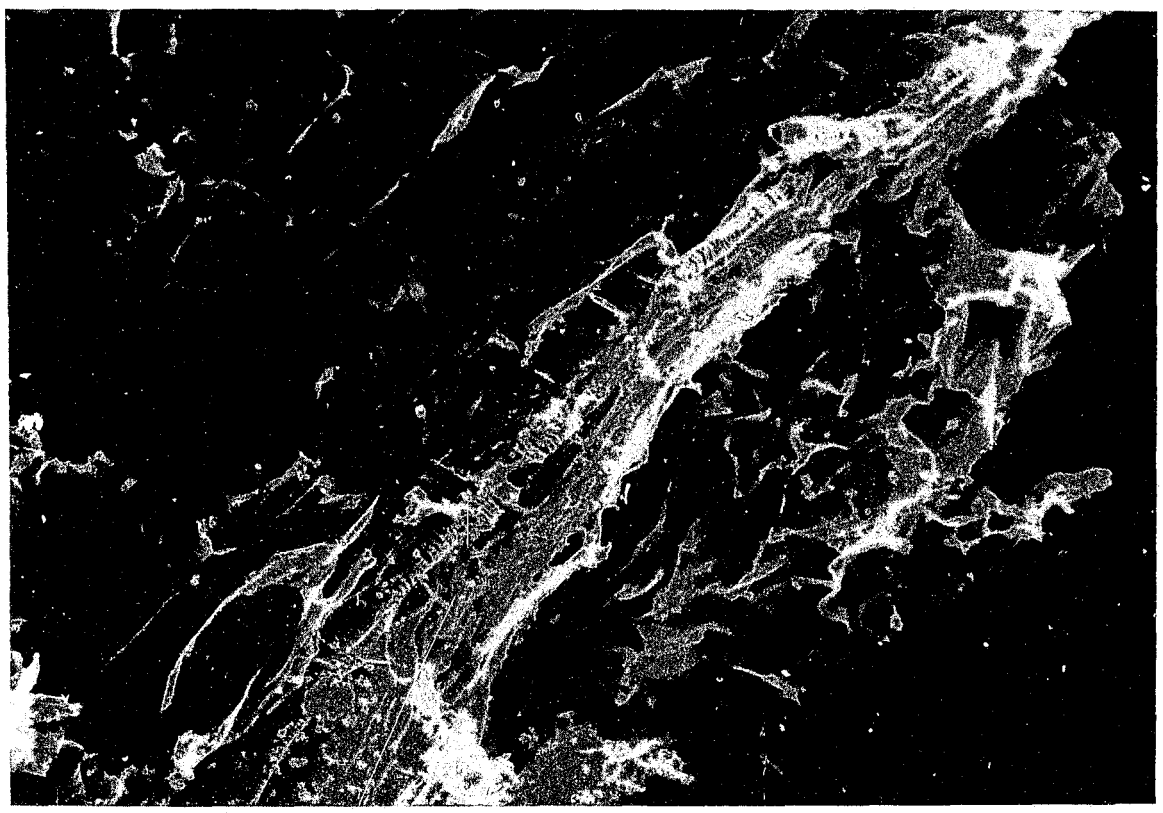


Figure 9.11 Caraway seed (X60 taken at 30X). Also see fig. 8.17.

Figure 9.12 Carrot pulp. Cells arranged in brick-like fashion. Also see fig. 8.18.

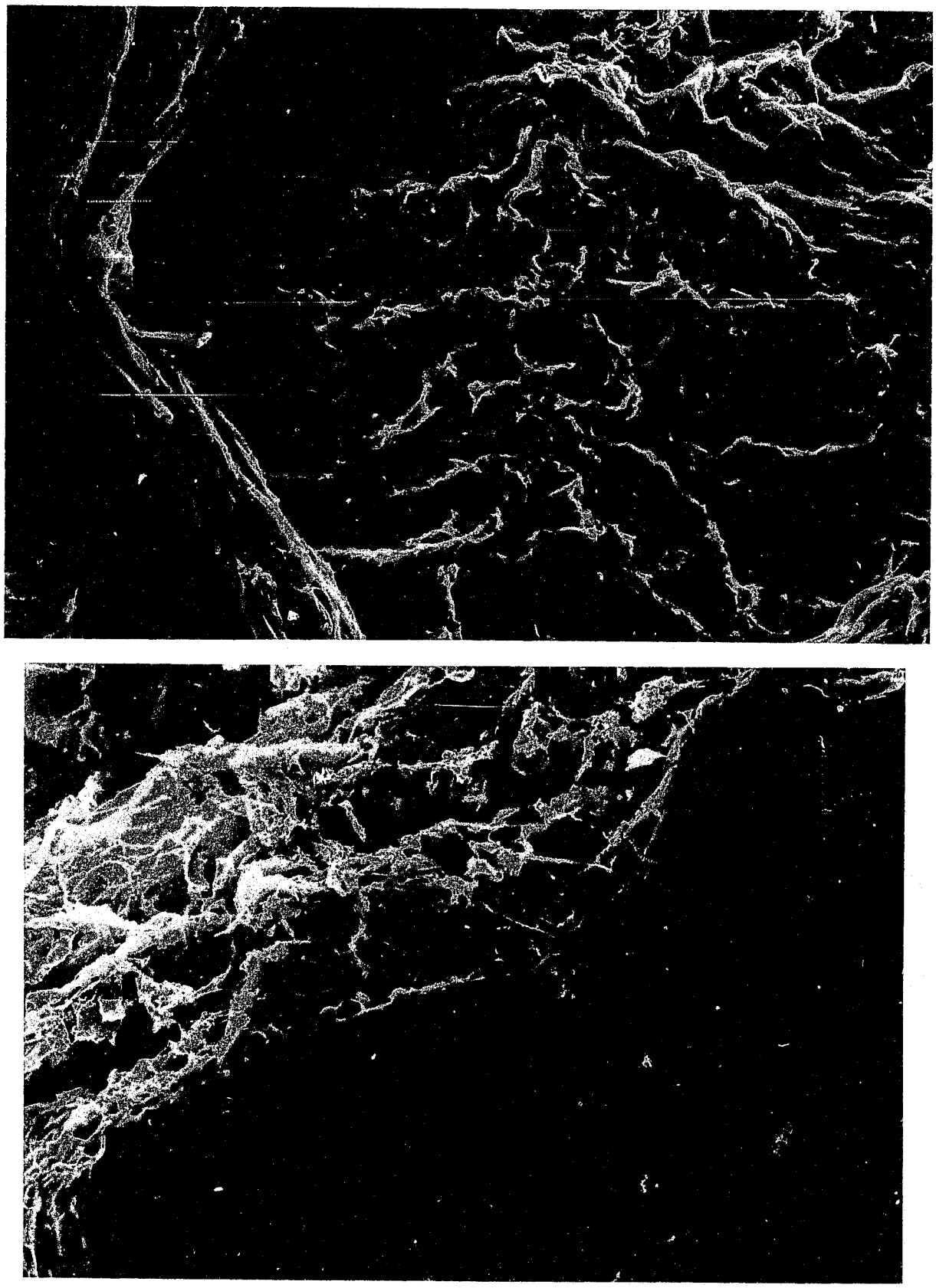
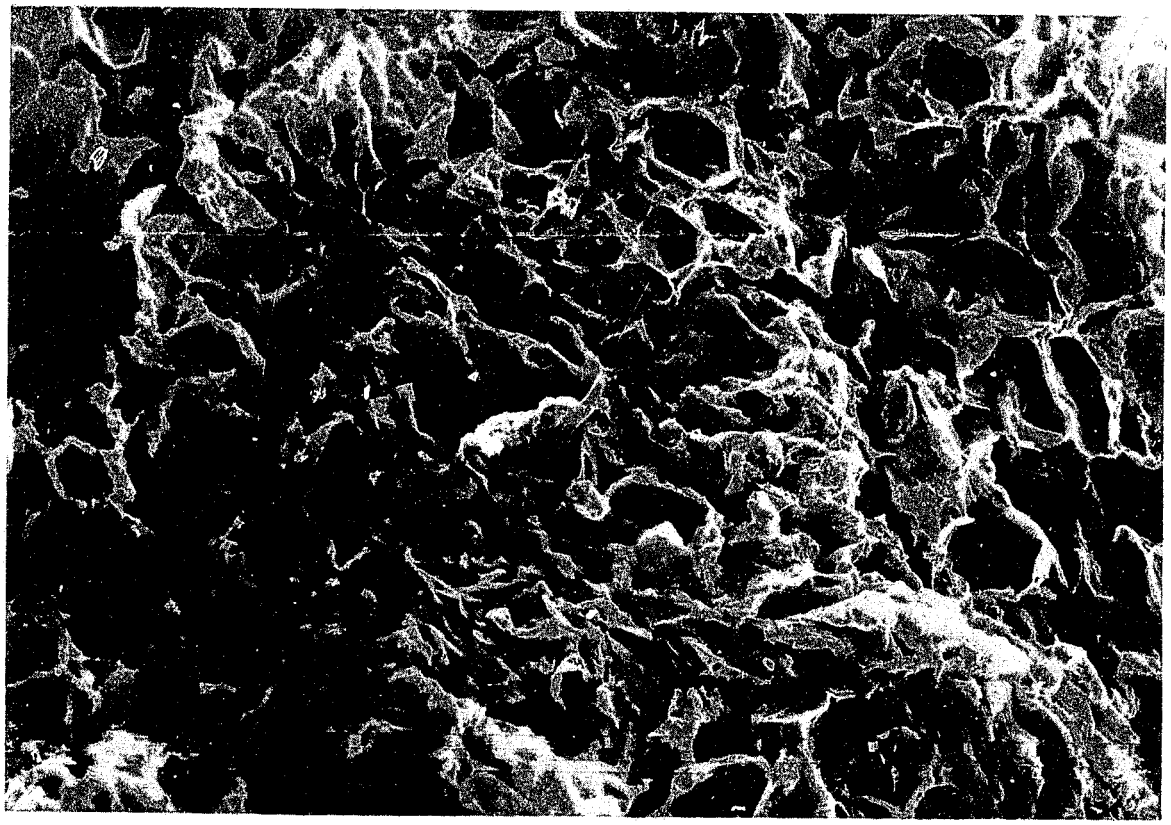


Figure 9.13 Celery. Note celery "string" (at left) and pulp. Also see fig. 8.19.

Figure 9.14 Cherry skin (lower right) and pulp (upper left). Note the thick-walled cells of the skin. Also see figs. 8.21 and 8.22.



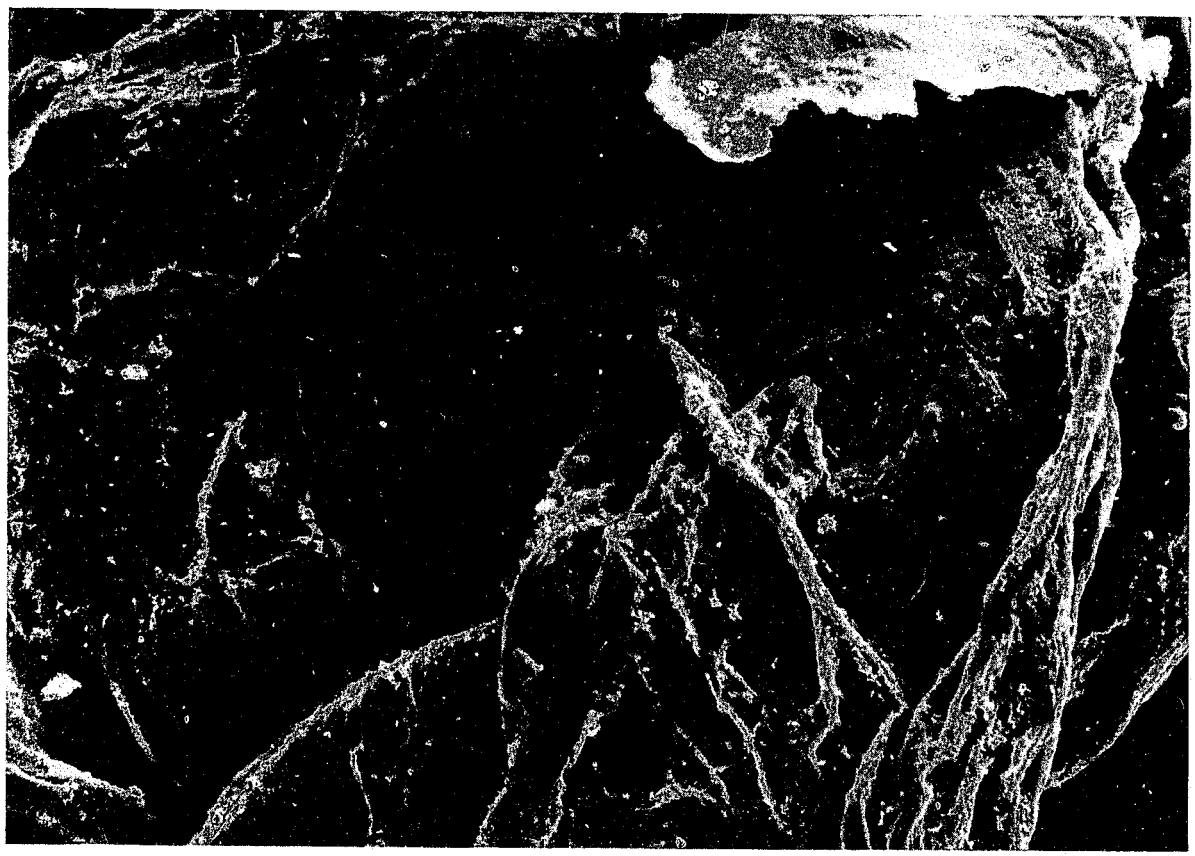
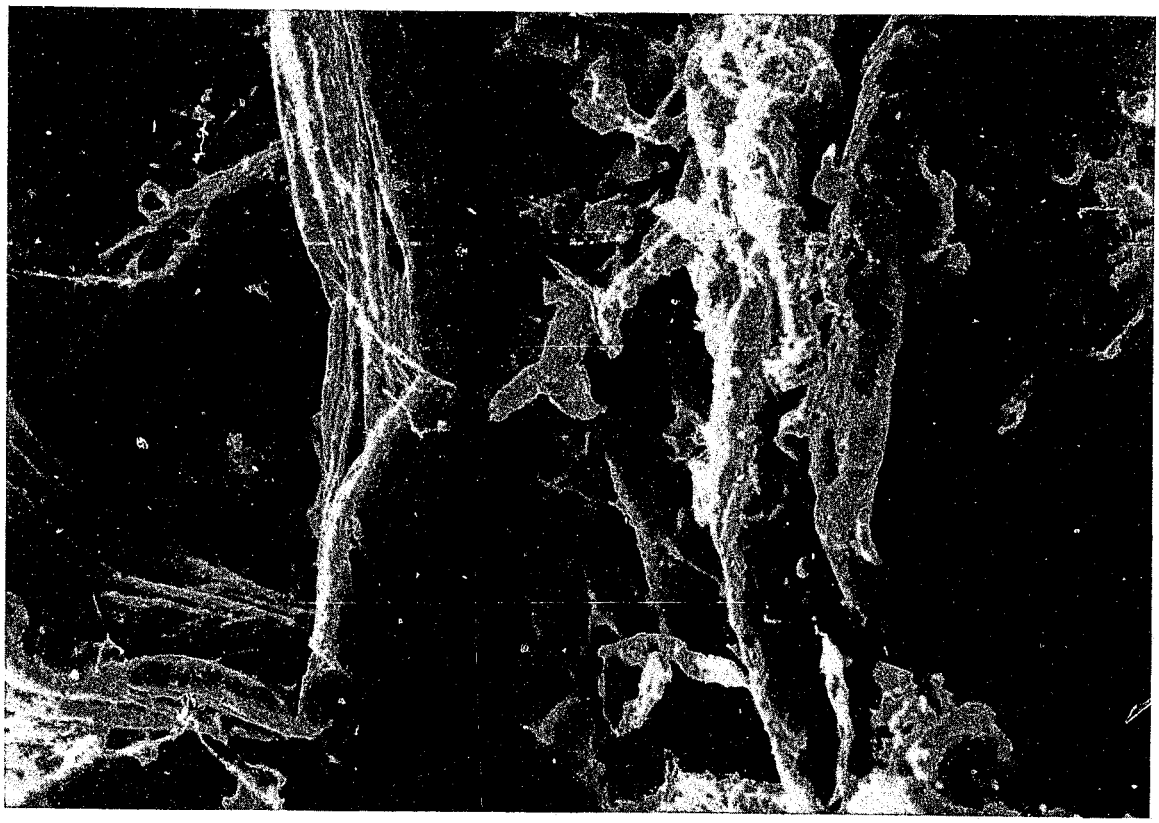


Figure 9.15 Citrus (lemon) pulp. Also see fig. 8.24.

Figure 9.16 Citrus (lime) section membrane. Also see fig. 8.25.



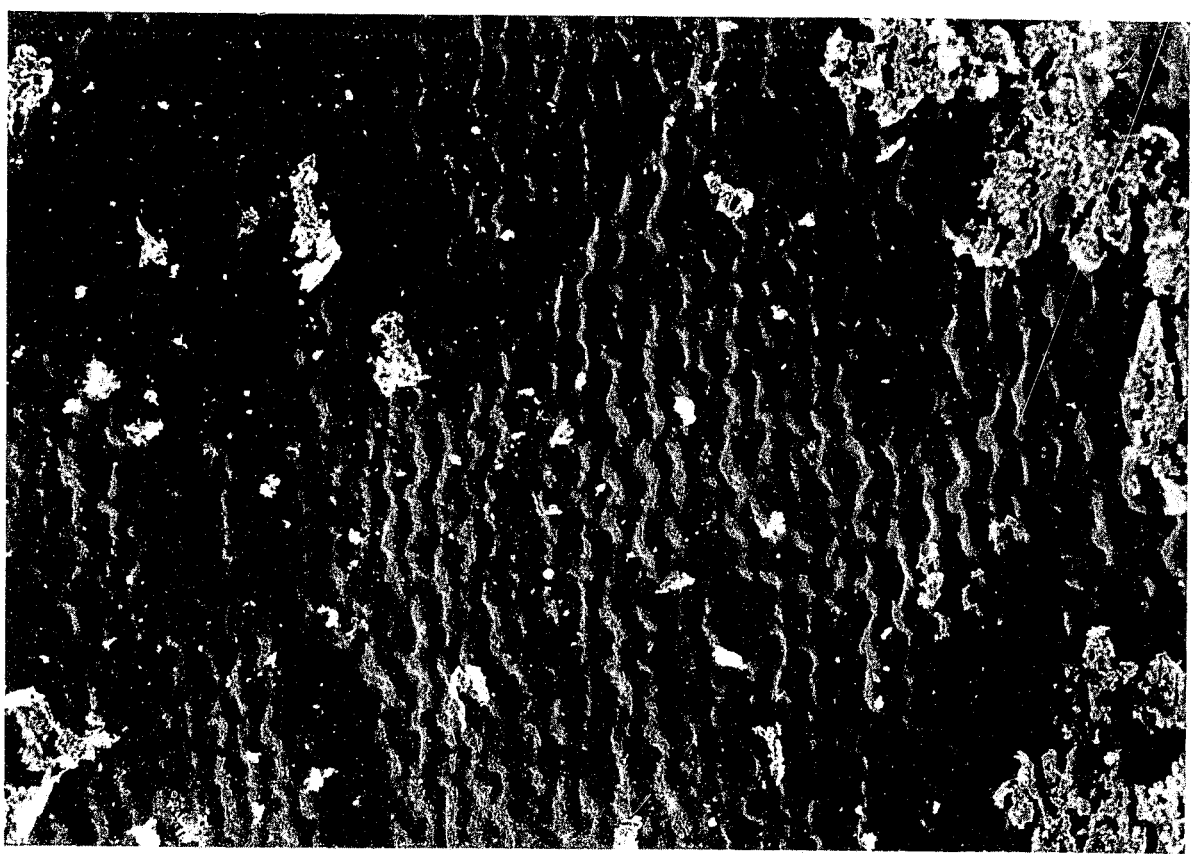
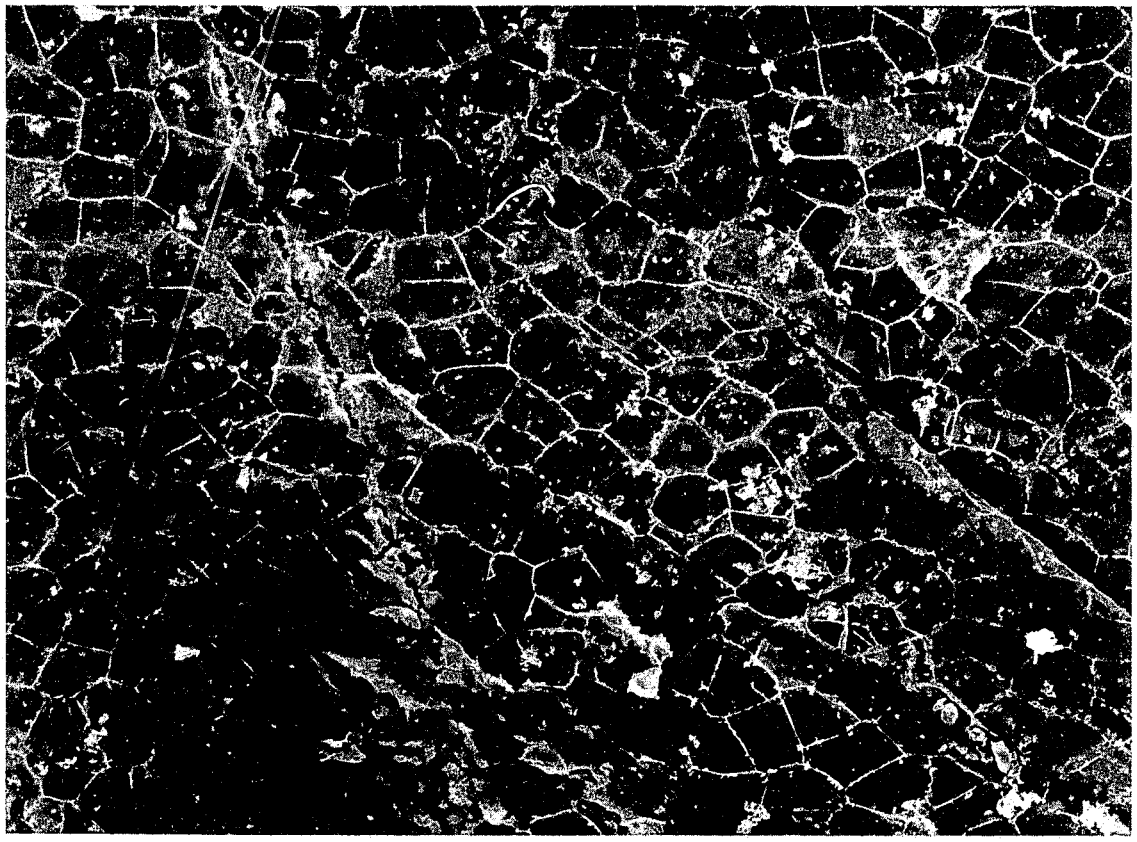


Figure 9.17 Citrus (orange) section membrane with pulp. Also see figs. 8.24 and 8.25.

Figure 9.18 Corn seed coat (outer).



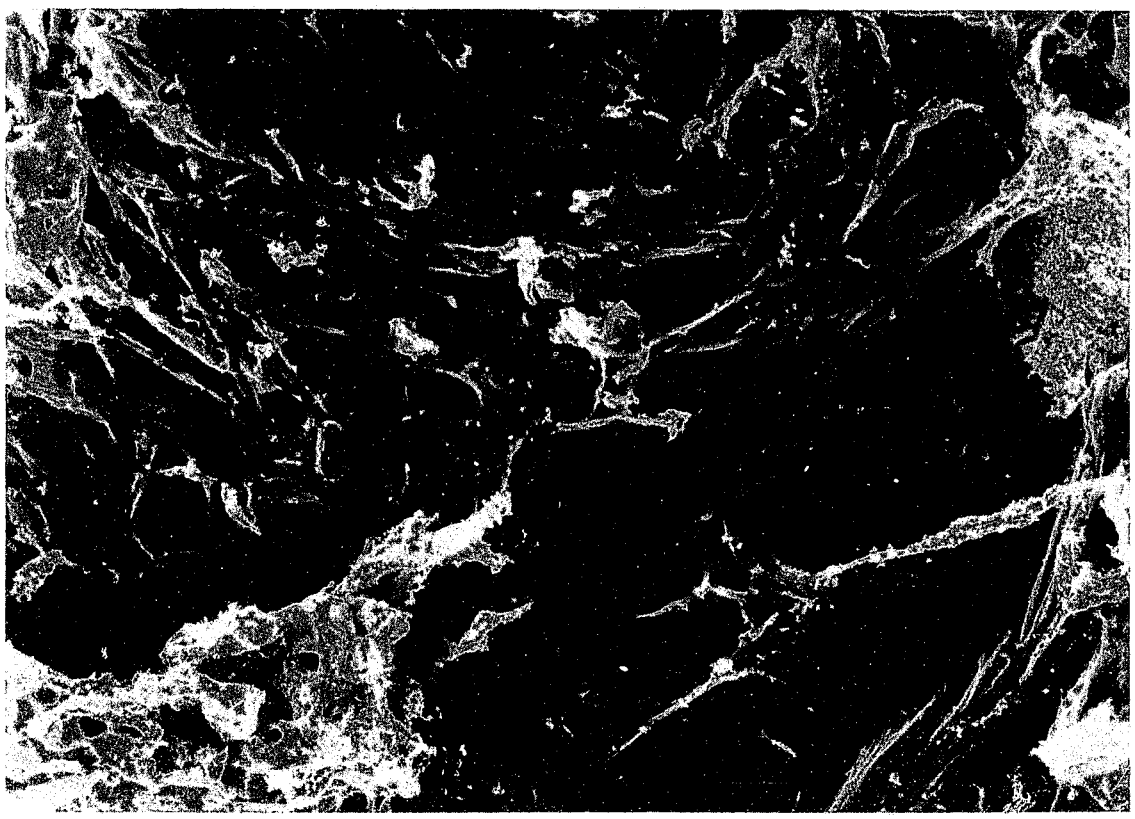


Figure 9.19 Corn seed coat (inner). Also see fig. 8.26.

Figure 9.20 Cucumber pulp. Note fragility of cell walls.

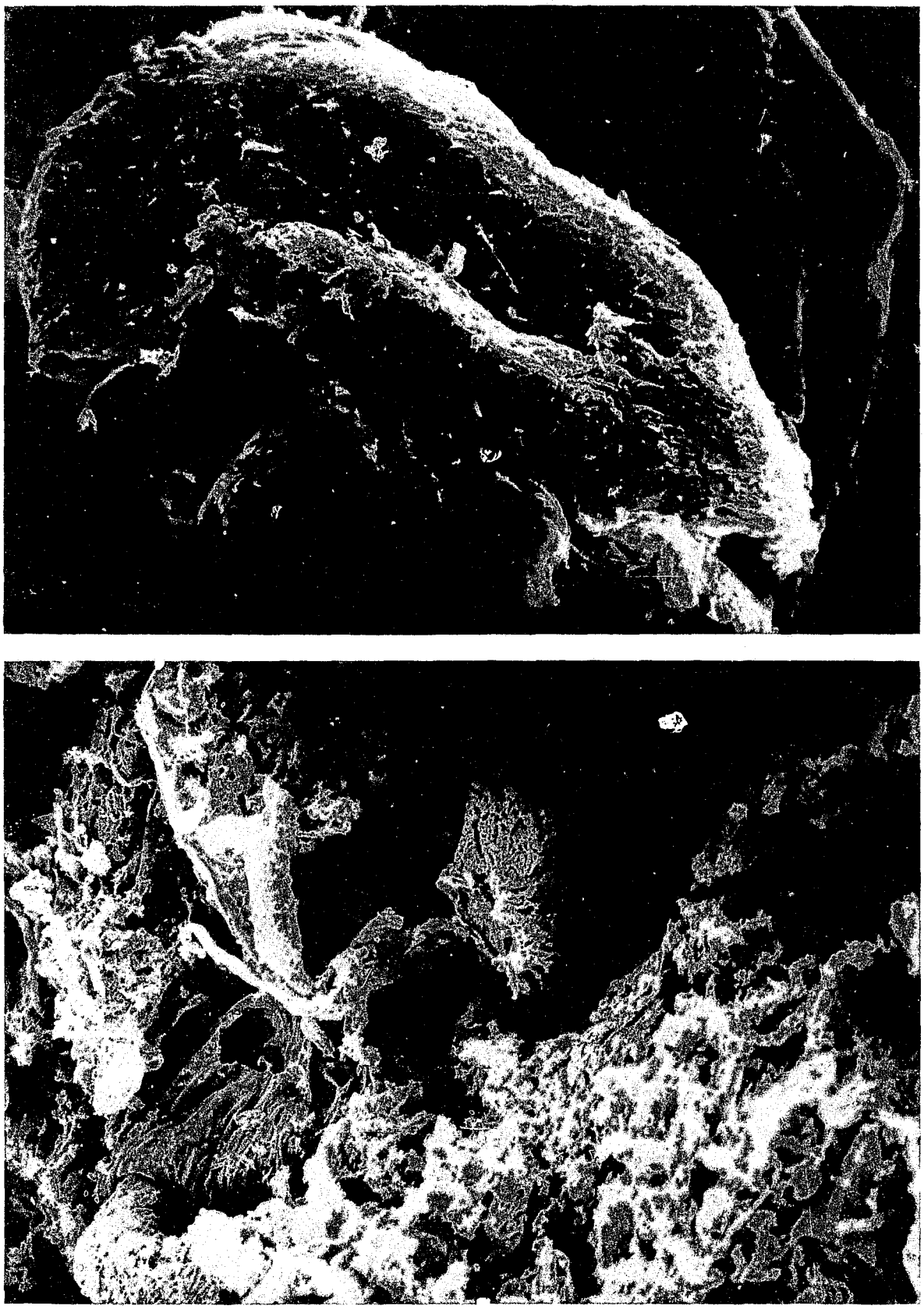


Figure 9.21 Cucumber seed (X60 taken at 30X).

Figure 9.22 Fig pulp. Also see figs. 8.29 and 8.30.

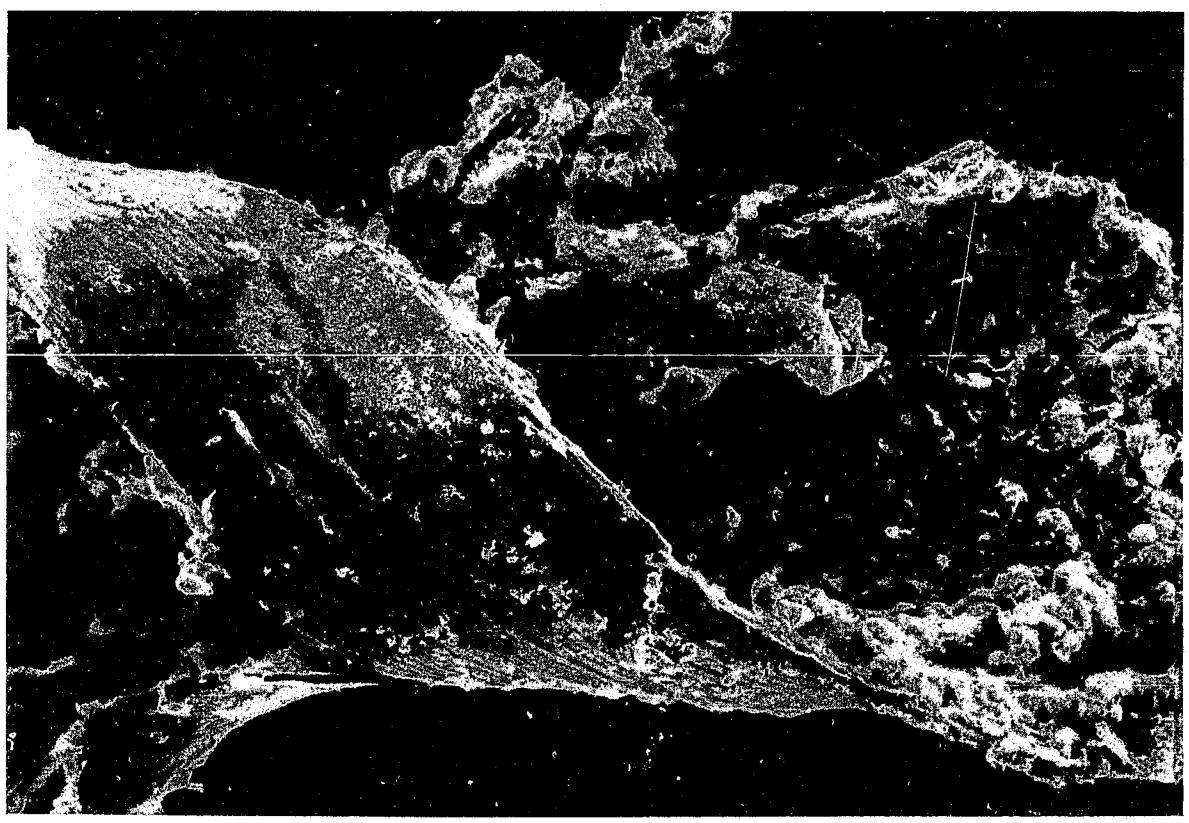


Figure 9.23 Fig seed (X120 taken at 60X).

Figure 9.24 Garlic epidermis. Outside (on left) and inside (to right) are botn visible. Also see figs.5.31 and 8.32.

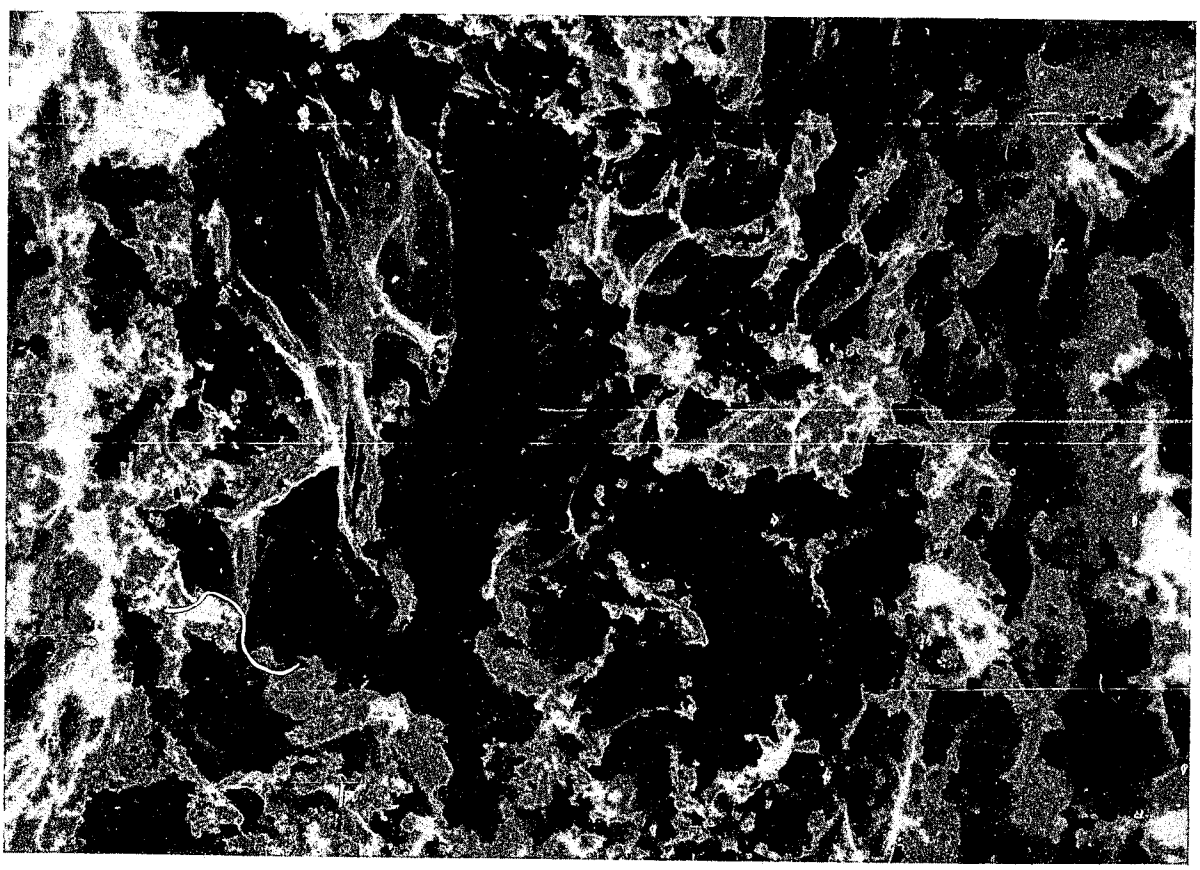


Figure 9.25 Grape or raisin epidermis (outer). Also see fig. 8.33.

Figure 9.26 Grape or raisin pulp. Also see fig. 8.34.

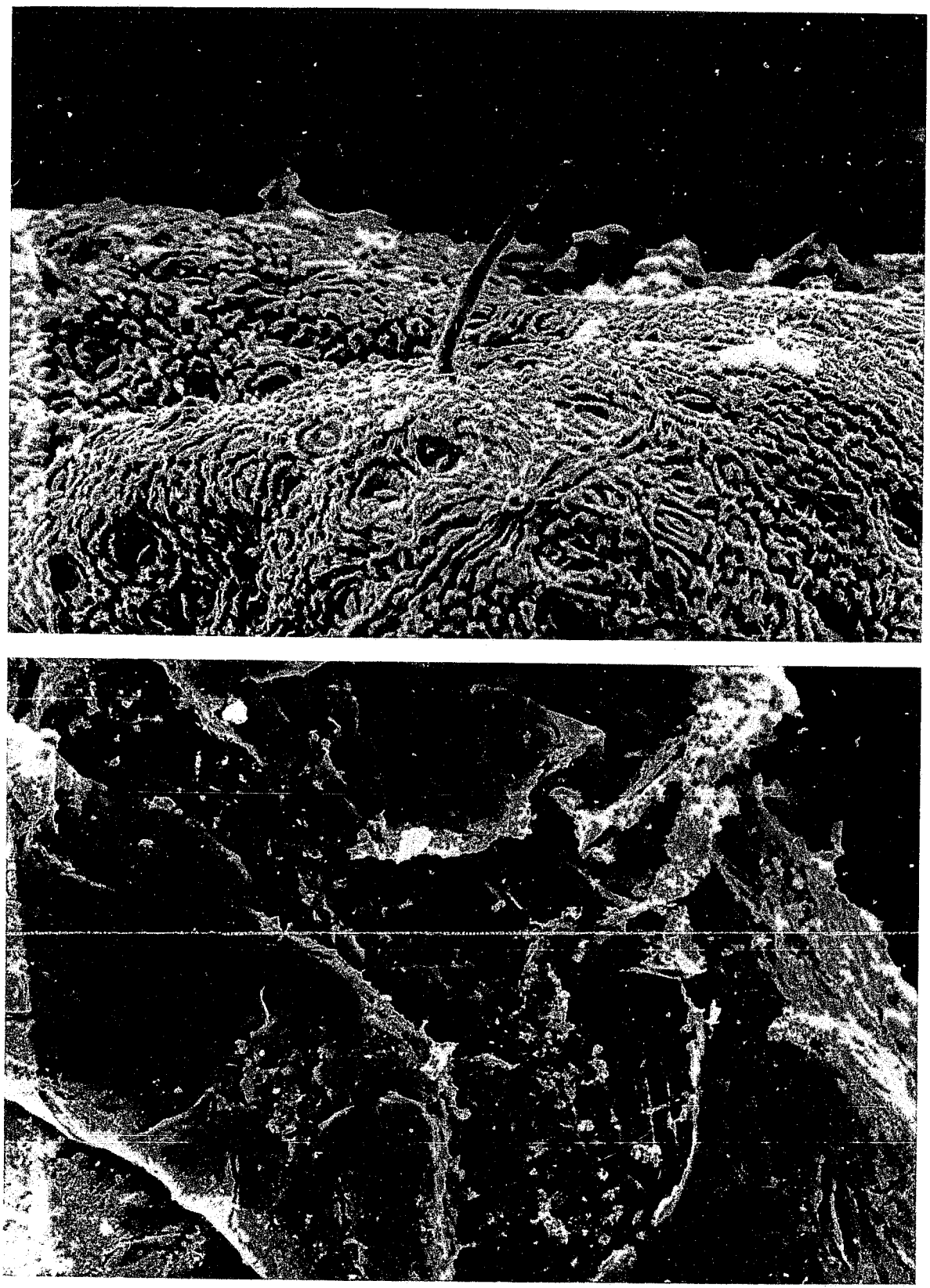


Figure 9.27 Green bean pod epidermis. Also see fig. 8.35.

Figure 9.28 Kiwi pulp. Also see fig. 8.37.

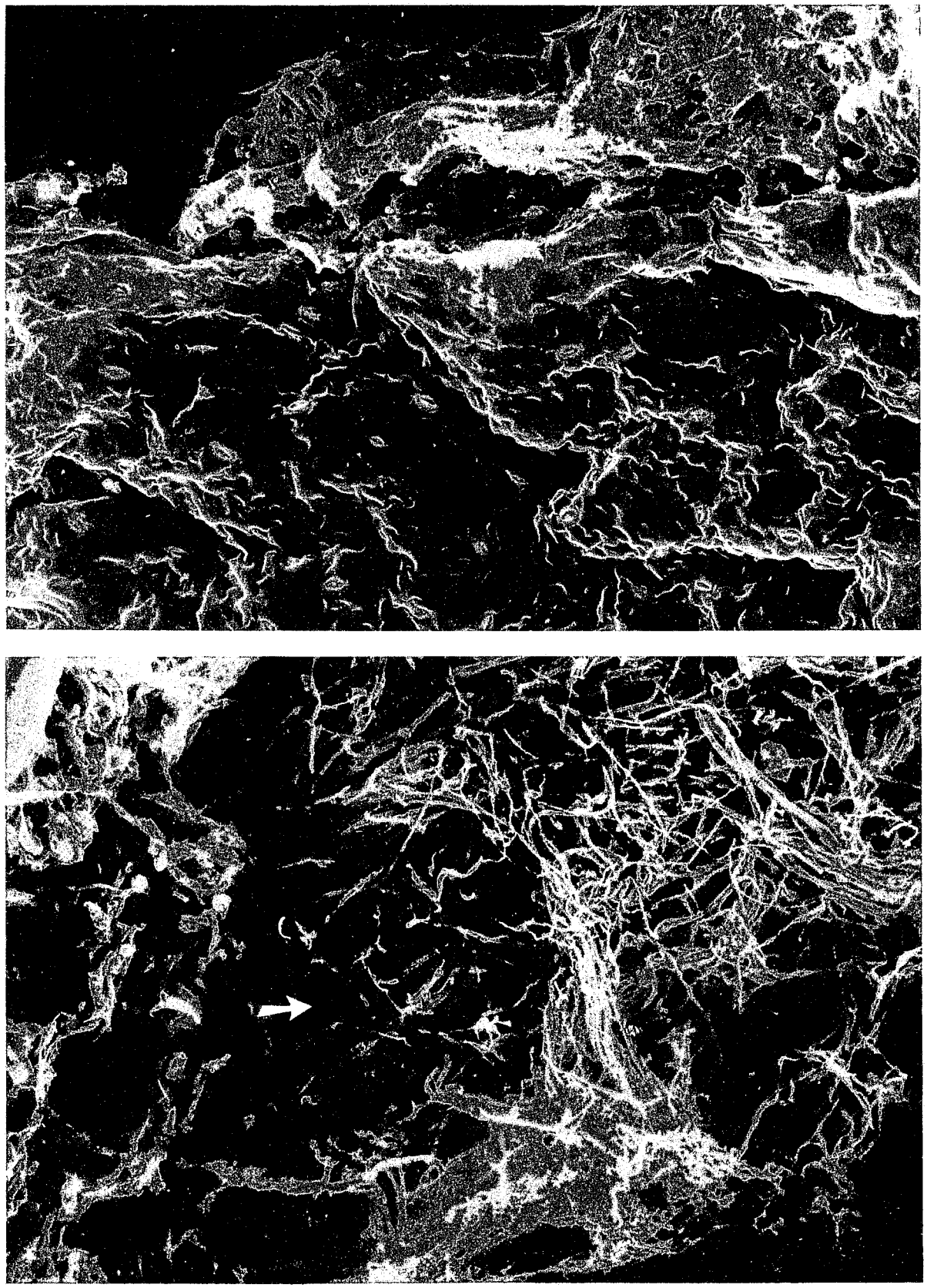
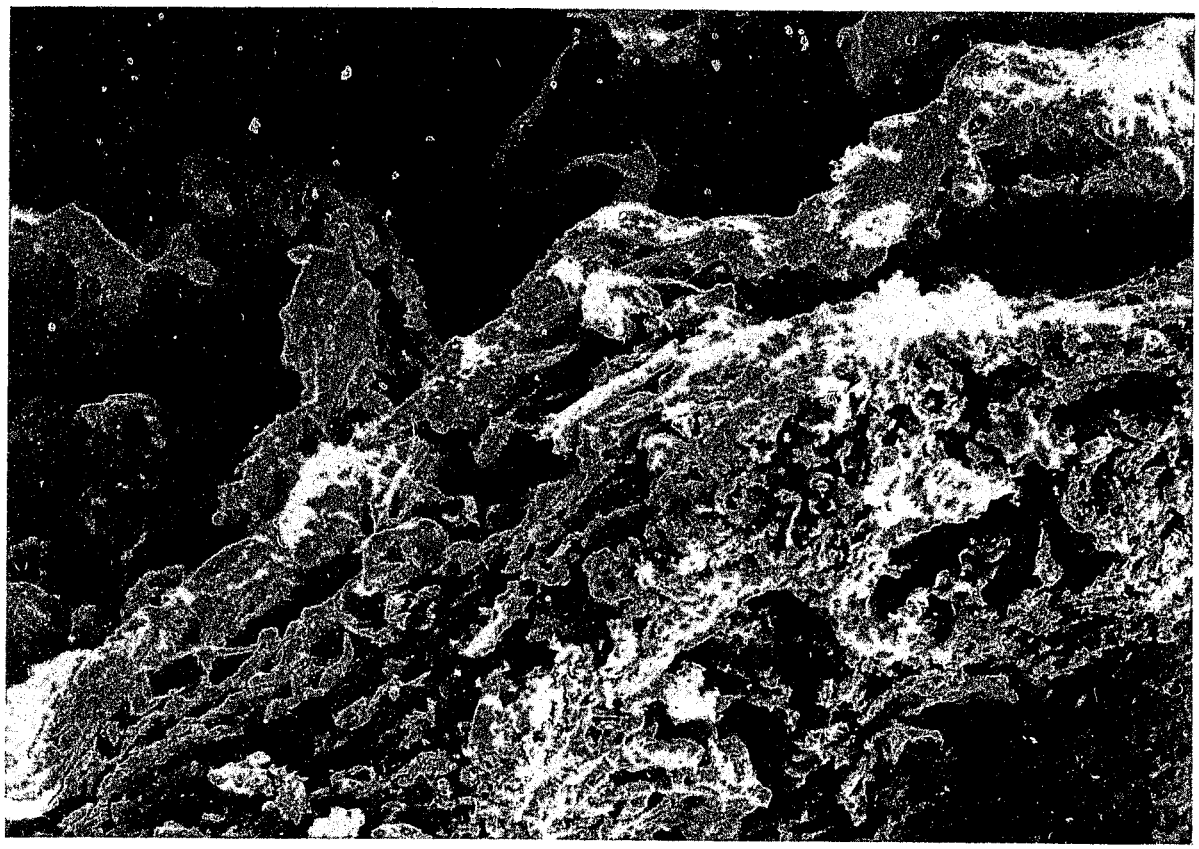


Figure 9.29 Lettuce lower epidermis (bottom) with some interior cells (top). Also see fig. 8.39.

Figure 9.30 Mushroom hyphae with spore (arrow). Also see fig. 8.40.



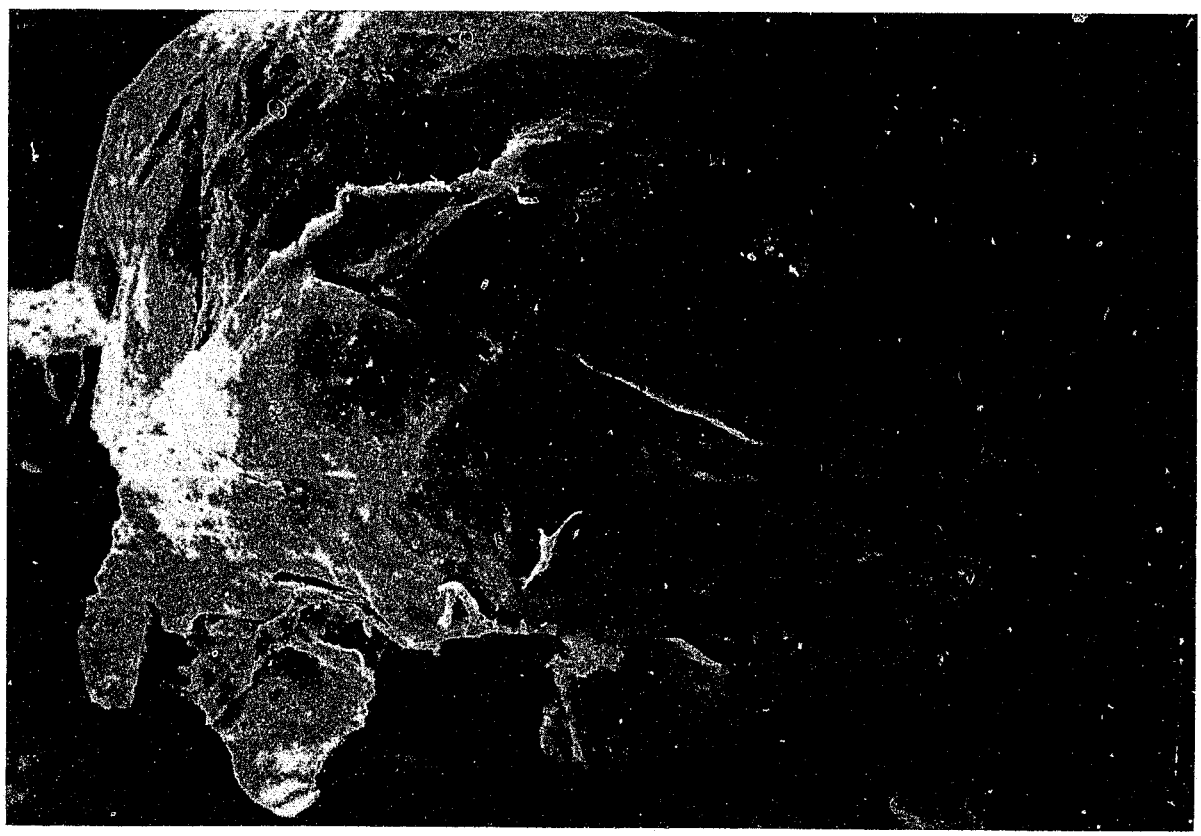
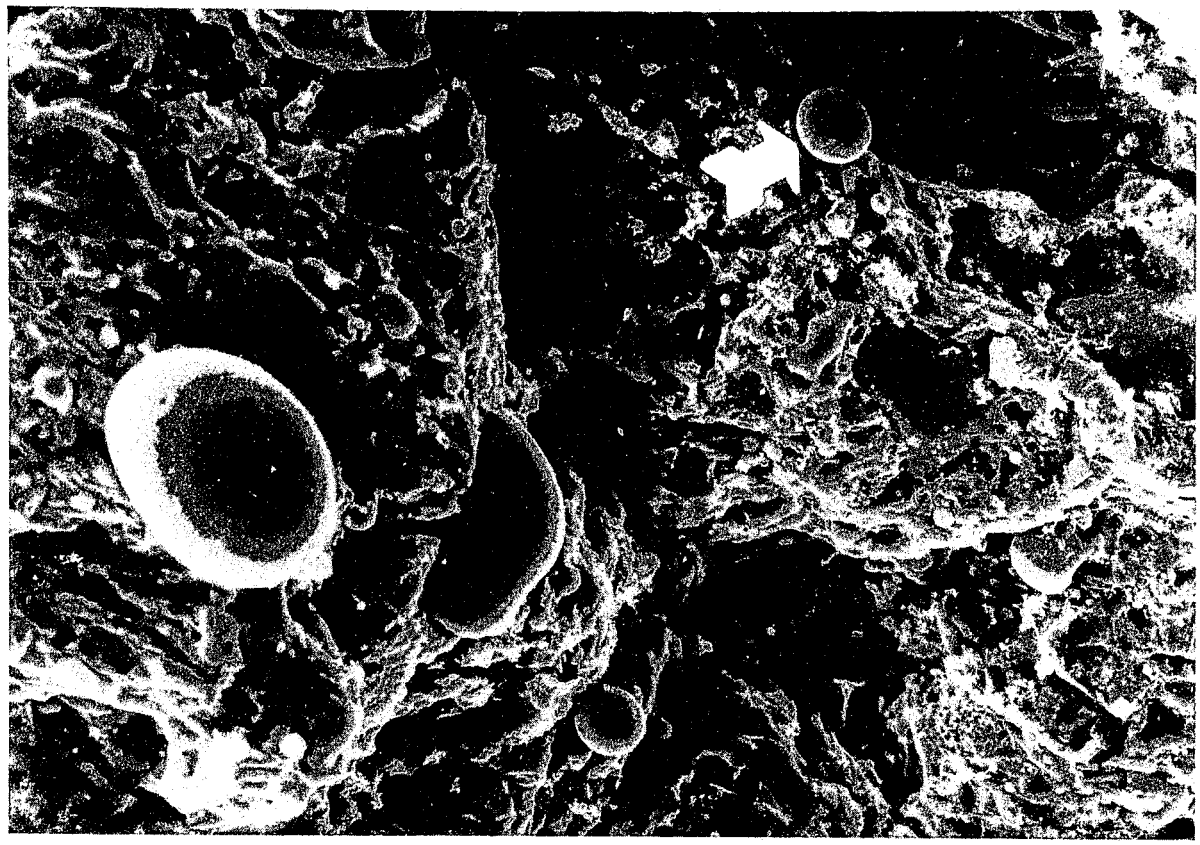


Figure 9.31 Okra pulp. Also see figs. 8.43 and 8.44.

Figure 9.32 Okra seed (X60 taken at 30X).



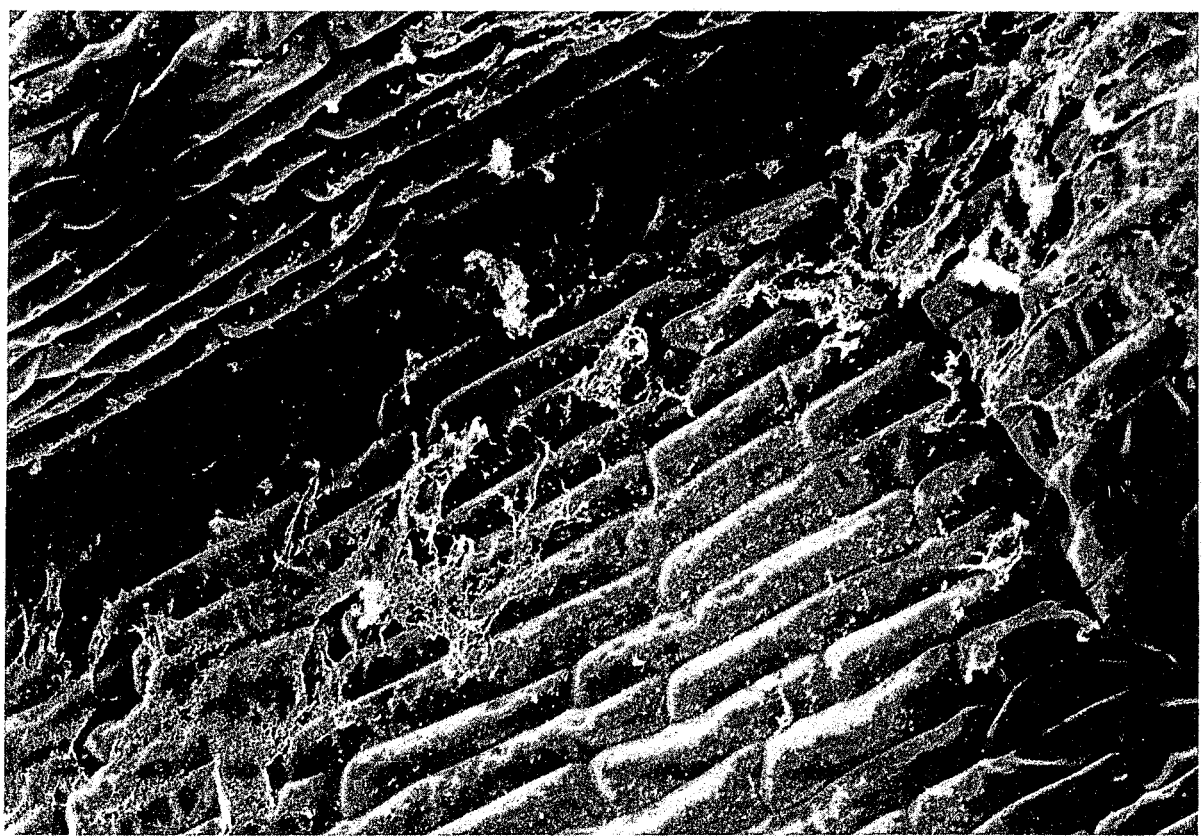
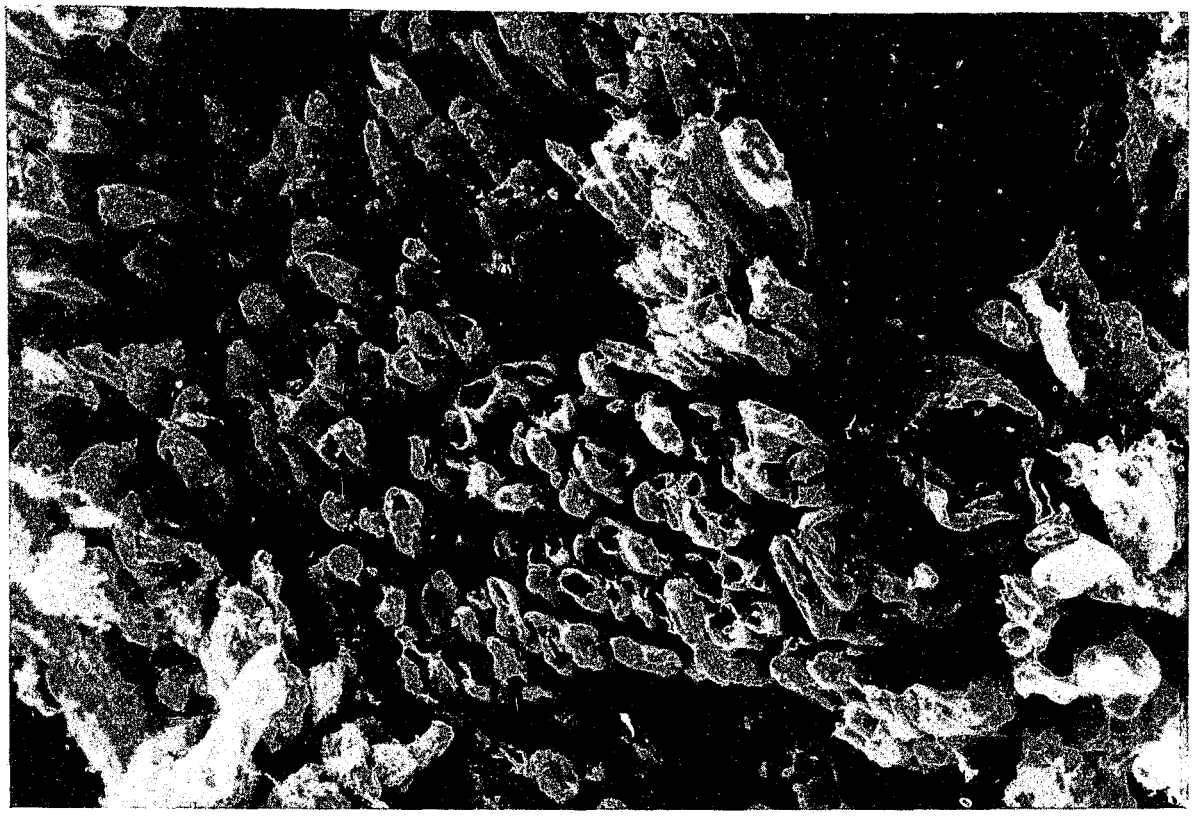


Figure 9.33 Olive. Note the large droplets of fat (arrow). Also see fig. 8.46.

Figure 9.34 Onion epidermis. Note the very regular, elongate cells. Compare to garlic epidermis (fig. 9.24). Also see figs. 8.47, 8.48, and 8.49.



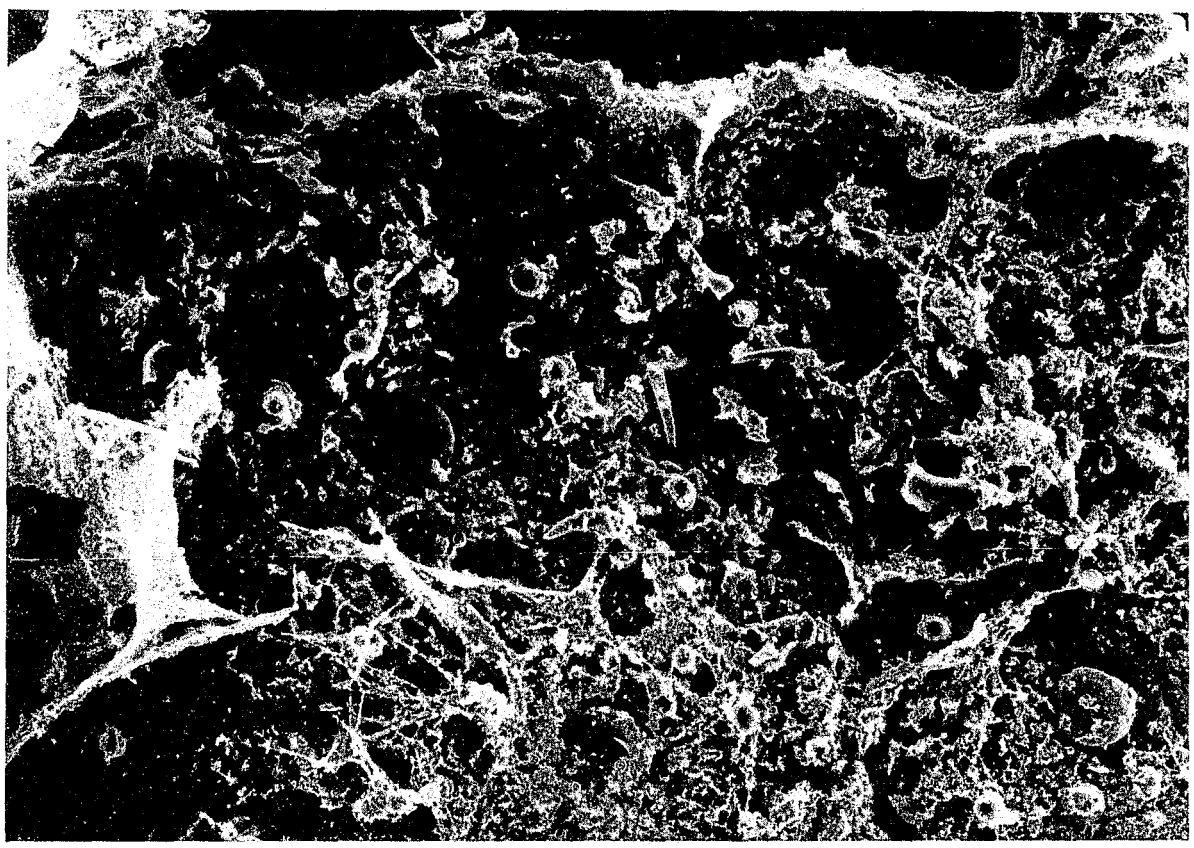
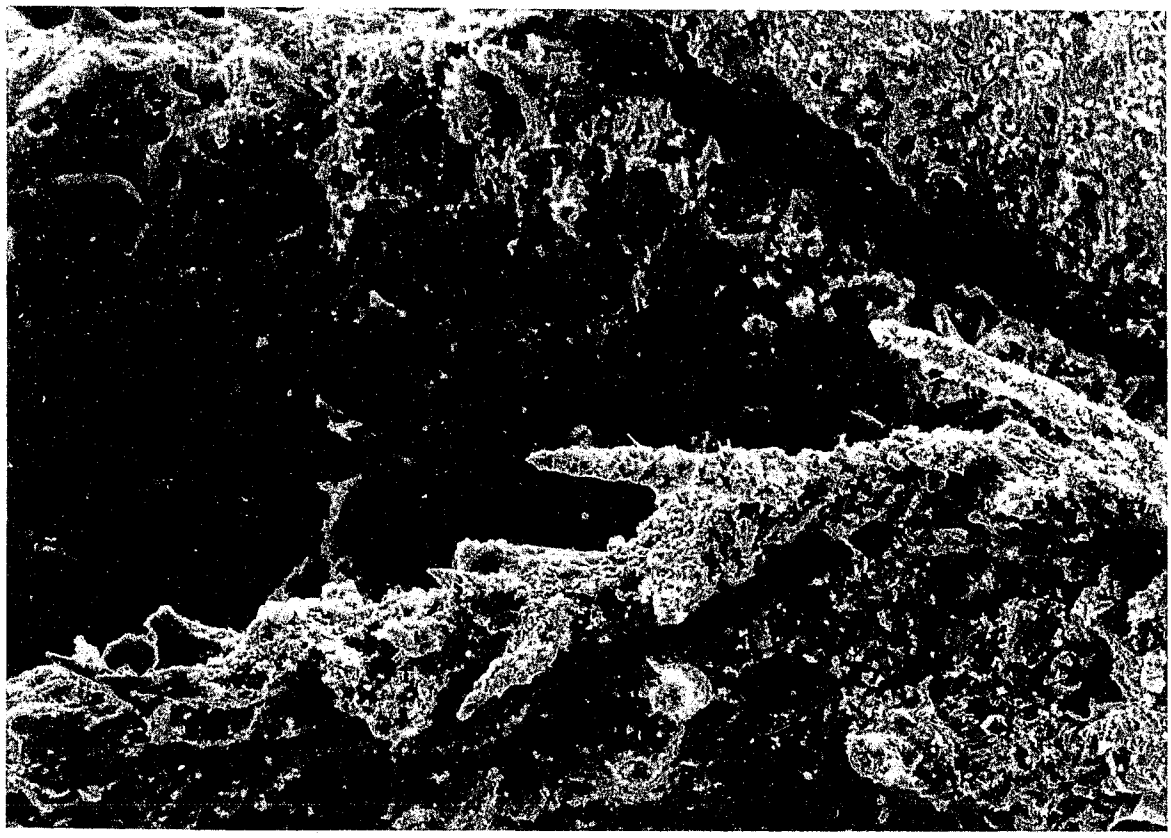


Figure 9.35 Onion pulp. Note tubular and spherical cells.

Figure 9.36 Oregano epidermis after chewing but with no artificial digestion. Note the unbranched hairs and large glands. Also see figs. 8.50 and 8.51.



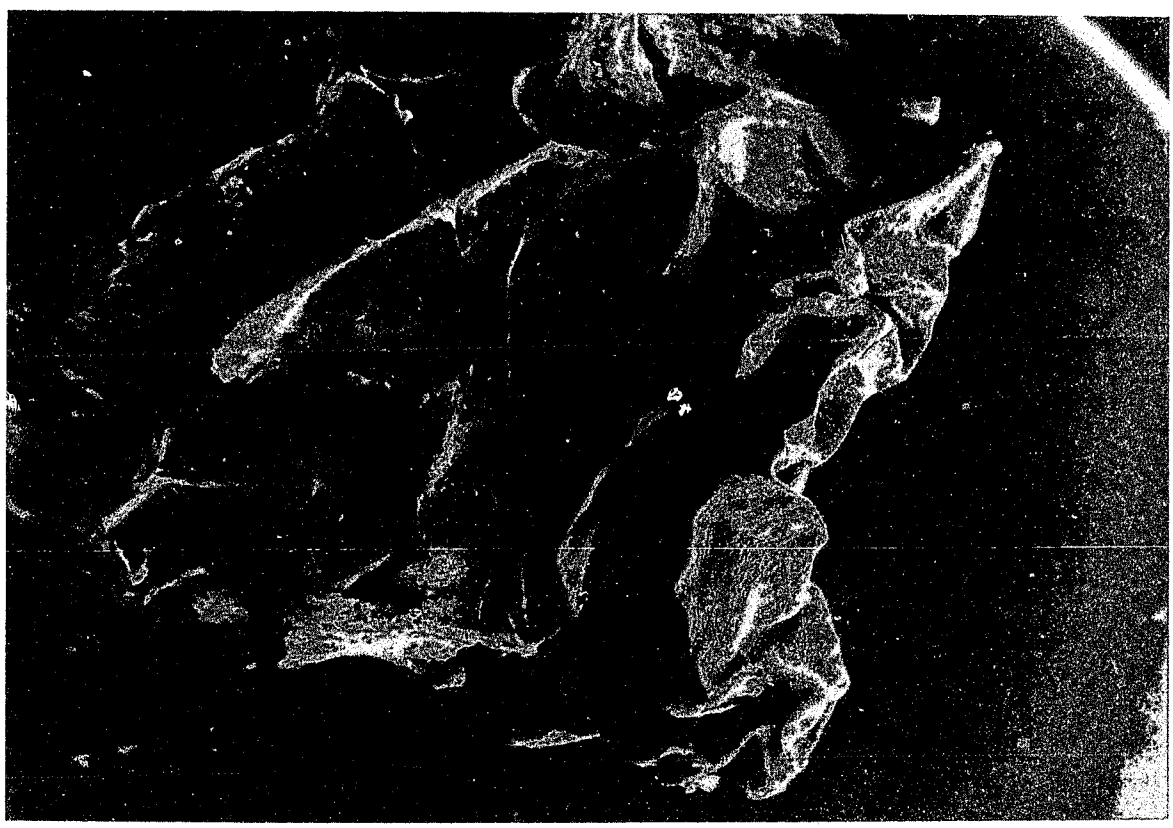
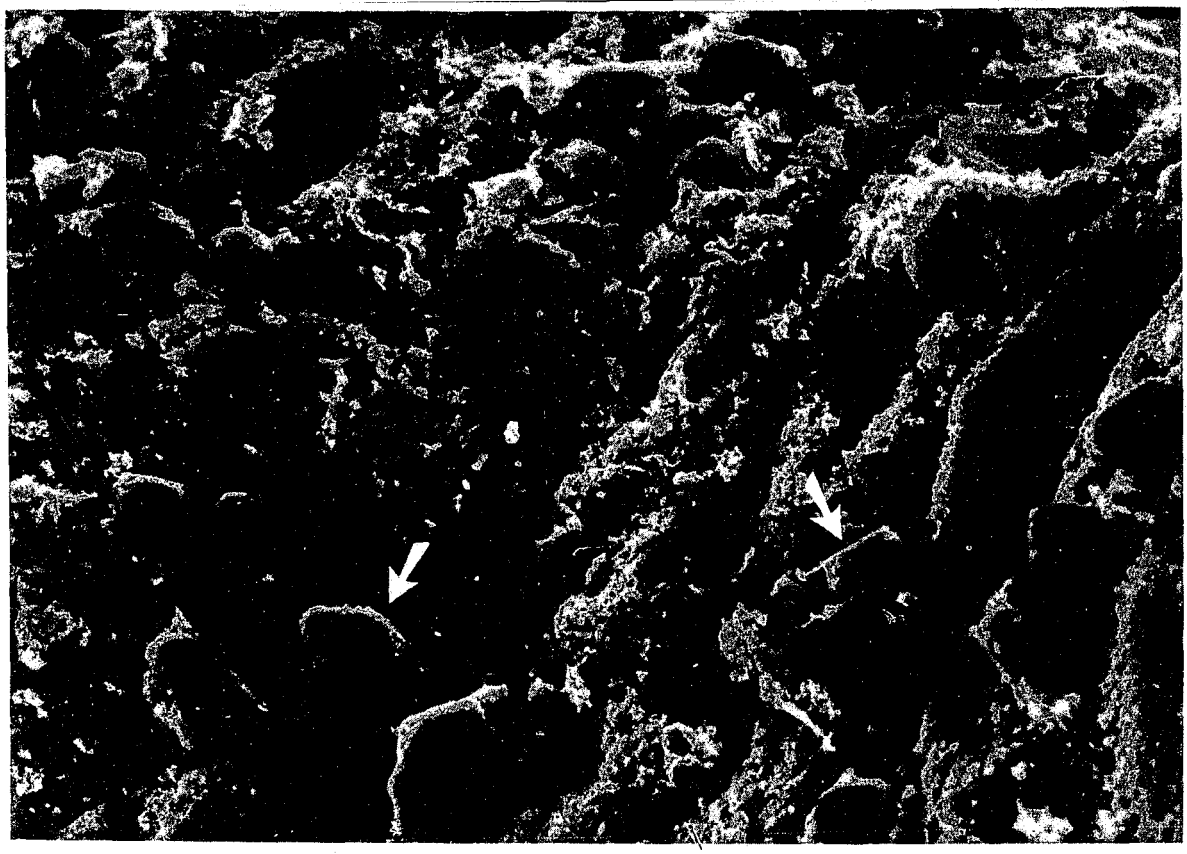


Figure 9.37 Oregano epidermis after chewing and 6 hours of artificial digestion. Note the unbranched hairs, large glands, and absence of as many disrupted cells and other material as in Figure 9.36.

Figure 9.38 Papaya seed (X40 taken at 20X).



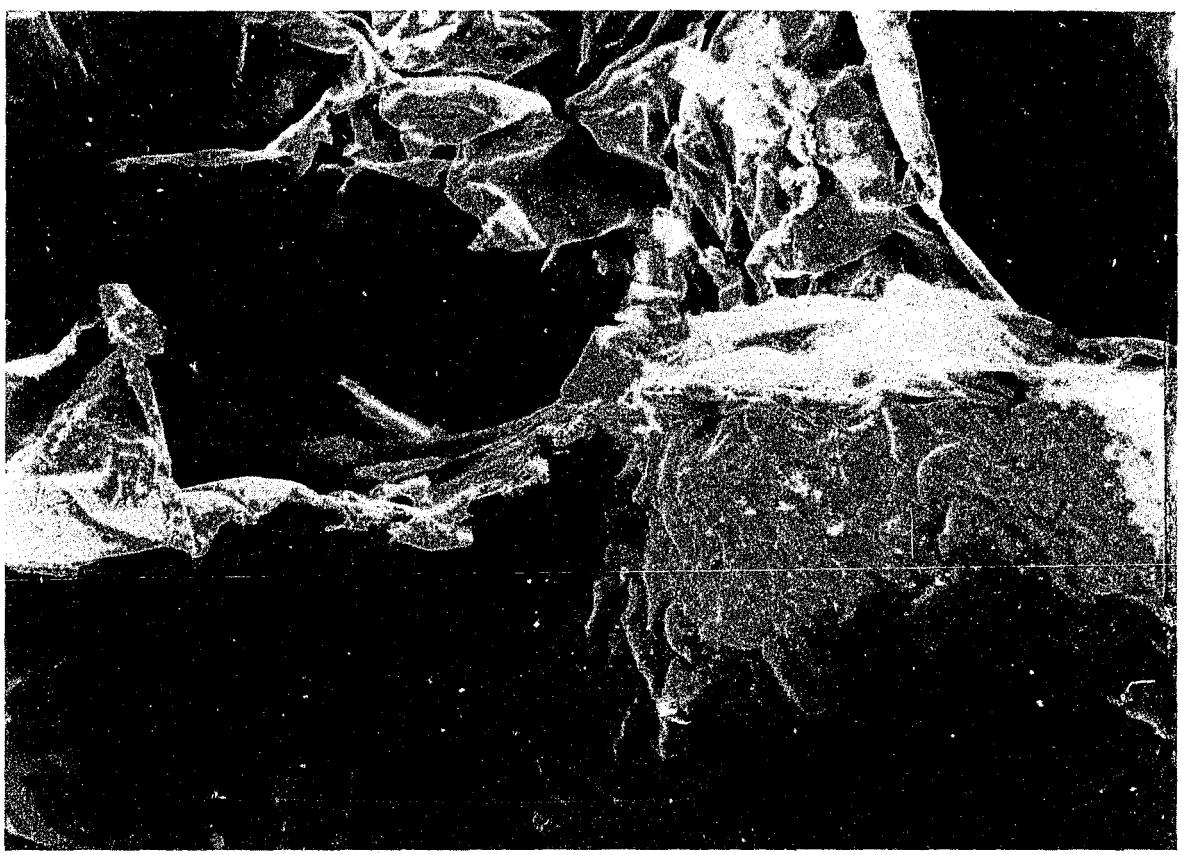
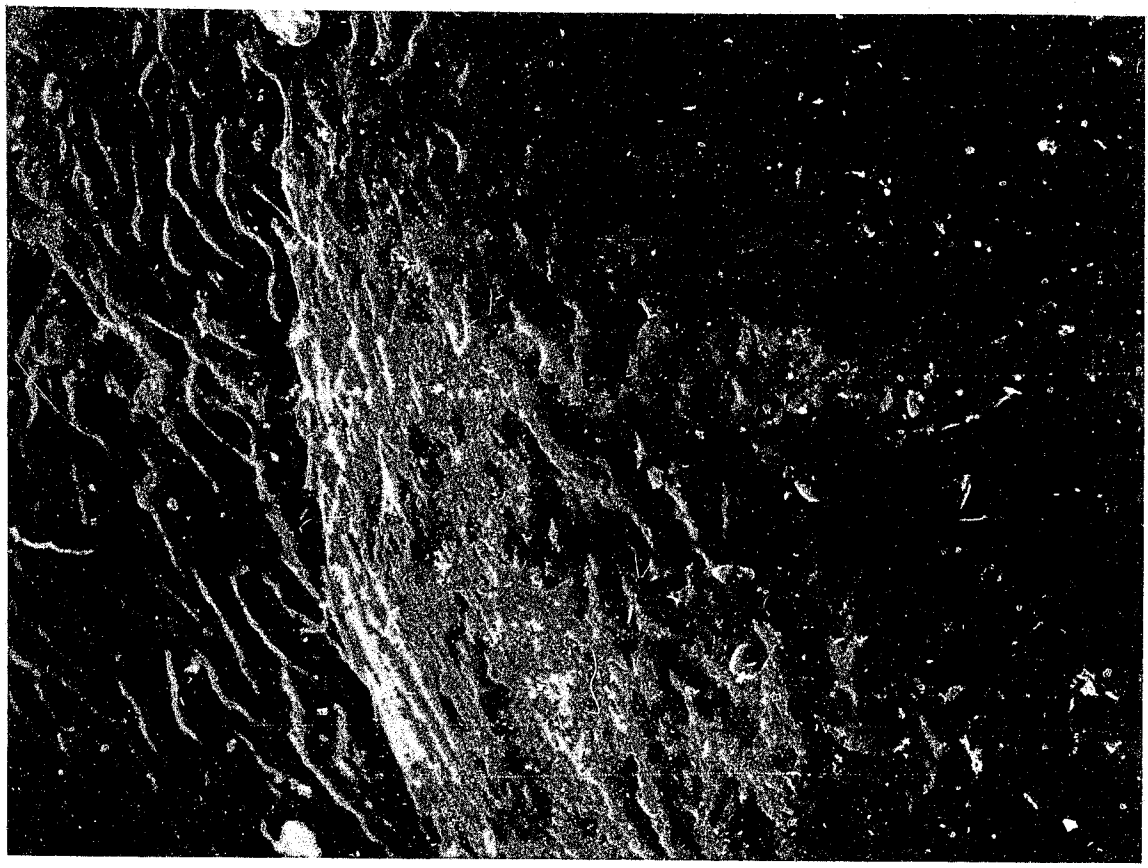


Figure 9.39 Peach peel and starch-grain filled pulp cells (arrow). Also see figs. 8.53, 8.54, and 8.56.

Figure 9.40 Pepper epidermis (bottom) and pulp (top). Also see figs. 8.58 and 8.59.



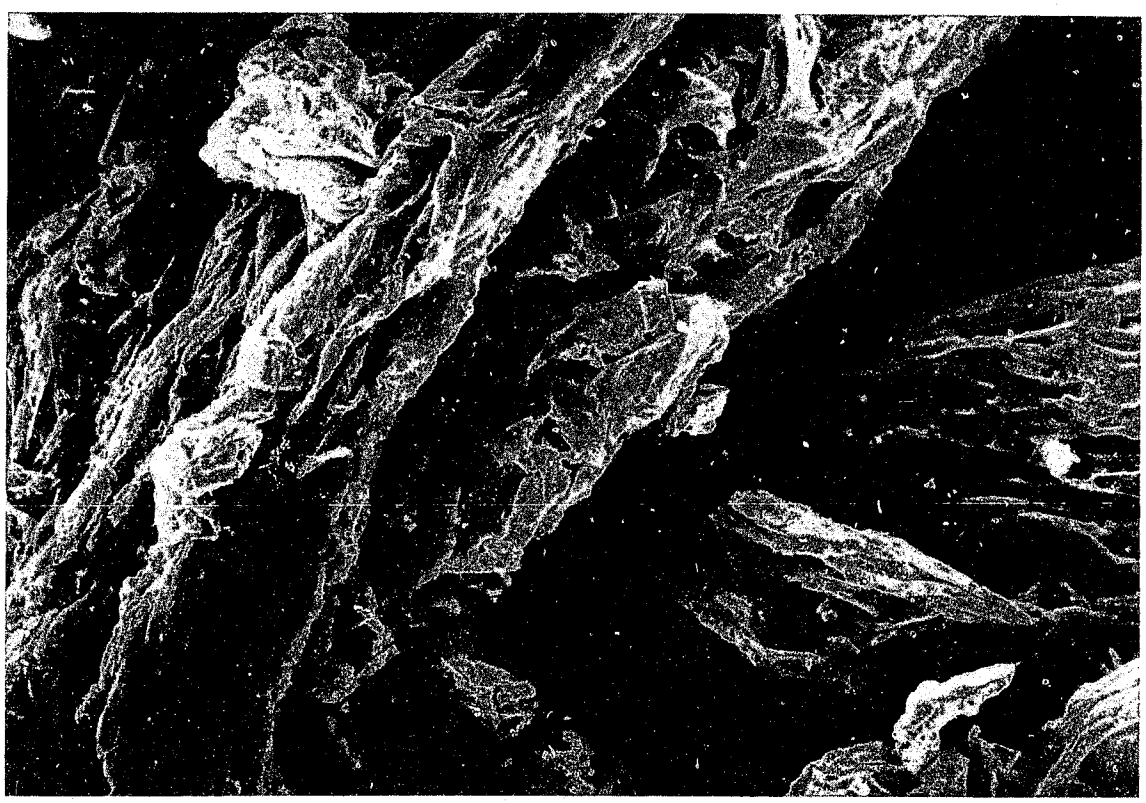
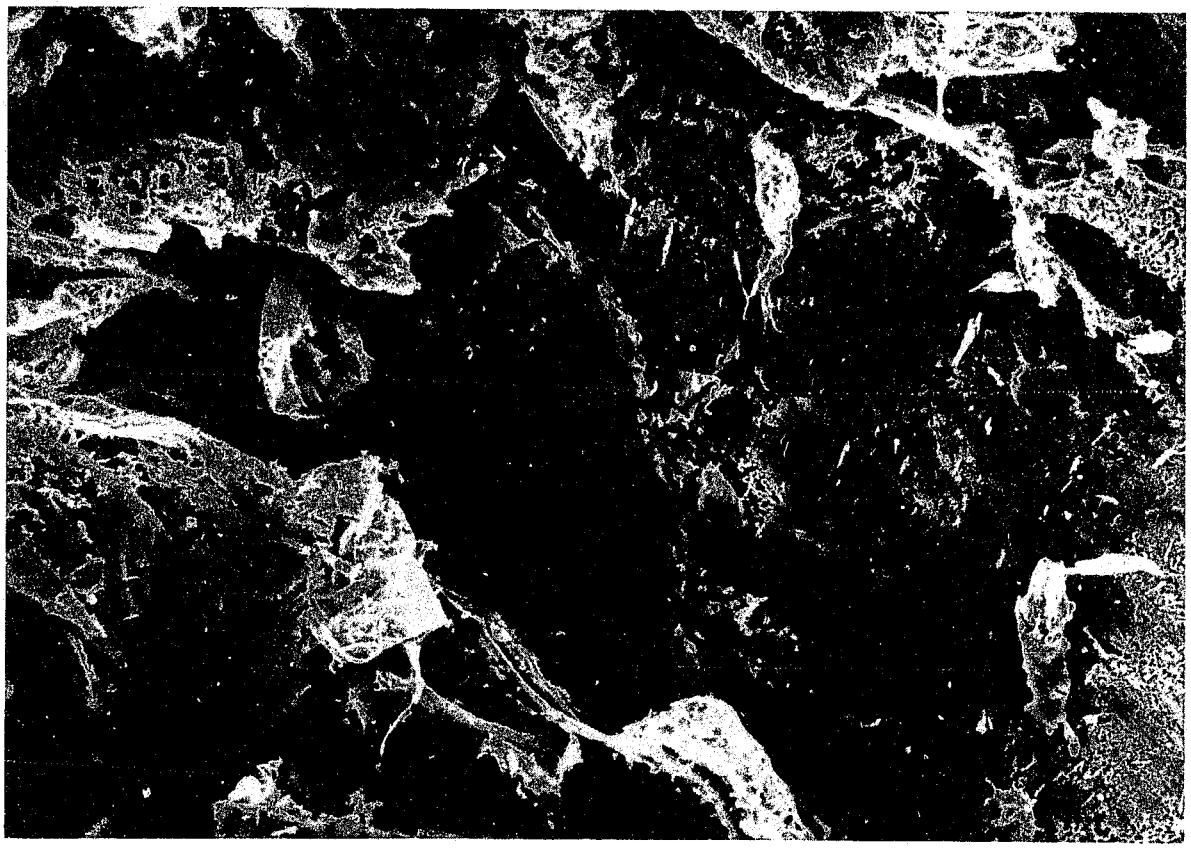


Figure 9.41 Pepper epidermis. Note the jigsaw puzzle-like cells. Compare to lettuce (fig. 9.29). Also see fig. 8.58.

Figure 9.42 Pineapple pulp. Also see figs. 8.60, 8.61, and 8.62.



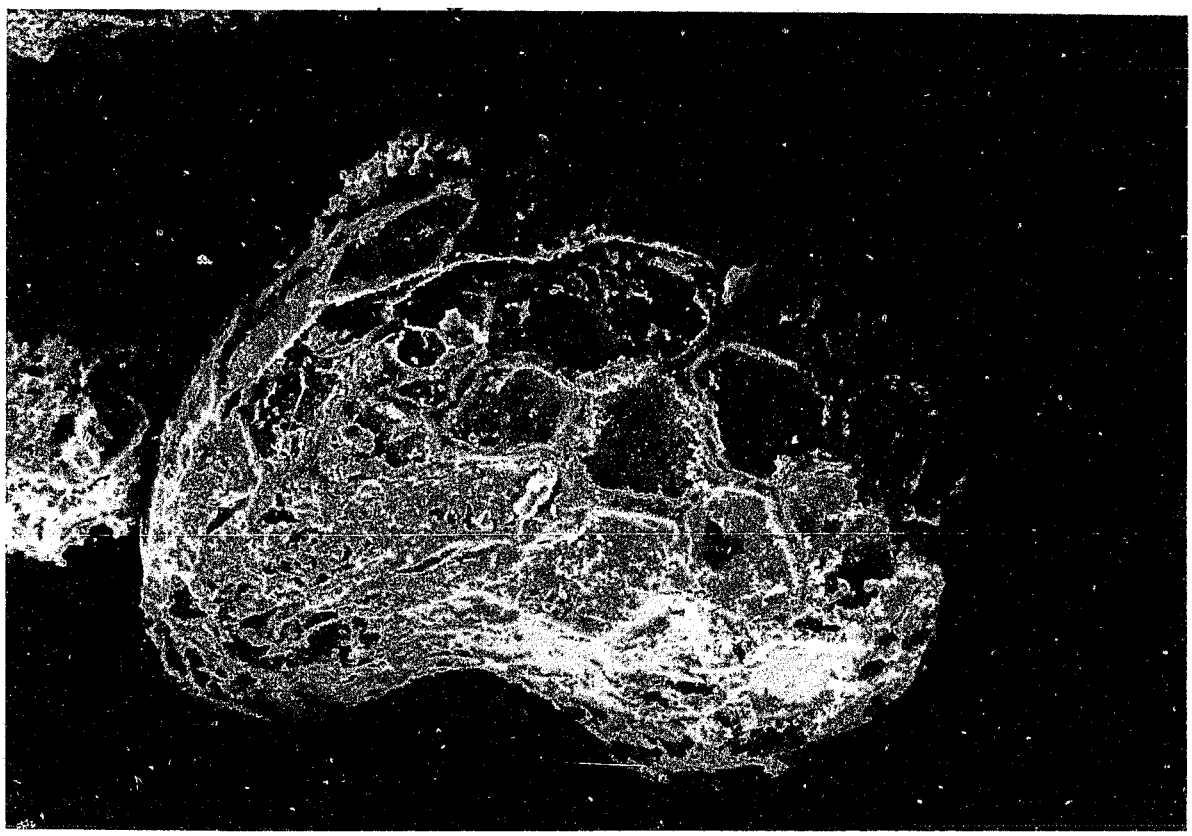


Figure 9.43 Plum and prune epidermis (center) and pulp. Compare to cherry (fig. 9.14). Also see figs. 8.63, 8.64, and 8.65.

Figure 9.44 Poppy seed (X120 taken at 60X). Also see fig. 8.66.

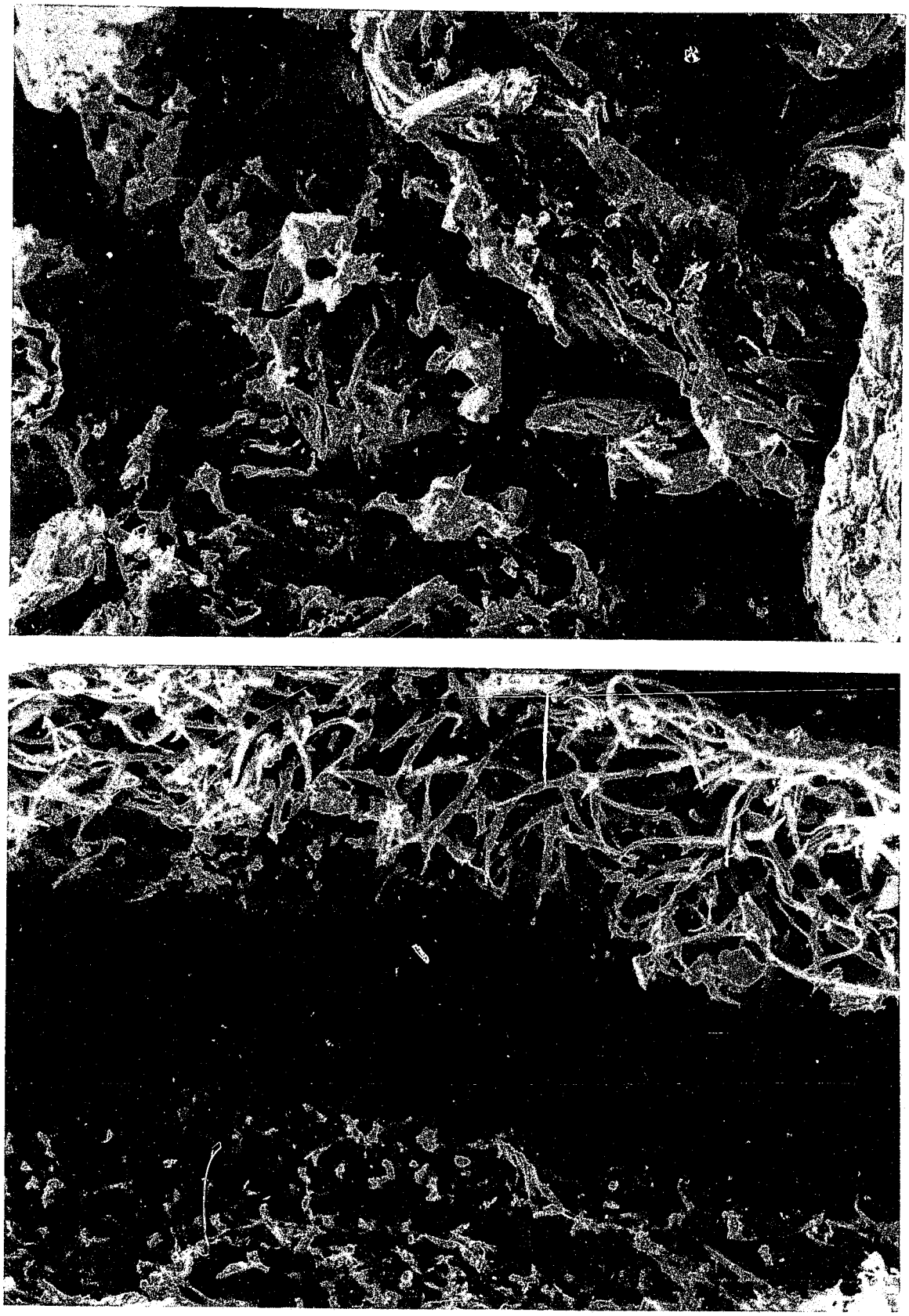
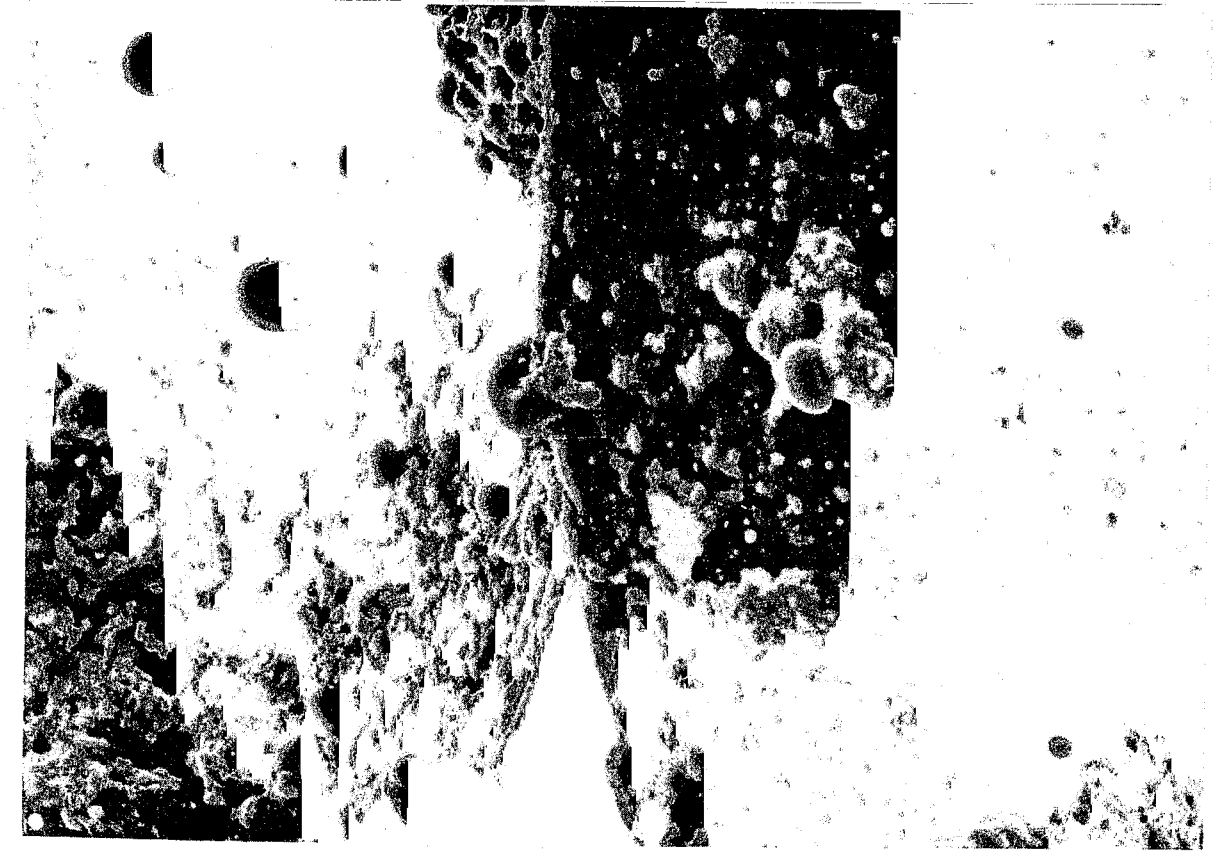


Figure 9.45 Radish pulp. Also see figs. 8.67 and 8.68.

Figure 9.46 Rosemary epidermis. Note the undamaged unbranched hairs (top) and broken hairs (bottom). Also see figs. 8.69 and 8.70.



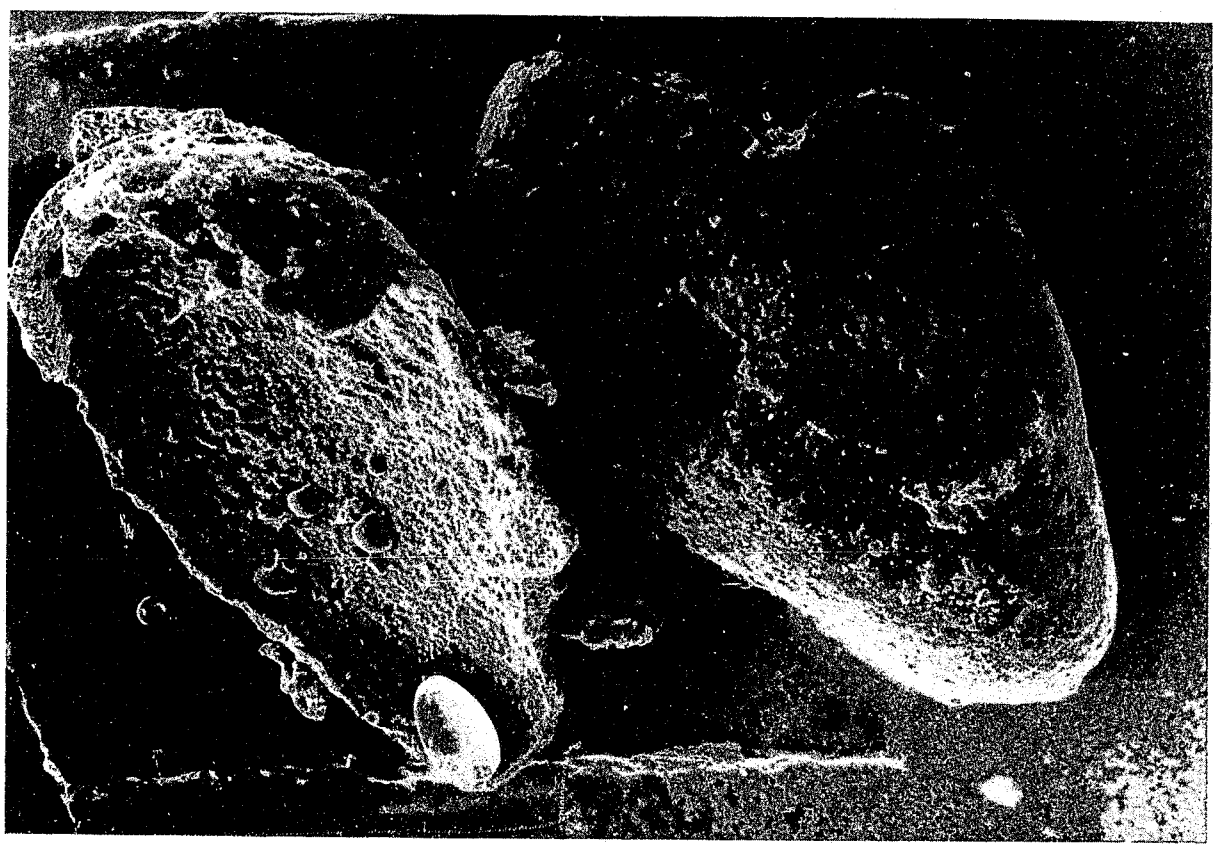


Figure 9.47 Sesame seed pulp. Note the droplets of fat. Compare to olive (fig. 9.6.33). Also see fig. 8.71.

Figure 9.48 Sesame seed coat (X60 taken at 30X) inside (left) and outside (right). Also see fig. 8.71.

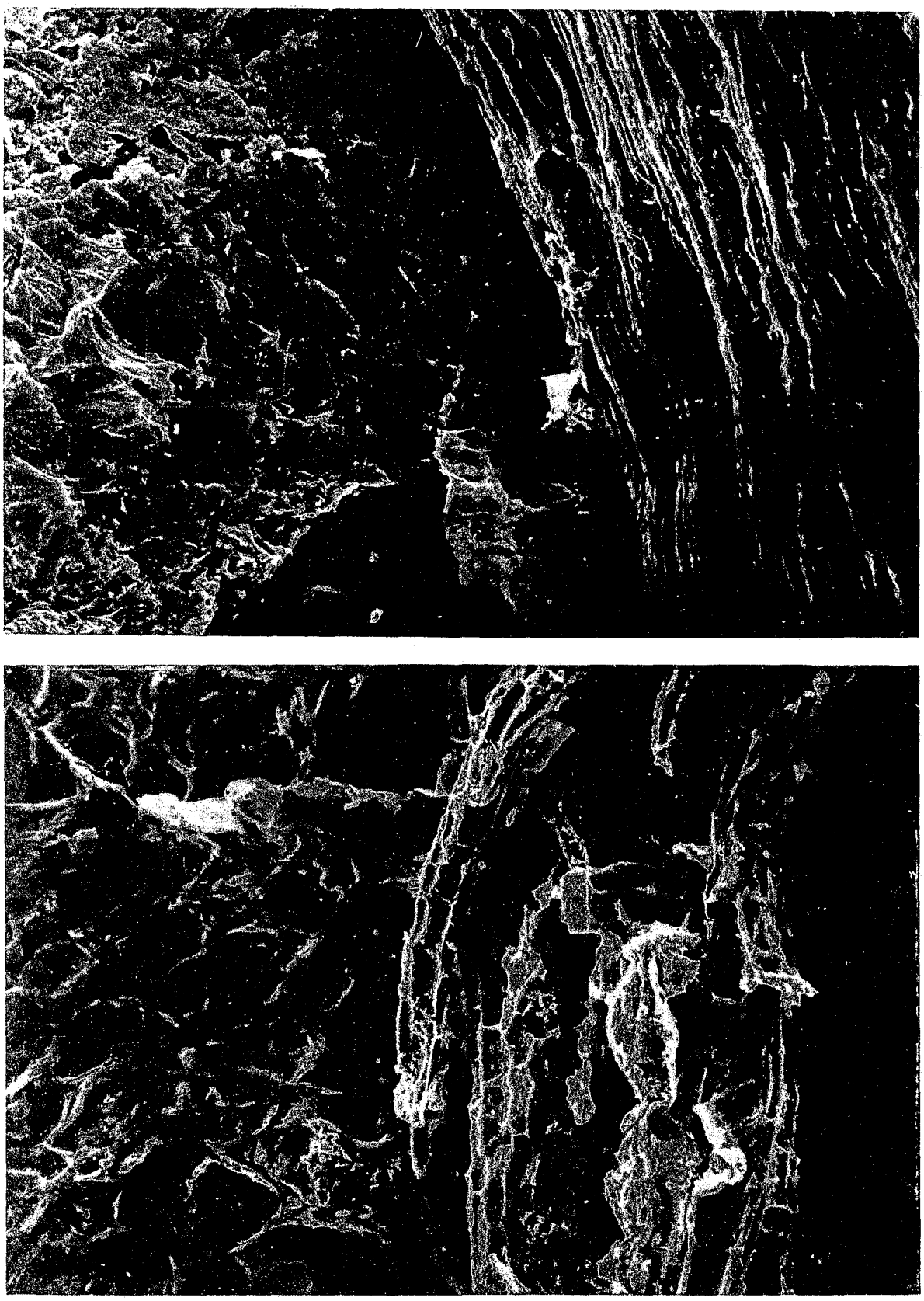
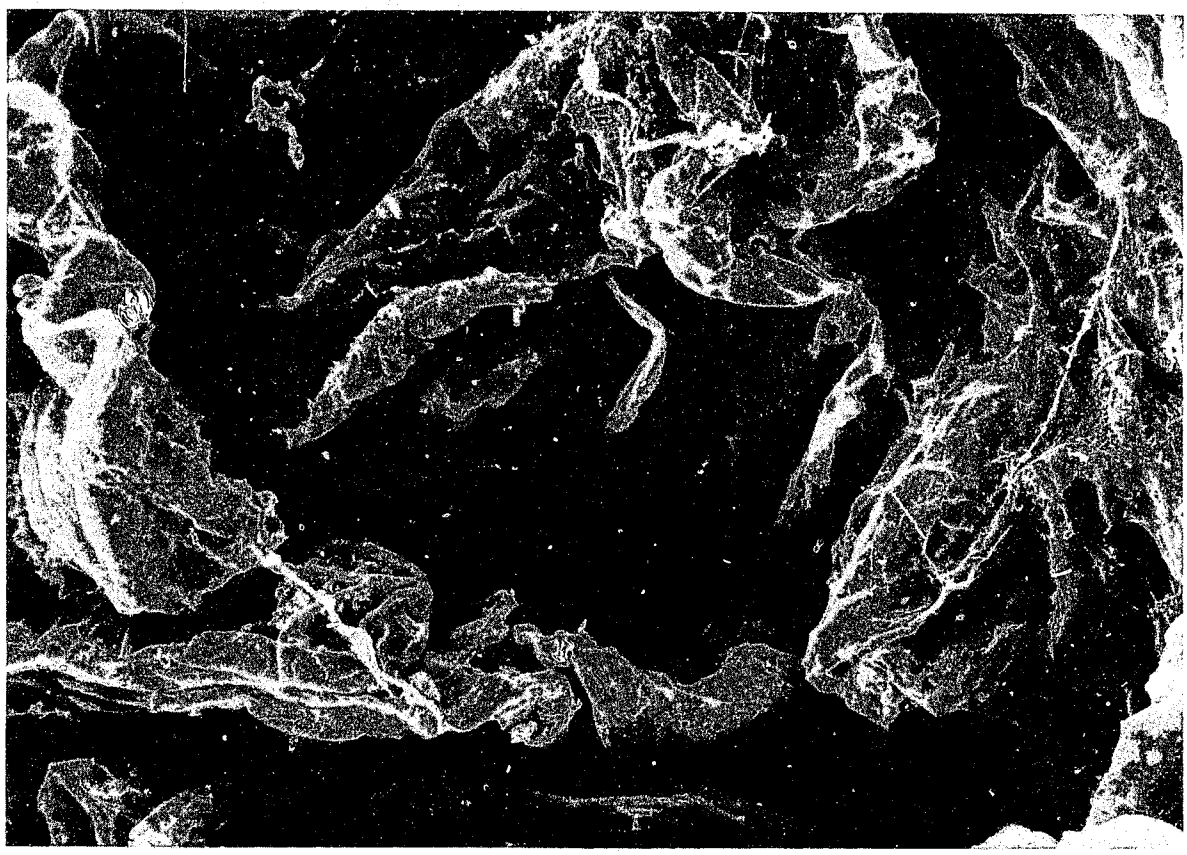


Figure 9.49 Spinach pulp (on left) and conducting cells (on right). Also see fig. 8.72.

Figure 9.50 Squash pulp. Also see figs. 8.74 and 8.75.



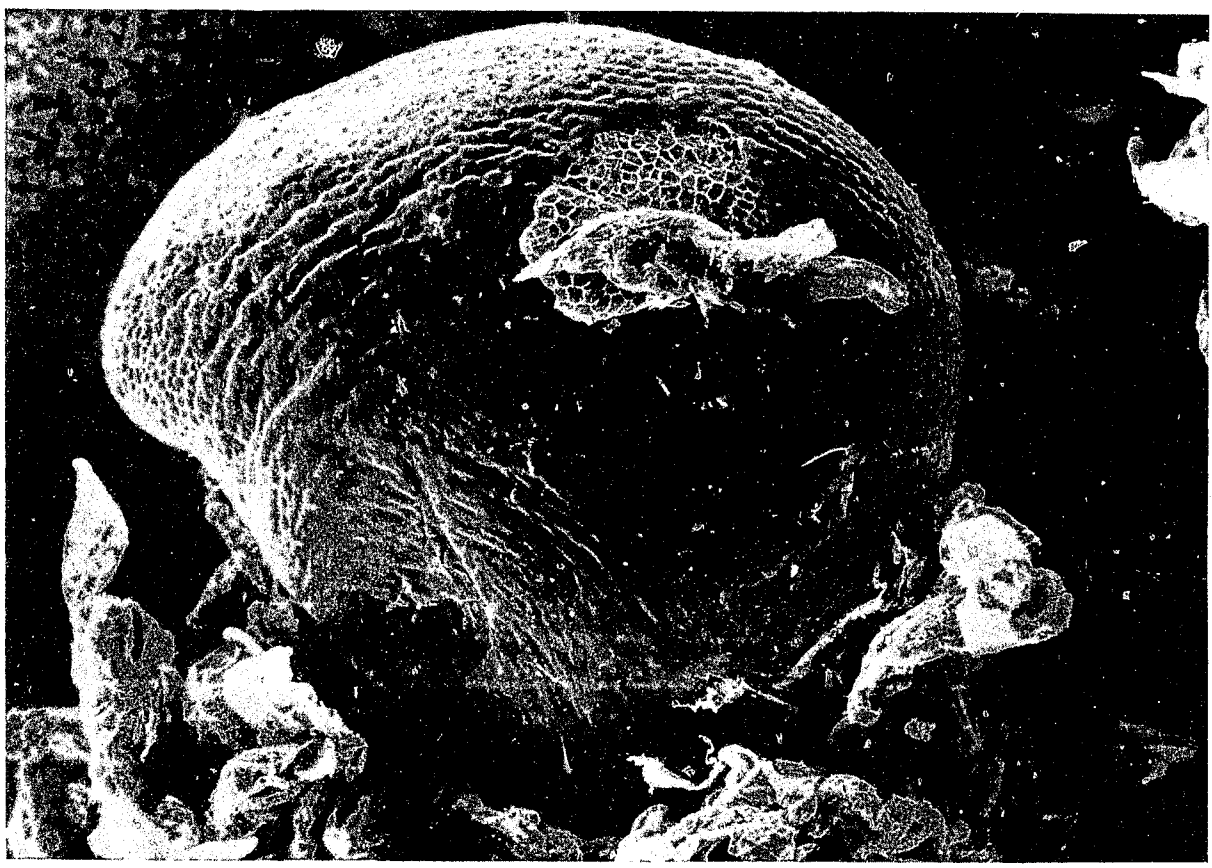
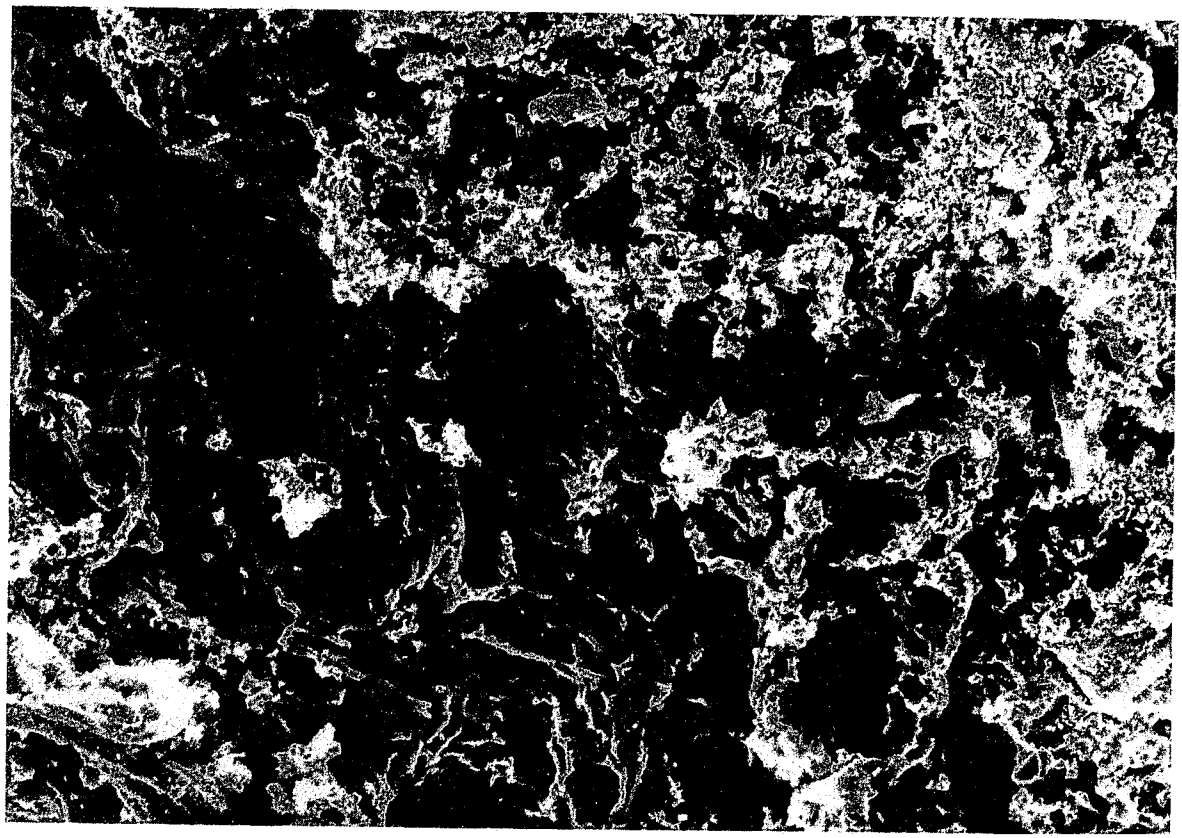


Figure 9.51 Strawberry pulp. Also see figs. 8.76 and 8.77.

Figure 9.52 Strawberry seed (X120 taken at 60X).



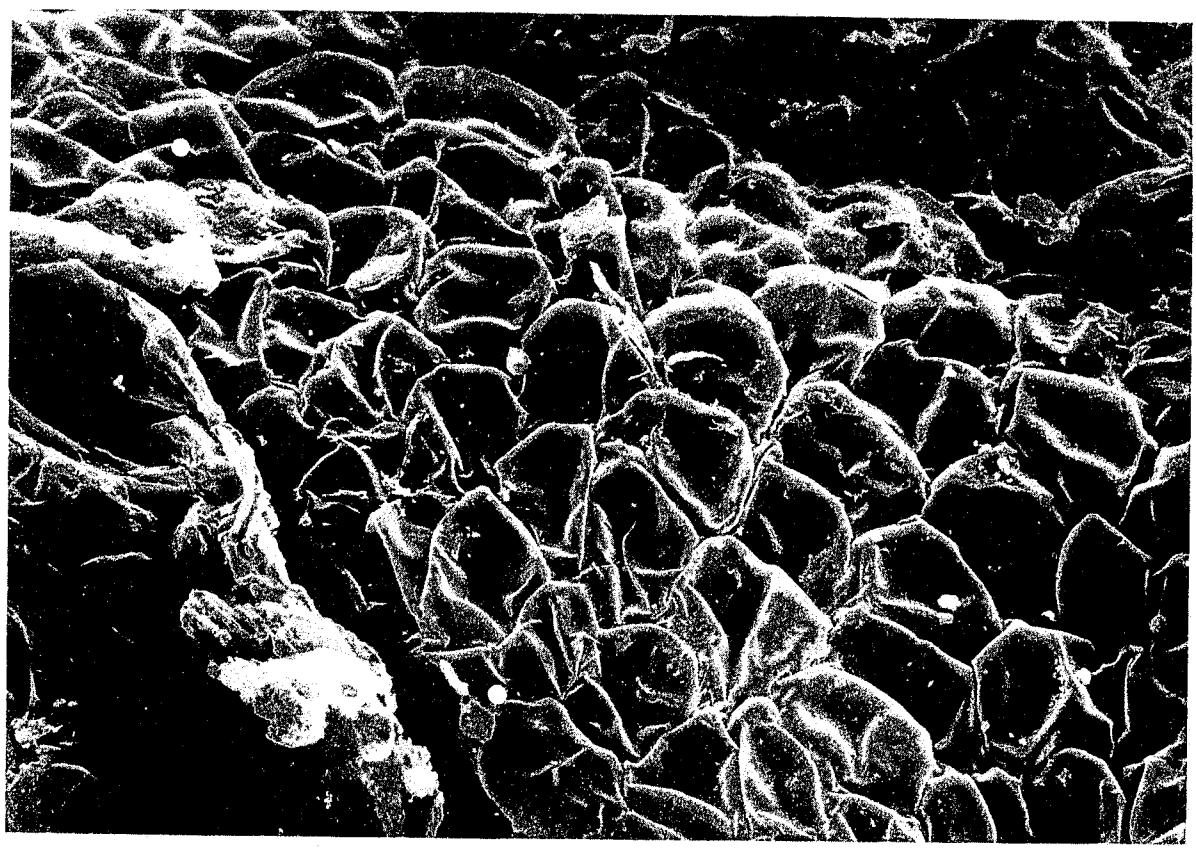
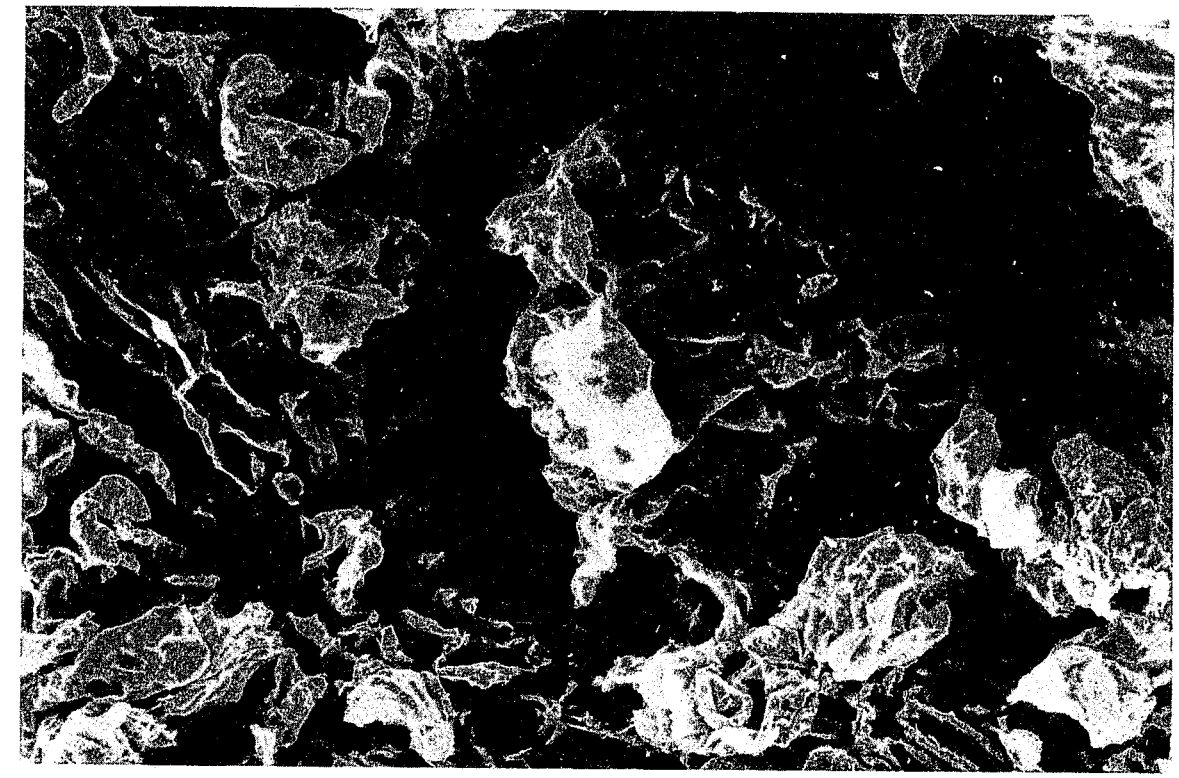


Figure 9.53 Sweet basil. Compare to oregano (figs. 9.36 and 9.37) and rosemary (fig. 9.46). Also see fig. 8.78.

Figure 9.54 Tomato peel. Also see fig. 8.79.



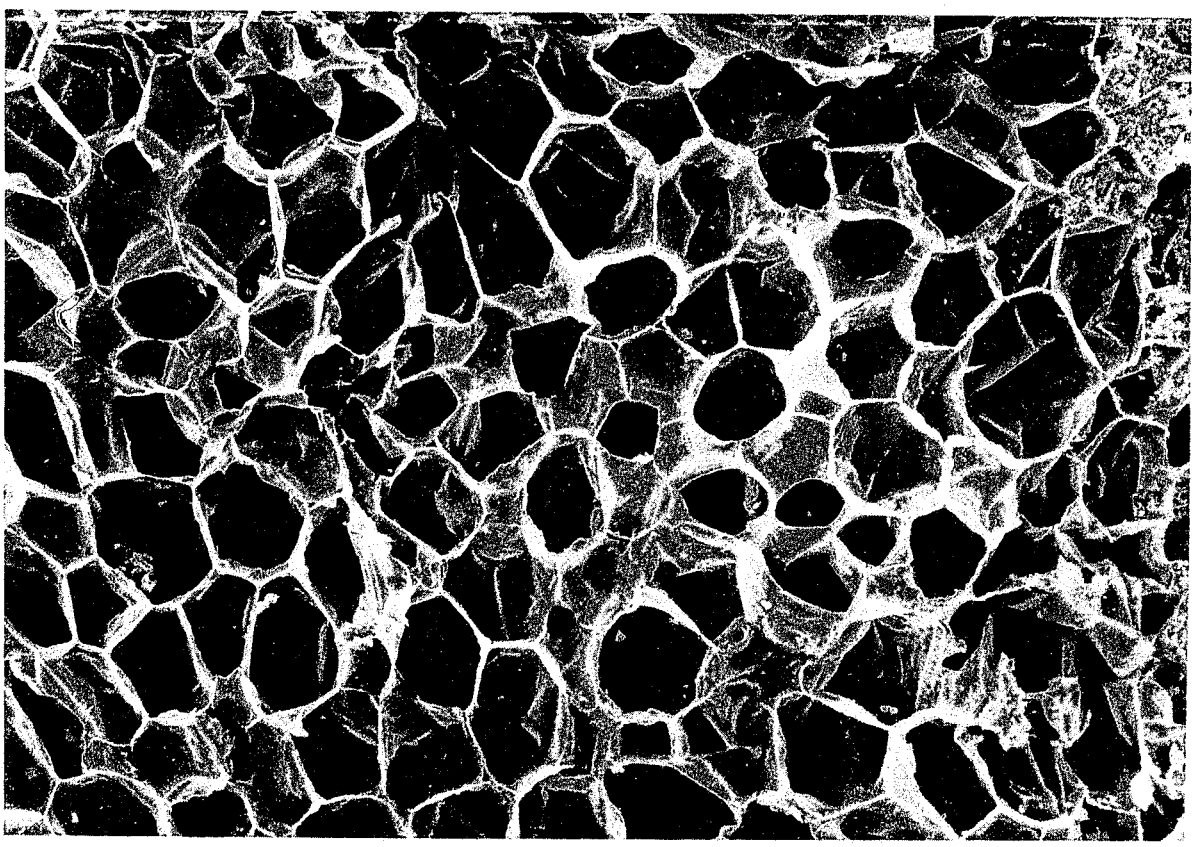


Figure 9.55 Turnip pulp. Compare to radish (fig. 9.45). Also see fig. 8.81.

Figure 9.56 Water chestnut pulp. Also see fig. 8.83.

# GLOSSARY

AUTOPSY examination of human body after death, especially to determine the cause of death

BOLUS a mass of masticated food (see Chapter 2)

CELLULOSE a polysaccharide, indigestible by humans, which composes the cell walls of all plants (see Chapter 1)

CLEARING AGENT a chemical used in the process of preparing microscope slides which makes the tissue more transparent (see Chapter 5)

COMPOUND MICROSCOPE an instrument used for magnifying small objects many times their actual size; see light microscope (see Chapter 6, especially figs. 6.1 and 6.2)

CONDENSER a combination of lenses on a light microscope used for focusing light rays (see Chapter 6, especially figs. 6.1 and 6.2)

CONDUCTING CELL hollow cells which transport liquids through the plant body

CONTRAST in our case, the amount of distinction between stained and unstained cells or parts of cells (see Chapter 5 and Chapter 6)

COUPLET a pair of options in a key used for identifying biological objects; in our case, cell types (see Chapter 7)

COVERSLIP a glass or plastic cover for materials on a microscope slide (see Chapter 5)

DEPTH OF FIELD thickness ('depth') of the focal plane (see Chapter 6)

DICHOTOMOUS branching in two (see Chapter 7)

DISSECTING MICROSCOPE a light microscope of low magnification and great depth of field through which whole, opaque objects can be viewed without the use of microscope slides (see Chapter 4)

ENZYME a protein which facilitates the chemical reactions of living cells (see Chapter 3)

EPIDERMIS the outermost layer of cells of a plant part; in our case, the "skin" of leaves, stems or fruits (see Chapter 1)

EYEPIECE synonym for "ocular" (see Chapter 6)

FECES human or animal excrement (see Chapter 2)

FLOWERING PLANTS a class of plants which bear flowers and fruits, as opposed to cones or other reproductive structures (see Chapter 1)

FOCAL LENGTH the distance between the lens and the focal plane (see Chapter 6)

FOCAL PLANE that position of adjustment of the microscope at which the object being observed through the microscope can be viewed most clearly, i.e. is 'in focus' (see Chapter 6)

FORCEPS tweezers (See Chapter 4, Appendix 1)

FORENSIC pertaining to criminal justice (see Chapter 3)

FUNGUS (FUNGI) a group of organisms which includes such things as mildews, molds, mushrooms and toadstools whose bodies are made up of intertwined hyphae (see Chapter 1)

HOMICIDE murder (see Chapter 3)

HYPHAE long, tubular cells lacking cross-walls which make up the bodies of fungi (see Chapter 1)

ILLUMINATOR a light source used in a light microscope (see Chapter 6, especially figs. 6.1 and 6.2)

INCLUSIONS objects included inside something else; in our case, certain non-living materials included within cells (see Chapter 1)

KEY a dichotomously branching guide used for identification biological objects (see Chapter 7, Chapter 8)

LIGHT MICROSCOPE a microscope which uses a combination of light and magnifying lenses to make very small objects visible (see Chapter 6, especially figs. 6.1 and 6.2).

LIGNIN cell wall-thickening compound common in conducting cells (see Chapter 1 and Chapter 5)

MASTICATION the process of chewing (see Chapter 2)

MECHANICAL STAGE a device which holds a microscope slide in place for viewing and moves it with great precision (see fig. 6.1)

MICROSCOPE SLIDES thin glass or plastic plates, usually rectangular in shape, about 1 inch wide and 3 inches long, used to hold objects to be viewed under a light microscope (see Chapter 4, Chapter 6, Appendix 1)

MILLIMETER (mm) 0.001 meters, about 0.04 inch (see Chapter 6)

MOUNTING MEDIUM an adhesive substance used to permanently affix a specimen and the coverslip to a microscope slide (see Chapter 5, Appendix 1)

OCULAR the lens or lenses through which one looks in a light or dissecting microscope (see Chapter 6, especially figs. 6.1 and 6.2)

OCULAR MICROMETER a calibrated, non-magnifying piece of glass which is inserted into an ocular to help determine the size of objects being examined with the microscope (see Chapter 6)

PATHOLOGIST one who diagnoses the causes of disease and death (see Chapter3)

PEPSIN an enzyme which is active in the digestion of proteins in the human stomach (see Chapter 3)

POLYSACCHARIDES chemical compounds made up of many joined sugar molecules; starch and cellulose are examples of plant polysaccharides (see Chapter 1, Chapter 3)

PULP the fleshy portion of a fruit or vegetable, or the layers of cells between the upper and lower epidermises of a leaf (see Chapter 1, Chapter 8)

PRESERVATIVE a substance which prevents decay (see Chapter 5)

PYLORIC VALVE the muscle which controls the opening between the stomach and the small intestine (see Chapter 3)

SALIVARY AMYLASE an enzyme involved in the digestion of starch which is secreted in the mouths of humans (see Chapter 3)

SAMPLE VIAL a small container used to hold specimens for scientific examination (see Chapter 5, Appendix 1)

SCANNING ELECTRON MICROSCOPE (SEM) a microscope that uses a beam of electrons focused by magnets as a mechanism for producing a magnified image (see Chapter 9)

SEED found within fruit and containing an embryo plant usually surrounded by nutritive material provided by the parent plant; humans consume seeds to obtain this nutritive material (see Chapter 8, Chapter 9)

SLIDE CLIPS devices for holding a microscope slide in position on a non-mechanical stage of a light microscope (see fig. 6.2)

STAGE the platform of a light microscope on which a slide is placed for microscopic examination (see Chapter 6)

STAGE MICROMETER a microscope slide, which has a millimeter scale subdivided into 0.01 mm units etched on its surface, used to calibrate a compound microscope (see Chapter 6, especially fig. 6.3)

STAIN a dye added to biological materials to increase contrast when viewed through the microscope (see Chapter 5)

STARCH a polysaccharide which is commonly found in certain sorts of plants, e.g. corn, potatoes (see Chapter 2, Chapter 5)

STONE CELL a type of sclerenchyma cell that is round or blocky in outline, and which have very thick walls and extremely small cell cavities (see Chapter 1)

SUBSAMPLE a portion of a sample (see Chapter 5)

VEIN a group of conducting cells visible among pulp or epidermal cells (see Chapter 1)

VOMITUS partially digested material which has been forcibly ejected from the stomach, vomited matter (see Chapter 2)

YOKED opposing statements of a couplet in a key appearing together and numbered with the same integer (see Chapter 7)

# APPENDIX 1

## Details of Procedures and Formulas for Solutions Discussed in Chapters 4, 5 and 6

The "Plant Cell Analysis Kit" mentioned in Chapter 4 may be obtained from:

Carolina Biological Supply Company
Burlington NC 27215
1-800-334-5551

or

Gladstone OR 97027
1-800-547-1733

The various components of the Kit (listed below), and other items such as stage and ocular micrometers, can also be purchased individually from Carolina Biological Supply Company or from other vendors of scientific supplies such as:

Fisher Scientific
1-201-379-1400
1-613-226-8874 (in Canada)
[call for nearest distributor]

VWR Scientific
1-415-468-7150
[call for nearest distributor]

Ward's Natural Science
1-800-962-2660

There may be other vendors in your area you could locate through the section of the telephone book usually under the heading "scientific apparatus and instruments".

The "Plant Cell Analysis Kit" contains the following items:

FAA Preservative
Safranin O Stain
Picric Acid Solution
Clearing Agent
Potassium Iodide Solution
70% Ethanol
95% Ethanol
100% Ethanol
Mounting Medium A
Mounting Medium B
Microscope Slides (1/2 gross)
Coverslips (1 oz. box of 22X22 mm #1)
Forceps (2 pair)
Eyedroppers (1 dozen)
Sample vials (one dozen 8 cc Wheaton glass snap-cap vials with polyethylene caps)
Pres-aply labels (7/8" X 1 1/4") for vials and slides (500)
Lens paper (1 booklet)
Slide storage (25 slides per box) boxes (2)
Clear plastic millimeter ruler (1)

Should you have the appropriate laboratory facilities, you may wish to purchase items from the kit separately, and prepare the solutions yourself. The formulas for the first ten items listed above are as follows:

FAA (Formalin-Acetic Acid-Alcohol) Preservative

5 ml 100% formalin (= 37% formaldehyde solution)
90 ml 70% ethanol
5 ml glacial acetic acid

Safranin O Stain

1 gm Safranin O
99 ml 70% ethanol

Picric Acid Solution

5 g picric acid (2,4,6-trinitrophenol)
95 ml 95% ethanol

Clearing Agent

Histo-clear, a low toxicity, low odor commercial product available from:

National Diagnostics
1013-1017 Kennedy Blvd.
Manville NJ 08835

This product should be acceptable for use in any well-ventilated laboratory without a fume hood, unlike most clearing agents.

Potassium Iodide Solution (1.5% aqueous)

0.3 g iodine
1.5 g potassium iodide
100 ml distilled water

## Ethanol Solutions

100% commercial preparation

95% commercial preparation, or dilute from 100%:

95 ml 100% ethanol
5 ml distilled water

70% dilute from either 100% or 95%:

70 ml 100% ethanol
30 ml distilled water

or

70 ml 95% ethanol
25 ml distilled water

## Mounting Medium A (Modified Hoyer's Medium)

50 ml distilled water
30 g Acacia (Gum Arabic)
0.1 g merthiolate (Thimerosol)
20 ml glycerin

Let Acacia sit overnight in distilled water. Add merthiolate and glycerin. Filter through glass wool or large Kimwipes. Filtering is a very slow process. Make only small batches as needed, because long-term storage is not recommended.

## Mounting Medium B (Permount)

commercially available from vendors listed on page 127