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Photomicrographs and autoradiograms of fresh and freeze-dried strawberries

Hung-yen Kao

To the Graduate Council:

I am submitting herewith a thesis written by Hung-yen Kao entitled "Photomicrographs and autoradiograms of fresh and freeze-dried strawberries." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Melvin R. Johnston, Major Professor

We have read this thesis and recommend its acceptance:

Jimmie L. Collins, Thomas S. Osborne, Ivon E. McCarty

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

May 5, 1966

To the Graduate Council:

I am submitting herewith a thesis written by Hung-yen Kao entitled "Photomicrographs and Autoradiograms of Fresh and Freeze-Dried Strawberries." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology.

M. R. Johnston
Major Professor

We have read this thesis and recommend its acceptance:

I. S. Osborne

J. L. Collins

H. McCarty

Accepted for the Council:

Hilton A. Smith
Dean of the Graduate School

PHOTOMICROGRAPHS AND AUTORADIOGRAMS OF FRESH AND FREEZE-DRIED STRAWBERRIES

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Hung-yen Kao

June 1966

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CHAPTER I

INTRODUCTION

Little information concerning the histology of the strawberry fruit is available. The reason for inadequate histological investigations is due primarily to the difficulty encountered in conducting such a study. The strawberry fruit is composed of delicate tissues; therefore, preparation for histological examination of such materials presents problems not always encountered with the more rigid structures.

One of the most recent processing methods utilized to preserve strawberries is freeze-drying. Inadequate data are available concerning the effect of this process on the histological changes of the berry. Knowledge of the cellular structure of fresh berries and freeze-dried (F-D) berries is believed essential for advancement of technology concerning strawberry processing.

Investigations concerning the effect of the freeze-drying process on physical alterations of the tissue have been conducted on several vegetable and fruit tissues. These studies indicate that rate of freezing tissue prior to

drying influences the amount of water taken up during rehydration. Use of radioactive tracers in the rehydrating media (autoradiography) coupled with the employment of histological examination should provide information showing the effect of freezing rate on structural and cellular distortion.

Objectives of this study are:

1. To make a histological study of the fresh strawberry fruit.
2. To study the effects of freezing rates on the structural and cellular changes of F-D strawberries.
3. To study the penetrability and distribution of different radioactive substances in the F-D strawberry fruit.

CHAPTER II

REVIEW OF LITERATURE

I. TISSUE OF THE STRAWBERRY FRUIT

The cultivated strawberry (Fragaria Chiloensis Ehrh.) bears an aggregate fruit, consisting of many enlarged ovaries scattered over the surface of the receptacle (20).* The receptacle, which is the edible part, consists of a fleshy pith, an even more fleshy cortex, and a narrow zone of fibro-vascular bundles located between the pith and cortex. These bundles ramify the cortex to the achenes. The surface of the receptacle has a tufted appearance, due to the somewhat regularly arranged depressions occupied by the achenes. The epidermal cells are usually polygonal and isodiametrically shaped. The cells of the hypoderm, or sarkogen, are usually tangentially elongated and are always without intercellular spaces. During development of the berry the cells of the cortical tissue, formed by the division of cells in the

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*The numbers in parentheses represent similarly numbered references in the Bibliography.

sarkogen layer, increase rapidly in size, become round in shape, and form intercellular spaces. The fibrovascular bundles consist of spiral, and annular vessels, and elongated, thin-walled cells. In relatively large berries the pith contains comparatively large intercellular spaces or cavities (42).

The edible portion of the fruit is composed primarily of parenchyma cells with some interlacing vascular tissue. Parenchyma cells are generally thin-walled and are "cemented" together by pectic substances. A parenchyma cell may be polygonal or cubical in shape. The exact shape depends on intercellular contact and pressures. Parenchyma cells do not fit together perfectly; this condition results in the formation of intercellular spaces filled with gases. Fleshy fruit tissue contains many large intercellular spaces (3, 11, 14, 41).

All parenchyma cells have a primary wall and some have an additional inner layer, called the "secondary wall." Distinguishing between the middle lamella and primary wall is sometimes impossible; therefore, the term "compound middle lamella" has been applied to include both the middle lamella and primary walls of two adjacent cells. The

secondary wall is formed after the volume of each cell has reached a maximum. The functions of the secondary wall are to strengthen the primary wall and to provide resistance to cellular injury (12, 14).

Cavities in primary and secondary walls are frequently observed in parenchyma tissue. Primary walls may have definite depressions, called "primary pit fields." When a cell wall is relatively thick, the entire cavity is called a "pit." A pit in one cell is usually opposite another pit in the adjacent cell. A pit membrane is composed of two primary walls and the middle lamella and it closes the opening between the pit pair. Sometimes a pit may adjoin an intercellular space with a pit membrane as the barrier. Presumably, the pit membrane is semipermeable and allows ions and low-molecular-weight components to migrate between cells. Pit membranes are very thin and can be broken easily by a slight pressure (12, 14).

The most common structural constituent of parenchyma cell walls is cellulose. Other carbohydrates such as hemicellulose and pectic substances occur in smaller quantities (12, 14).

It is believed that the middle lamella is composed of insoluble pectic substances, probably calcium (Ca) pectates. In some cases, Ca salts, applied during the canning process, improve firmness of fruit and vegetables (9, 13). Softening of tissues is due primarily to separation of cells and to loss of rigidity in the individual cell walls. Any agent or process which hydrolyzes the cementing substances can cause cell separation. The cell wall becomes softer as pectic materials are degraded (36, 37).

When the pectic substances are degraded in the middle lamella, the cells gradually loosen and separate readily. When these substances diminish, the cell walls become thinner and more readily punctured (29, 31).

II. HISTOLOGICAL TECHNIQUES

Jensen (23) indicated that the standard histological procedure involves the following steps:

1. The tissue is killed and fixed in a fluid designed to preserve cell structure.

2. Water is removed from the cell by gradually replacing it with alcohol. This step is termed tissue dehydration.

3. The tissue is infiltrated with paraffin and then embedded in a block of the same material.

4. The infiltrated and embedded tissue is sectioned with a microtome, and the sections are affixed to slides.

5. Paraffin is removed and the tissue is stained.

6. Finally, the tissue is covered with a cover slip which is held in place with a permanent mounting medium.

Davenport (10) noted that the greatest change in tissues subjected to the action of fixing agents is the denaturation of the proteins. Fats and carbohydrates may be leached out during fixation. How these components are affected depends upon the proportion of water to alcohol in the fixing fluid.

Usually, material placed in the killing and fixing fluid will not sink, but floats upon the surface because of the occluded gases in the tissues. To insure thorough infiltration of the fixative and paraffin, the gases should be removed (6).

Brooks, Bradley, and Anderson (6) suggested that the fixative, FPA (formalin-propionic acid-ethyl alcohol), may be used with almost any plant material intended for anatomical or morphological study. FPA fixative is preferred over

other fixative agents in that FPA results in less shrinkage. The length of time required for fixation will depend on the size and density of the tissue, the rate of penetration of the fixing fluid, and the temperature at which the tissue is fixed (32).

Tissues must be dehydrated thoroughly after fixation, since neither paraffin nor celloidin is miscible with water. Dehydration should be performed gradually. Tissue transferred directly from water or an aqueous solution to highly concentrated alcohol will undergo distortion due to the mixing of the alcohol and water. Treating for too long a period of time in the higher concentrations of alcohol (above 80 per cent) makes tissue brittle and difficult to cut. Treatment for an excessive time in lower dilutions of alcohol (under 70 per cent) macerates the tissue. Without proper dehydration, efficient tissue infiltration by the embedding medium is improbable (33).

Paraffin infiltration usually requires 12 hours for satisfactory results. For most soft or delicate materials, exposure to hot paraffin should be limited to as short a period as possible. The temperature should be kept as low as possible to avoid cooking of the material (23).

Jensen (23) indicated that the thickness in which tissue should be sectioned is an important consideration and depends completely on the nature of the material and the objective of the investigation. The majority of plant materials can be sectioned at 10 to 20 microns (μ) when used for morphological studies, although the precise thickness must be determined by preliminary sectioning.

Safranin and fast green dyes comprise an excellent stain of brilliant contrast. The technique of staining is easily mastered. Lignified tissues, cuticle and cork stain red; cellulose and cytoplasm, green (6, 35).

Ham and Leeson (17) stated that the celloidin procedure required no heat and could be handled at room temperature. Hence, celloidin embedding does not distort the tissue as does paraffin embedding. Relatively large objects can be sectioned more advantageously by using the celloidin method because celloidin holds the cells firmly in place. However, preparation of celloidin-embedded tissue sections requires a longer time and they are usually thicker than paraffin sections (21).

Unlike paraffin and celloidin, carbowax is water-soluble; consequently, no dehydration of the tissue is

required. The carbowax method has been used successfully to demonstrate the presence of fat in tissue. Because water will dissolve the embedding medium, care must be taken to keep the blocks free from water. Greater care is required to obtain satisfactory carbowax-treated sections than is required for paraffin-treated sections. Tissue treated with carbowax is more difficult to section than tissue treated with paraffin. The formula usually given for the embedding mixture consists of carbowax 4000 and carbowax 1500 (9:1). This proportion is not rigid and may be varied (32). Little (25) stated that glycerol is a necessary component and its proportion with the carbowax is critical. Some of the difficulties encountered in cutting may be avoided by controlling humidity and temperature. For fragile tissue, a special flotation solution can be used, which consists of carbowax 1000 and glycerin (1:3)(43).

III. FREEZING RATES AND CELL INJURY

Mechanical Damage

Fruit and vegetable tissues are damaged appreciable by freezing. The delicate cells in many fruits are very susceptible to rupture during freezing (14). Love (26)

reported that slow-frozen tissue always contains larger ice crystals than those tissues quick-frozen. Under more rapid freezing conditions, all ice crystals formed were intracellular. The ice mass was relatively small and it did not deform or damage the cells appreciably. With relatively slow freezing, cells were damaged. Cell damage results from the mechanical action exerted by the expanding ice crystals of a certain critical size growing within the cell (27, 39).

Extracellular ice crystals which form during slow freezing presumably have a lower vapor pressure than does water present at cell surfaces. This vapor pressure differential allows water to migrate out of the cell where it can be deposited on the ice crystals. The slower the freezing rate, the greater the opportunity for water migration. Cell shrinkage and a decrease in the freezing point of protoplasm accompany cellular "dehydration," perhaps to the extent that intracellular freezing never occurs. Conversely, rapid freezing apparently provides little opportunity for cellular dehydration; as a result, intracellular crystallization occurs (14).

The protoplasm in intact cells tends to remain supercooled above -10°C., even when the extracellular medium is

frozen. This phenomenon immediately establishes a higher vapor-pressure inside the cell than outside, a condition that is thermodynamically unstable. There are two ways that equilibrium can be reestablished: supercooled water can either flow out of the cell and freeze externally, or it can freeze within the cell (1, 28).

Damage Caused by the Solute Concentration

Mazur (28) indicated that water in a suspension of cells begins to freeze at some temperature below 0°C. As it freezes, pure ice crystals separate out of solution; concomitantly, the solutes in the residual liquid solution become increasingly concentrated. With a continued drop in temperature, more and more water is withdrawn from the solution and deposited as crystals. The solution eventually becomes saturated with solute and a further lowering of the temperature causes all the solutes to precipitate, permitting all of the water to freeze. If only one solute is present, complete solidification occurs at a specific temperature, known as the eutectic point. If many solutes are present, each precipitates out at its particular eutectic point. However, some liquid water remains until the temperature has dropped

below that of the lowest eutectic point for the solutes present.

The concentration of solutes and suspended materials which occurs during the transformation of water to ice is another factor which can lead to damage during the freezing process. Changes in such properties as pH, titratable acidity, and ionic strength result from dehydration. These changes affect the stability of hydrophilic colloids, emulsions, and perhaps even larger cellular and intercellular structures. The extent of these changes will vary with the product and the rate of freezing. Slow freezing allows a greater opportunity for concentration damage than does rapid freezing (1, 14).

Webster (40) reported that the marked decrease in ice crystal size is related to a reduction in product destruction, drip loss, and other quality defects normally associated with conventionally frozen berries. The most obvious benefit from freezing with liquid nitrogen is the tremendous improvement in product quality compared with the conventional or air-blast frozen product.

IV. FREEZE-DRYING

F-D fruits have an improved quality over fruits atmospherically dehydrated. Most F-D fruits rehydrate readily to produce a high-quality product, similar to fresh-frozen fruits. F-D peaches and apricots are light in color, have low density, are highly porous, and do not change in volume during drying. F-D vegetables have the following quality attributes: (1) they are not damaged by high temperatures since they are not subjected to heat; (2) they undergo little or no change in shape; (3) they rehydrate readily; and (4) they exhibit an excellent storage potential due to the low moisture content (16, 18, 34).

Harper and Tappel (18) stated that the freeze-drying process is simple in principle. The material is frozen and water vapor is removed by sublimation. The temperature must be kept low enough to prevent the ice crystals from melting. The rate of sublimation is greatly increased when the material is held under a vacuum well below the vapor pressure of the ice.

The various methods used for freezing may be classified in two main groups: (1) prefreezing, and

(2) evaporative freezing. Prefreezing means that the material is frozen before it is placed under vacuum. Freezing may be done in conventional equipment or on refrigerated shelves in the freeze-dryer. When evaporative freezing is used, the unfrozen material is placed in the dryer, the chamber is evacuated, and freezing is effected by evaporative cooling. Evaporative freezing is unsatisfactory for meat products. Surface drying takes place in unfrozen meat and causes "case-hardening," which results in a lower rate of drying and a product of poor quality (18).

V. CELL MEMBRANE AND ITS PENETRATION AND REHYDRATION

Cell Membrane and Penetration

The cell is the unit of structure of tissue. Each cell possesses a thin cell membrane which serves as the site of contact between cells and their environment. The membrane regulates the transfer of water, ions and other nutritive material between the outside and inside of the cell. An equilibrium between cellular components and the surrounding fluid affects the physiology of cells. This equilibrium may still influence physiological properties for a short

time after death. Chemical fixation or drying destroys the regulating powers of cell membranes. Maintenance of proper fluid pressure in living cells is governed by the laws of osmosis. A fluid with the same osmotic pressure as intracellular solutions is isotonic with the cell. Solutions whose osmotic pressure is greater than that inside a cell are hypertonic, while those having a smaller pressure are hypotonic. The membrane of a cell allows water, certain ions and some non-ionized substances to pass into and from the cell, but it retains the cell contents. Fluid surrounding a cell is isotonic when it contains certain dissolved substances in an amount which prevents exchange of water between the fluid and the substances inside the cell. Under this condition no osmotic pressure differential is exerted across the cell walls (10).

Hechter (19) indicated that a relatively thin lipoprotein layer influences translocation of solutes into and from the cell. The unit-membrane concept is an hypothesis which indicates that all biological membranes consist of two lipid monolayers sandwiched between two fully spread monolayers of nonlipid components. The two nonlipid layers are generally thought to be composed of protein.

Hechter (19) also indicated that various treatments may depolarize the cell membrane, resulting in changes of molecular configuration in the lipoprotein layers. The hexagonal protein subunits in these layers may become globular or partially helical; the lipid phase may develop a micellar arrangement. According to the unit-membrane concept, pores or channels extend through the lipoprotein layers. These openings regulate the permeability of the cell membrane. There are fixed sites which are electrostatically charged, although they no longer exhibit an orderly arrangement. As a result of depolarization, the water in the membrane channels becomes less organized in relation to the peptide surface. It also becomes more mobile and increasingly more available as solvent for ionic diffusion. The modified water creates new channels which allow sodium (Na) and potassium (K) ions to diffuse freely into and from the cell, depending on the electrochemical gradient (19).

Steward (38) showed that the selective permeability of protoplasm to salts, sugars, and other substances is characteristic only as long as the cells are alive. Dead protoplasm is permeable to almost all dissolved substances. The increased permeability does not always occur immediately

upon death. It may occur gradually to allow protoplasm to become permeable first to ions and relatively small molecules. Later, they become more permeable to increasingly larger molecules. Such plasma membranes assume the properties of molecular sieves of gradual increasing pore size.

These selective pores and channels permit cell membranes to exhibit high selectivity for both ions and non-electrolytes. Selectivity of the membrane for various cations depends upon the size and shape of the cation after hydration. The K ion has about one or two water molecules in its immediate hydration shell, whereas Na has at least six (19, 24).

Pederson and Albury (30) demonstrated the vascular system of cucumbers by soaking them in a brine-dye solution. Cucumbers absorb salt and acid from the brine during fermentation and curing. The brine enters by diffusion through various openings in the skin, that is, the stomata, the vascular system, and the calyx tube. According to these authors, a proportionately larger amount of brine enters the mature cucumbers through these organs than through the epiderm.

Rehydration

Baker, Kulp, and Miller (4) stated that pectins are probably the most important class of substances affecting rehydration and that their action varies with the degree of methylation.

The degree and rate of rehydration apparently depend upon the directness and distance of water routes into the tissue. The level of rehydration decreased as the rehydrating solution was made more hypertonic. The highest level of rehydration was observed in demineralized water. Another factor influencing rehydration is pH. In all cases, regardless of salt concentration, the highest level of rehydration occurred at approximately pH 7 (2).

VI. AUTORADIOGRAPHIC TECHNIQUES

The autoradiographic technique is based on the incorporation of radioactive isotopes in biological materials. The site of the incorporated isotope is detected by use of a photographic emulsion. The site of activity is ascertained by a deposit of silver grains remaining on the emulsion after it has been exposed to the radioactive specimen and has been developed. Autoradiographs are developed on several scales.

For example, on the macro scale whole plants can be used, where the localization is usually observed in terms of organs. On the micro scale localization can be established in the components such as cells and cell parts. The most widely used radioactive isotopes (H^3, C^{14}, S^{36}, P^{32}) emit beta particles upon disintegration. A beta particle ionizes matter through which it passes. The number of silver grains activated by a beta particle is determined by the velocity of the ionizing particle, the thickness of the emulsion, and the size and number of silver halide crystals (5, 23).

In autoradiography the amount of radioisotope exposure necessary to produce an autoradiogram within a specified period of time must be determined. The isotope concentration and the exposure time are inversely related: the higher the activity, the shorter the exposure time, and vice versa. The exposure time is usually determined by counting the radioactivity of the specimen with a Geiger counter (7, 8, 15).

Jensen (23) stated that resolution is affected by the following factors:

1. Energy--the energy level of the ionizing particles affects the resolution. The higher the energy level,

the further the particle will penetrate into the emulsion and the larger the halo formed.

2. Emulsion--the size, number and uniformity of the silver bromide crystals will affect the resolution. The greater the number of crystals present, the greater the possibility that a beta particle will ionize a crystal in close proximity to the site of the isotope, thus yielding a more precise image of the specimen. Relatively large crystals are more sensitive than smaller crystals.

3. Thickness of the emulsion and the specimen--the thicker the emulsion, the larger the halo formed. The geometry between emulsion and radioactive isotope influences the resolution; an increase in geometry produces poorer resolution.

4. Self-absorption--biological tissue absorbs many of the beta particles, particularly those possessing a relatively low energy level. As thickness is progressively increased, there is concomitantly greater absorption.

CHAPTER III

MATERIALS AND METHODS

I. PLANT MATERIAL UTILIZED

The Ozark Beauty variety of strawberries was used in this study. The berries were supplied by a commercial grower in Dayton, Tennessee, and were shipped to Knoxville. Upon arrival, the strawberries were placed in storage at 40°F. until processed.

II. TISSUE SECTION PREPARATION OF FRESH BERRIES

The berries were removed from storage, washed thoroughly, and all unsound and malshaped berries removed. The berries were sliced into both transverse and longitudinal sections (2 mm. thick) with a sharp razor blade.

Paraffin Method

The following procedure was utilized to prepare paraffin-embedded specimens for microscopic study. The procedure of Brooks, Bradley, and Anderson (6) was used with modifications.

Killing and fixing. The sections were immersed and held in FPA solution for three days. This solution consisted of formalin, propionic acid, and 70 per cent ethyl alcohol (1:1:18).

After fixation, the sections were transferred to 70 per cent ethyl alcohol for temporary storage. The achenes were removed before further treatment.

Dehydration. Tissue sections were dehydrated by immersing and holding them in a series of ethyl alcohol solutions of increasing concentrations for 3 hours each. The percentages of alcohol were 85, 95, and 100, respectively.

Clearing. After dehydration, the specimens were immersed and held in toluene for 3 hours and then transferred to a mixture of toluene and paraffin oil (1:1) for 3 hours.

Infiltration. The specimens were removed from the clearing solution to melted paraffin (56 to 57°C.). A vacuum of 28 in. Hg was applied for 3 hours. A paper "boat" was used to prevent direct contact between the specimen and the hot metal walls of the paraffin oven.

Embedding. The specimen was embedded in a block of paraffin. The block was made in a lead mold with a copper bottom. After paraffin was poured into the mold, the specimen was quickly oriented and placed in contact with the copper bottom. A label identifying the specimen was placed on the paraffin surface before it solidified. The finished block was stored in a refrigerator.

Sectioning. Sections (15 μ) were made on a transverse and a longitudinal plane with a rotary microtome.

Affixing. Sections were affixed to slides in the following manner. First, the section was placed on a dry slide. Then, the slide and section were submerged in water, containing a small amount of gelatin, to wet the section. The slide was then placed in an oven and held at 62°C. overnight.

Staining. The section was stained with safranin and fast green dyes. The staining procedure follows:

Safranin and fast green staining schedule

Xylene - 10 minutes
↓
Xylene - 5 minutes
↓

Safranin and fast green staining schedule (continued)

Ethyl alcohol, absolute - 2 minutes

↓

Ethyl alcohol, 95 per cent - 2 minutes

↓

Ethyl alcohol, 85 per cent - 2 minutes

↓

Ethyl alcohol, 70 per cent - 2 minutes

↓

Ethyl alcohol, 50 per cent - 2 minutes

↓

1 per cent safranin in 50 per cent ethyl alcohol and placed in oven at 62°C. for 25 minutes.

↓

Washed in very slow running water until the red color completely disappears from the overflow water.

↓

Ethyl alcohol, 50 per cent - 5 dips

↓

Ethyl alcohol, 70 per cent - 5 dips

↓

Ethyl alcohol, 85 per cent - 5 dips

↓

Ethyl alcohol, 95 per cent - 5 dips

↓

Fast green staining solution for 2 minutes

↓

The staining solution contains the following substances:

1. Solution of ethyl alcohol and methyl cellosolve (1:1 v/v) saturated with fast green dye - 1 part
2. Clove oil - 5 parts
3. Ethyl alcohol - 5 parts

↓

Rinse solution - 2 minutes

↓

Safranin and fast green staining schedule (continued)

The rinse solution consists of absolute ethyl alcohol, clove oil, and xylene (2:1:1).

↓

Xylene and absolute ethyl alcohol (6:1) - 2 minutes

↓

Xylene - 2 minutes

↓

Xylene - 2 minutes

↓

Mount with Permount*

Celloidin Method

The procedure utilized to prepare celloidin-embedded berries was the same as that employed in the paraffin method with the following exceptions (21):

Dehydration. The sections, which were storaged in 70 per cent ethyl alcohol, were placed in 95 per cent ethyl alcohol and held for 24 hours. After this period of time, the sections were transferred to two successive containers of absolute ethyl alcohol and held for 24 hours in each. After soaking in the alcohol, the material was removed and immersed in a mixture of ether and absolute ethyl alcohol (1:1) for 24 hours.

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*Permount is a permanent mounting medium sold by Fisher Scientific Company.

Clearing. The clearing step was omitted.

Infiltration and embedding. The sections were treated in the following manner:

1. After dehydration, the sections were placed in a 10 per cent nitrocellulose (celloidin) medium and held in an air-tight jar for one week. The medium was prepared by dissolving dry nitrocellulose in a mixture of ether and absolute ethyl alcohol (1:1).

2. The sections were then transferred to 35 per cent nitrocellulose medium and held for three weeks. During this period, the ether and alcohol gradually evaporated and the celloidin material became thicker and eventually hard.

3. Celloidin-embedded material was trimmed and affixed to a wooden block using 35 per cent nitrocellulose medium.

4. The block was sealed in a jar containing a small amount of chloroform. The celloidin block became hard in approximately three days.

5. The block was soaked in a mixture of cedar oil and chloroform (1:1) for 24 hours and then in pure cedar oil for one week.

Affixing. After microtoming, the specimen was albumenized and carefully pressed on the slide. A small amount of clove oil was put on the slide and allowed to stand for 5 minutes. The slide was then placed successively in three different containers of 95 per cent ethyl alcohol and held for 5 minutes in each. Nitrocellulose was removed by holding the slide for 5 minutes in each of two successive changes of ether and absolute alcohol (1:1). The slide was then placed in 70 per cent ethyl alcohol and held for 5 minutes. After this treatment, the slide was dried at room temperature in preparation for staining.

III. PREPARATION OF TISSUE SECTIONS FROM FREEZE-DRIED BERRIES

Tissue sections for microscopic study were made from F-D strawberries furnished by a commercial firm* and F-D berries prepared in the laboratory.

The following information was provided by General Foods Corporation concerning preparation of the berries that they supplied.

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*General Foods Corporation, Tarryton, New York.

The variety was probably Shasta, grown in the Salinas Valley of California. It would have been tunnel (blast) frozen in 6-10 minutes, sliced in the frozen state and freeze-dried. The date of preparation would be June or July 1965.

The berries were washed, selected and sliced in a manner similar to that used for preparing fresh berries.

Techniques Utilized for Freeze-Drying the Fruit

Prior to drying, the strawberries were frozen by the following methods:

Air-blast. Sliced tissue (2 mm.) was frozen on a tray in an air-blast freezer (-30°F.).

Freon 12 immersion. Sliced tissue was frozen by immersing and holding in Freon 12 (-180°F.) for 2 minutes.

The frozen tissue was immediately placed in a freeze-dryer.* After the berries were placed in the dryer, a vacuum was applied and the pressure was maintained between 0.15 and 2.00 mm. Hg for 20 hours. The shelf temperature was raised to 90°F. and held. After drying, the berries were stored in jars with a desiccant.

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*Del-Vac, Model 11212 RVM, American Sterilizer Company.

Procedure Utilized for Preparing Specimens for Microscopic Study

Slides from F-D berries were prepared by the paraffin method with the following exceptions:

1. The freeze-drying process was utilized in lieu of the fixing and dehydration steps.

2. The clearing step was omitted.

3. Tissue sections were infiltrated with melted paraffin, under a vacuum of 28 in. Hg for 10 hours.

4. Specimens of 25μ, 30μ, and 35μ thick were obtained from F-D berries previously frozen in Freon 12 and in the air-blast freezer, and supplied by a commercial firm, respectively.

IV. PHOTOMICROGRAPH PREPARATION

From the specimens (slides) photomicrographs were made with magnifications at 5X, 52X, 100X, and 700X.

V. AUTORADIOGRAPHIC STUDY

Material Used for Preparing Autoradiograms

F-D berries previously frozen in the air-blast freezer and by immersion in Freon 12 were used in this

study. Fruit frozen in Freon 12 was cut into halves on a transverse and a longitudinal plane. The berries frozen in the air-blast freezer were not cut.

Preparation of Radioisotope Solutions

Four solutions, each containing a different radioactive substance, were used to study rehydration of the F-D berries. Solutions containing the following substances were prepared and utilized.

1. $NaCl^{36}$. A 10 per cent NaCl solution containing 4 microcuries (μc) of $NaCl^{36}$ per 100 ml.

2. $Ca^{45}Cl_2$. A 0.5 per cent $CaCl_2$ solution containing 4 μc of $Ca^{45}Cl_2$ per 100 ml.

3. C^{14}- citric acid. A 2 per cent citric acid solution containing 4 μc of C^{14}- citric acid per 100 ml.

4. C^{14}- sucrose. A 15 per cent sucrose solution containing 4 μc of C^{14}- sucrose per 100 ml.

Rehydration

The tissue was placed in small cheesecloth bags. The berry halves were immersed and held in the radioactive solutions for the indicated periods of time: $NaCl^{36}$, 3 minutes; $Ca^{45}Cl_2$, 10 minutes; C^{14}- citric acid and C^{14}- sucrose,

30 minutes. The whole berries were held in $NaCl^{36}$ for 3 minutes; $Ca^{45}Cl_2$, 5 minutes; C^{14}- citric acid, 15 minutes; and C^{14}- sucrose, 20 minutes.

Freeze-Drying of the Rehydrated Material

The rehydrated tissue was refrozen in Freon 12 and freeze-dried again.

Specimen Preparation

After drying, the berries were removed from the cheesecloth bag. A section (2 mm. thick) of surface tissue of the berry halves, which had been in direct contact with the radioisotope solution, was removed and discarded. The remaining tissue was sliced into 2 mm. sections on both transverse and longitudinal planes. The berry halves were also cut into 2 mm. transverse-longitudinal and in longitudinal-longitudinal sections. The whole berries were cut in a similar manner; however, no surface section was discarded. Each section was placed on an index card (ca. 3.5 x 4.5 in.) with double-stick tape. The sections were pressed gently with a flat surfaced object. The sections treated with $NaCl^{36}$ and $Ca^{45}Cl_2$ were covered with a single

thickness of household grade Saran wrap; those treated with C^{14}- labelled substances were not covered.

Autoradiographic Technique

The proper exposure time was determined by counting the activity of the specimen (counts per minute per square centimeter of specimen) with a Geiger counter. An aluminum shield with an opening of 0.5 square centimeter was placed over the specimen during counting (7, 8).

In the darkroom, the specimen was placed in contact with Kodak No-screen Medical x-ray film. This film was supplied in "ready packs." The film remained in contact with the specimen long enough to accumulate 2×10^6 beta particles per square centimeter of film (7, 8).

After exposure, the film was developed with Kodak x-ray developer and fixer. Developing time was that recommended by the manufacturer.

Procedure for Preparing Photographic Prints from the Autoradiograms

A "positive" image was obtained by photographing the autoradiogram negative. This step was necessary in order to portray the image on the photograph as it appeared on the

autoradiogram negative. A print was made from the positive, showing the image of the radioactive specimen in 2X magnification. The dark areas on the print indicated the presence of radioisotopes in the specimens.

CHAPTER IV

RESULTS

The results represent two areas of investigation: (1) histological study of strawberry fruit tissues, and (2) autoradiographic study of the distribution of radioactive substances in F-D berries during rehydration.

I. RESULTS OF HISTOLOGICAL STUDY

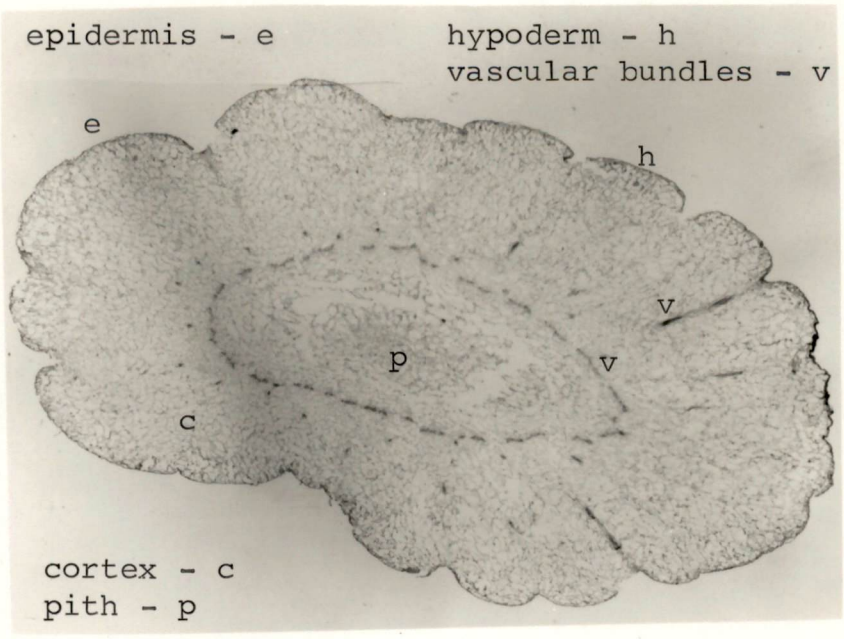
Fresh and F-D berries were utilized. Twenty-eight photomicrographs were prepared to present the cellular organization of berries which were cut on a longitudinal and a transverse plane. Prints show the sections enlarged to 5X, 52X, 100X, and 700X magnifications. By use of the lower magnification the entire section is presented; by utilizing the higher magnifications the individual tissues are shown in more detail.

Fresh Berries

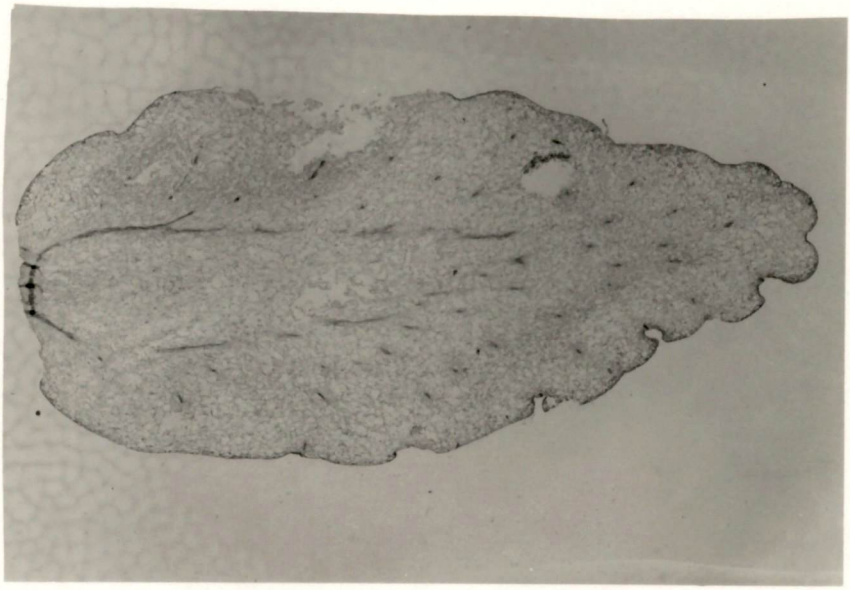
Photomicrographs of tissue prepared with paraffin and with celloidin are presented.

Plate I. Berries were prepared with paraffin and the prints magnified 5X. Epidermal and vascular tissues stained darker than the others. No distinguishing characteristics can be ascertained between the cells of the hypoderm, cortex and pith. On the longitudinal plane the vascular tissue exists as dark areas in a conical formation, tapering toward the blossom end; a circular formation, on the transverse plane. Vessels extend from the vascular tissue and ramify the cortex and hypoderm to the achenes. Observations indicate the presence of relatively large thin-walled cells and large cavities in the cortex and pith. The quantity of structural materials appears limited.

Plate II. The tissue was embedded with paraffin. The prints are enlarged to 100X magnification. The photomicrographs present the epidermal and hypodermal tissues. Epidermal cells are arranged in a single layer and are irregularly rectangular or polygonal in shape. Comparatively, the epidermal cells are much smaller than the parenchyma cells. Also, walls of epidermal cells are thicker than the walls of hypodermal cells. Epidermal cells are closely packed without apparent intercellular spaces.



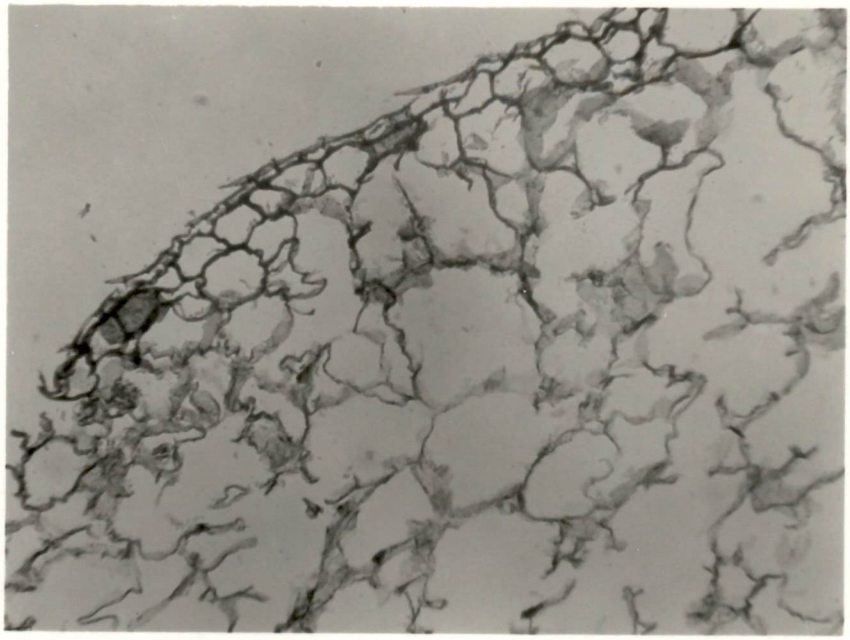
a. Transverse section



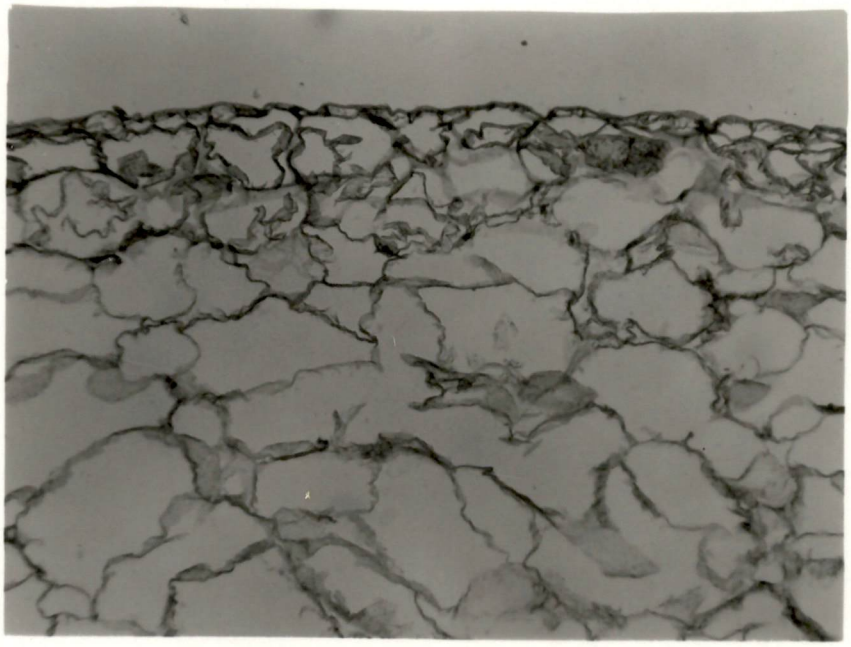
b. Longitudinal section

PLATE I

PHOTOMICROGRAPHS OF FRESH STRAWBERRY TISSUE SHOWING THE WHOLE SECTION--PARAFFIN EMBEDDED. 5X.



a. Transverse section



b. Longitudinal section

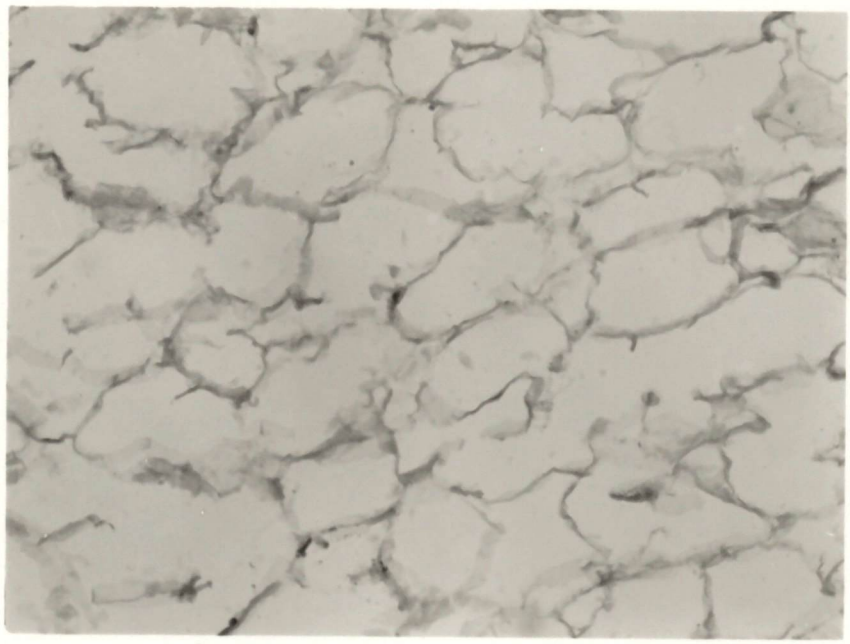
PLATE II

PHOTOMICROGRAPHS OF FRESH STRAWBERRY TISSUE SHOWING EPIDERMIS AND HYPODERM--PARAFFIN EMBEDDED. 100X.

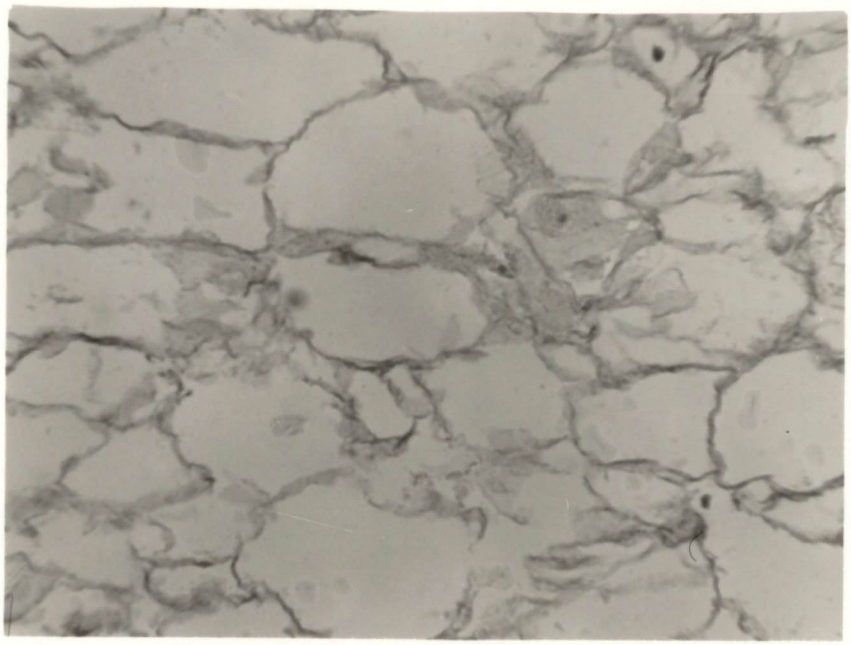
Hypodermal cells are polygonal shaped. There is an increasing gradient of cell size from the epidermis through the hypoderm. There appears to be little or no intercellular spaces in this area. Cell walls of both the epidermis and hypoderm show no damage from embedding with paraffin.

Plate III. The prints, with 100X magnification, present the cortical tissue. The fruit section was embedded with paraffin. Cells are relatively large and thin-walled and they do not appear uniform in size. The cortical cells do not fit together tightly; therefore, intercellular spaces are formed. Cell walls appear distorted and ruptured. The dark spots present appeared to be nuclear material.

Plate IV. These prints present the vascular tissue at 700X magnification. The fruit was embedded with paraffin. Spiral, and annular vessels and large tapering cells arranged end to end are present. These vessels have relatively thick walls. Phloem tissue cannot be distinguished in these photomicrographs. The print showing the "transverse" section does not present the vascular tubes on a true transverse plane.



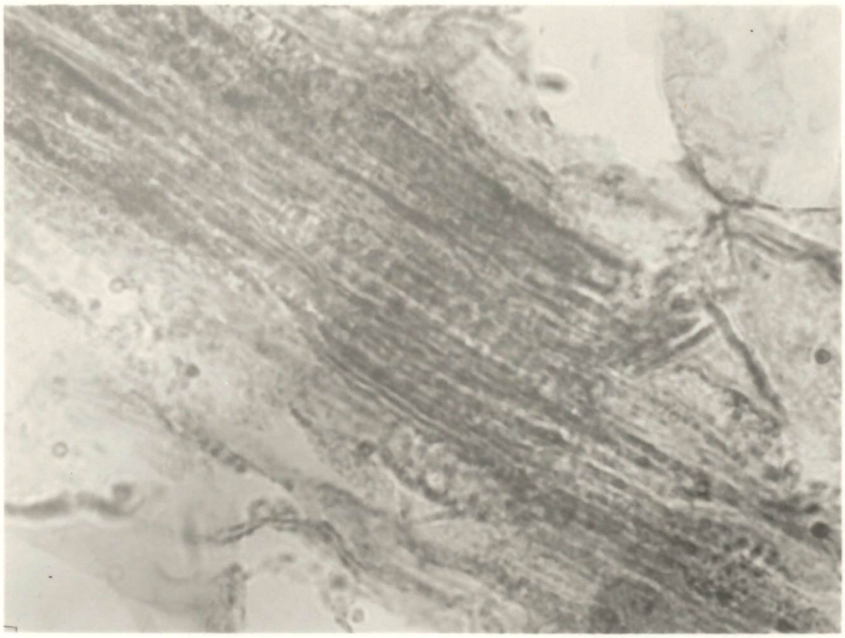
a. Transverse section



b. Longitudinal section

PLATE III

PHOTOMICROGRAPHS OF FRESH STRAWBERRY TISSUE SHOWING CORTEX--PARAFFIN EMBEDDED. 100X.



a. Transverse section

b. Longitudinal section

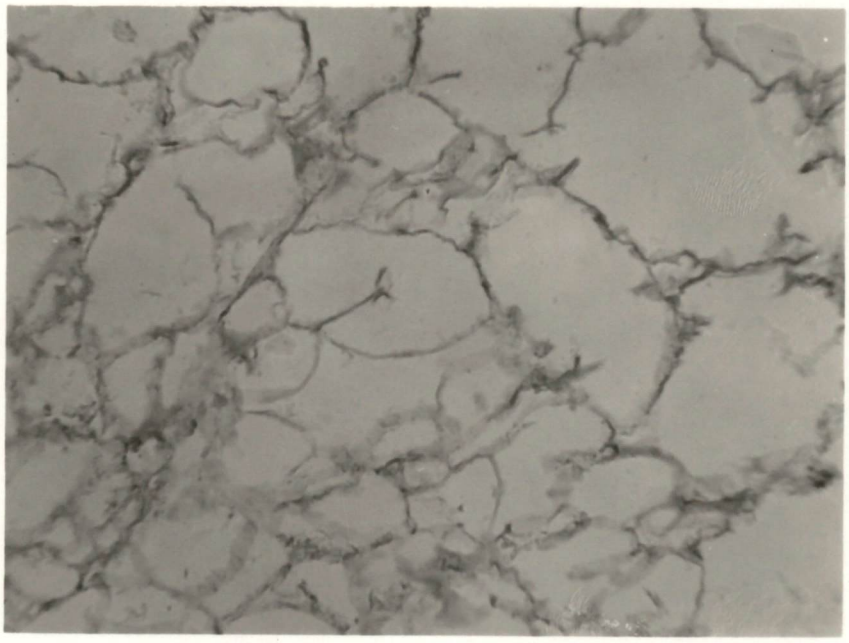
PLATE IV

PHOTOMICROGRAPHS OF FRESH STRAWBERRY TISSUE SHOWING VASCULAR BUNDLES--PARAFFIN EMBEDDED. 700X.

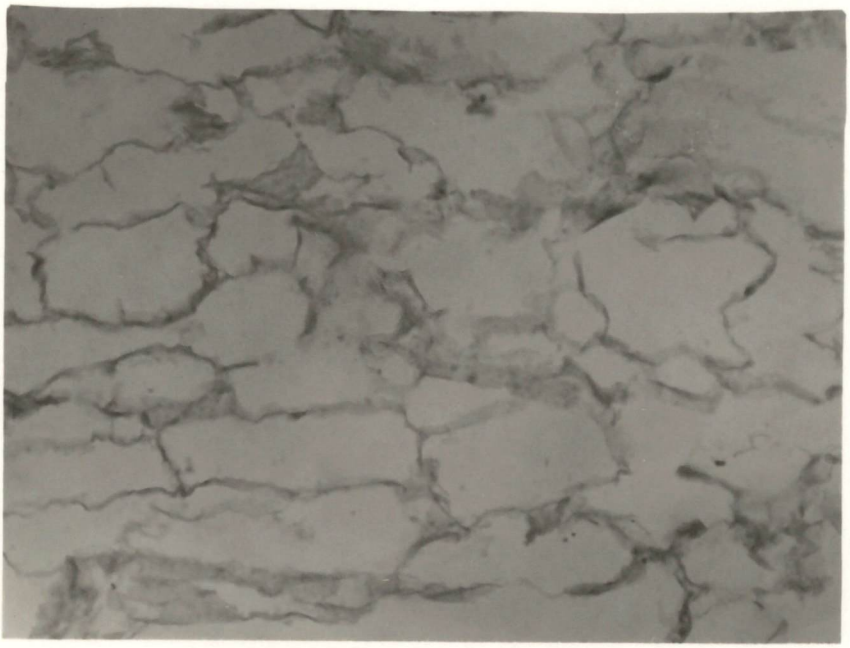
Plate V. The prints, enlarged to 100X magnification, present the pith tissue of strawberry fruit embedded with paraffin. Pith cells appear to be similar to those in the cortex, except they are slightly larger and they have no nuclear material. The cells appear longitudinally elongated, and intercellular spaces are present. Cell walls were broken.

Plate VI. Photomicrographs show whole sections cut on a longitudinal and on a transverse plane with 5X magnification. Celloidin was used to embed the section. Cellular material from celloidin embedding stained somewhat darker than material prepared with paraffin. Large cavities are present in both the cortex and pith areas; there is no evidence that these voids were formed as a result of utilizing the celloidin-embedding technique.

Plate VII. Prints, showing 100X magnification, present the cortex. The fruit was embedded with celloidin. Cell walls appear thicker than those of the paraffin-embedded tissue. Nuclear material appears as dark, dense spots and intercellular spaces are evident.



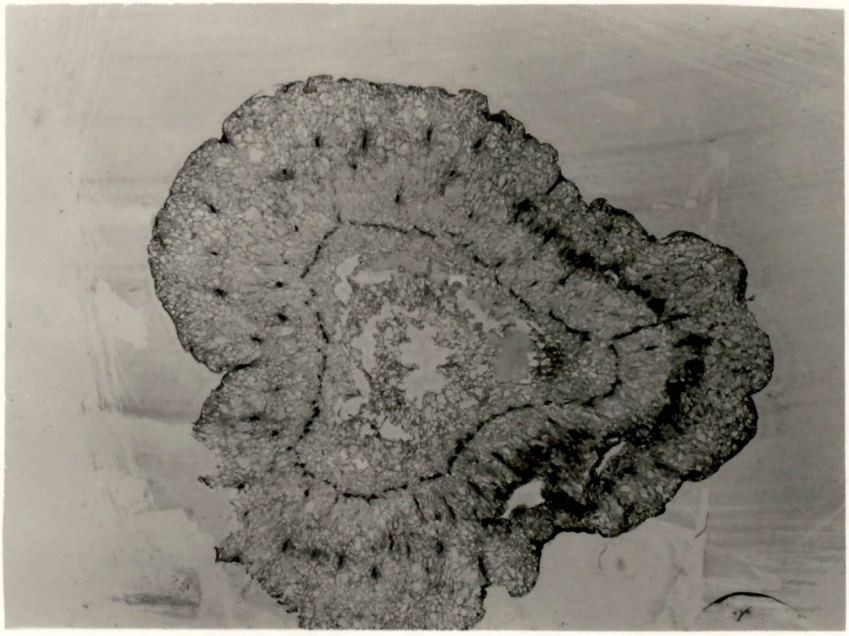
a. Transverse section



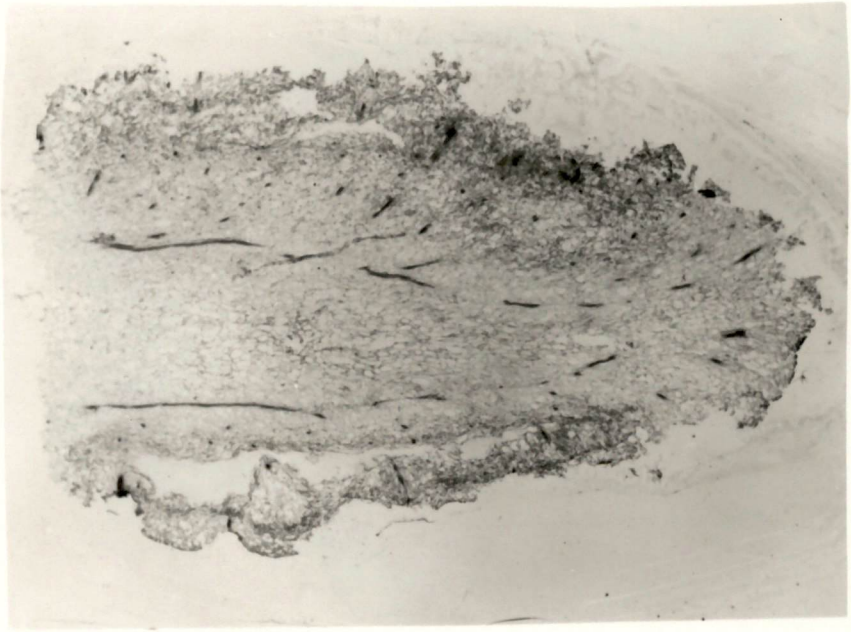
b. Longitudinal section

PLATE V

PHOTOMICROGRAPHS OF FRESH STRAWBERRY TISSUE SHOWING PITH--PARAFFIN EMBEDDED. 100X.



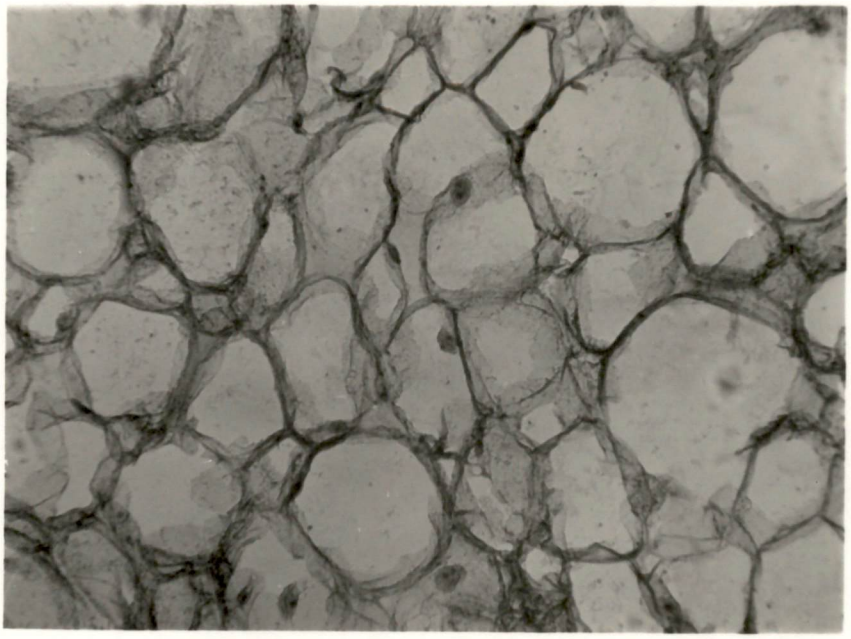
a. Transverse section



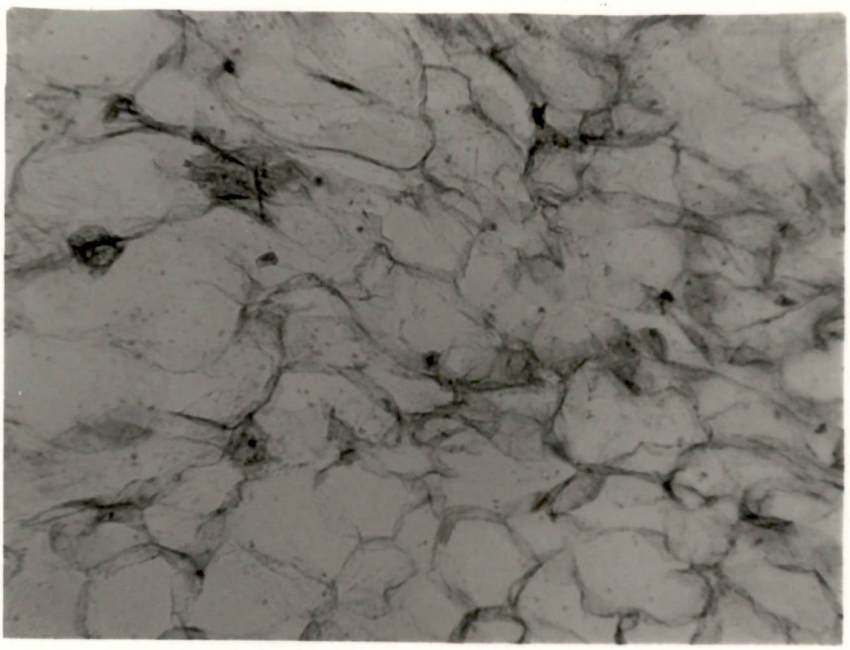
b. Longitudinal section

PLATE VI

PHOTOMICROGRAPHS OF FRESH STRAWBERRY TISSUE SHOWING THE WHOLE SECTION--CELLOIDIN EMBEDDED. 5X.



a. Transverse section



b. Longitudinal section

PLATE VII

PHOTOMICROGRAPHS OF FRESH STRAWBERRY TISSUE SHOWING CORTEX--CELLOIDIN EMBEDDED. 100X.

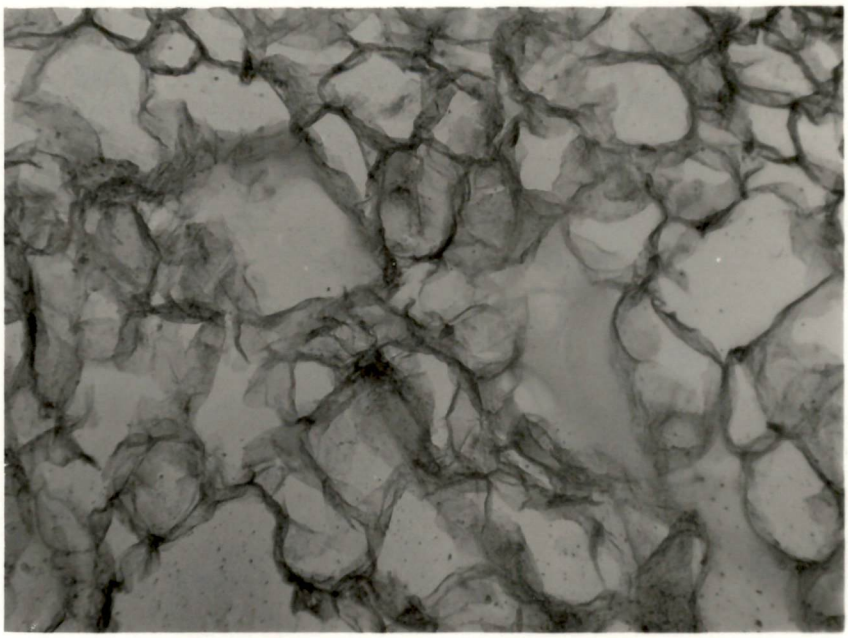
Plate VIII. Prints enlarged to 100X magnification present the pith cells. The fruit section was embedded with celloidin. Cell walls appear thicker than the walls of paraffin-embedded material. Cells are slightly longitudinally elongated. Relatively large intercellular spaces and cavities are evident. No nuclear material is present.

Freeze-Dried Berries

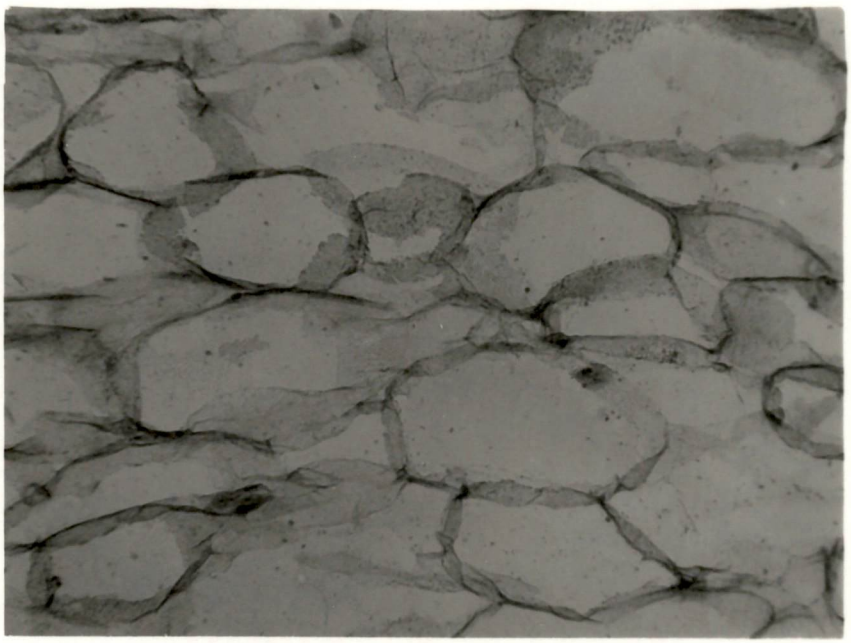
F-D tissues for histological examination were embedded with paraffin. Photomicrographs of F-D tissues from three different methods are presented: (1) berries previously frozen in Freon 12, (2) frozen in air-blast freezer, and (3) prepared by commercial method.

Plate IX. Berries were frozen with Freon 12 and then freeze-dried. Prints were enlarged to 5X magnification. The fruit tissues stained similar to those in fresh berries.

Plate X. Photomicrographs, enlarged to 52X magnification, present three different tissues of F-D berries previously frozen with Freon 12: cortical, vascular and pith tissues. In the print of tissue cut on the transverse plane, the cortical cells are located in the upper part of the



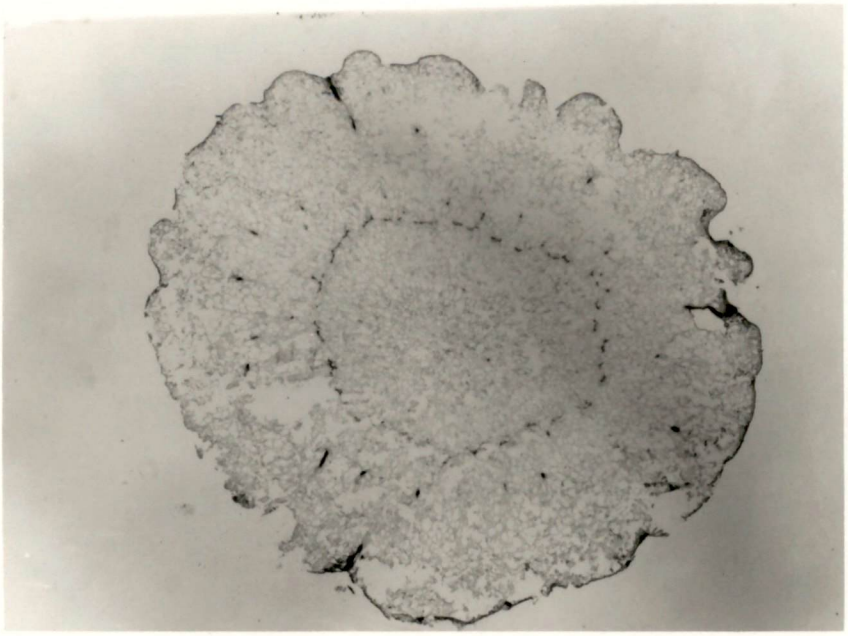
a. Transverse section



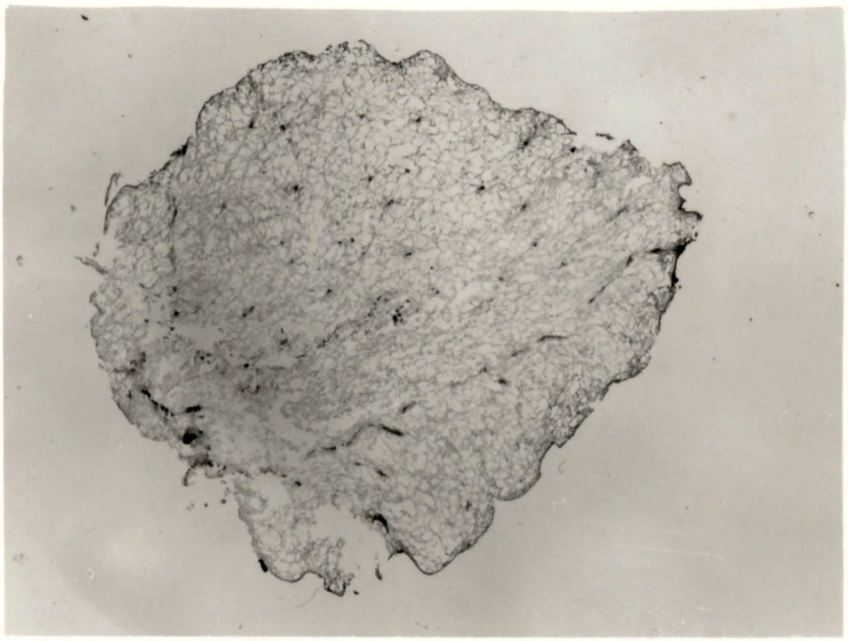
b. Longitudinal section

PLATE VIII

PHOTOMICROGRAPHS OF FRESH STRAWBERRY TISSUE SHOWING PITH--CELLOIDIN EMBEDDED. 100X.



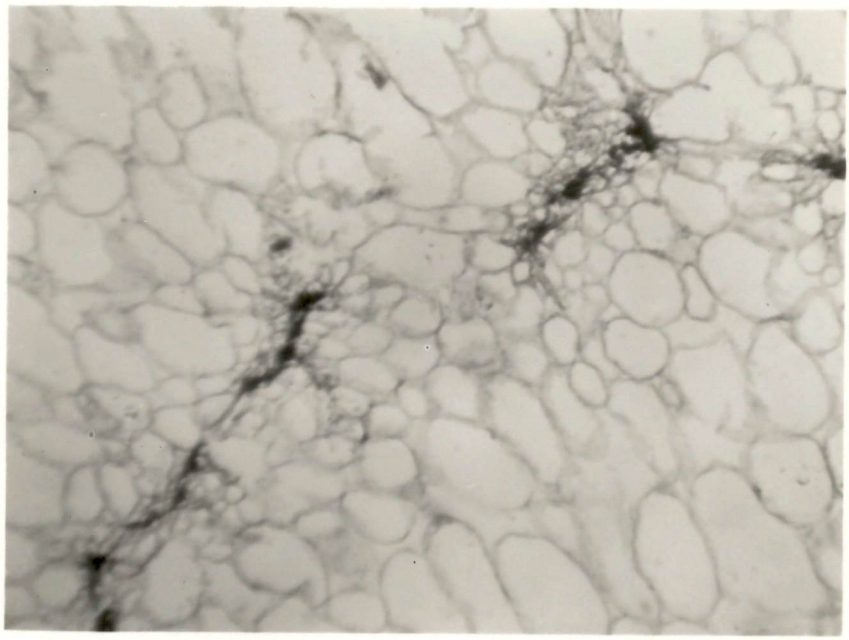
a. Transverse section



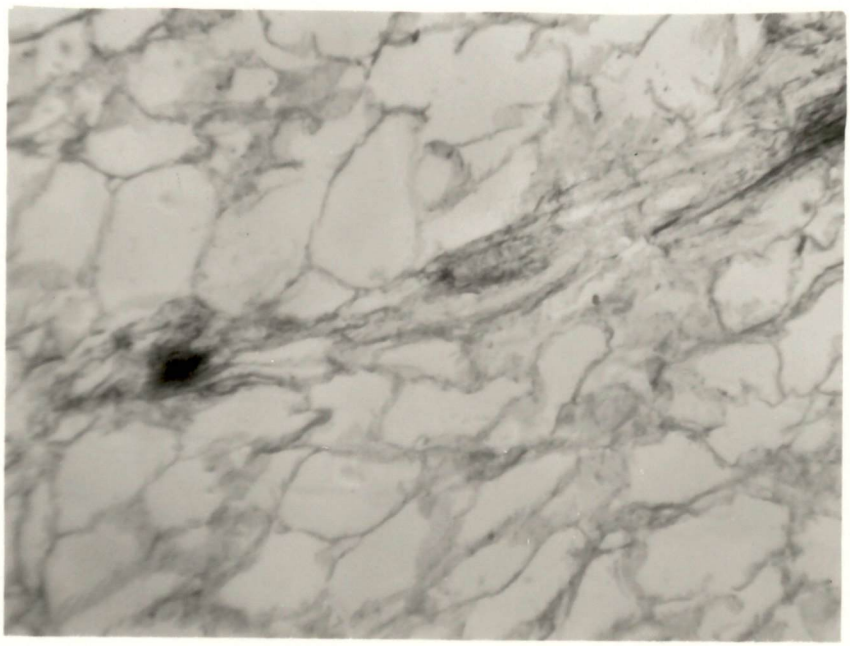
b. Longitudinal section

PLATE IX

PHOTOMICROGRAPHS OF FREEZE-DRIED STRAWBERRY TISSUE (FROZEN IN FREON 12) SHOWING THE WHOLE SECTION--PARAFFIN EMBEDDED. 5X.



a. Transverse section



b. Longitudinal section

PLATE X

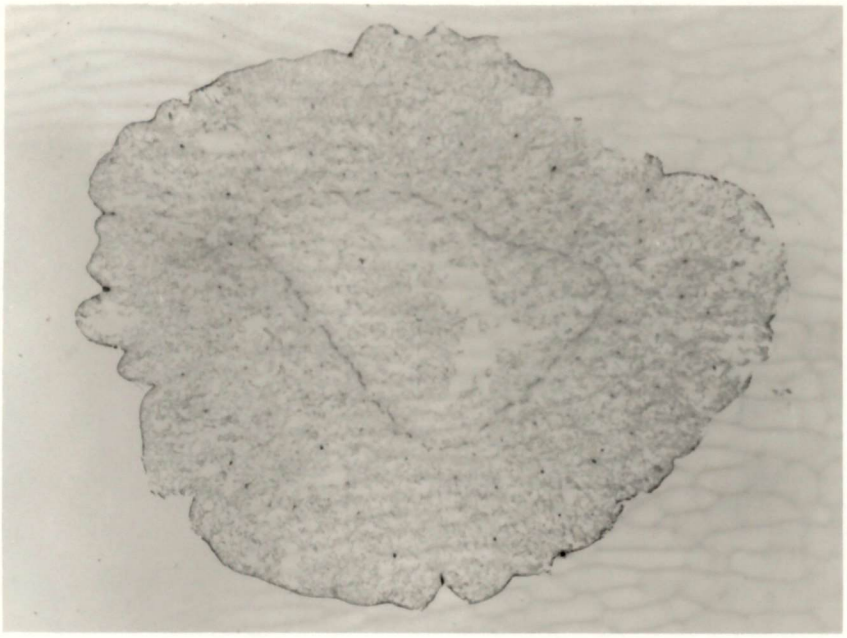
PHOTOMICROGRAPHS OF FREEZE-DRIED STRAWBERRY TISSUE (FROZEN IN FREON 12) SHOWING CORTEX, VASCULAR BUNDLES AND PITH--PARAFFIN EMBEDDED. 52X.

print and vice versa in the print showing the longitudinal section. The parenchyma cells appear to have retained their normal conformation. Transversely, cells are round to oval shaped. On the longitudinal plane the cells appear elongated. Vascular tissue is present in bundles.

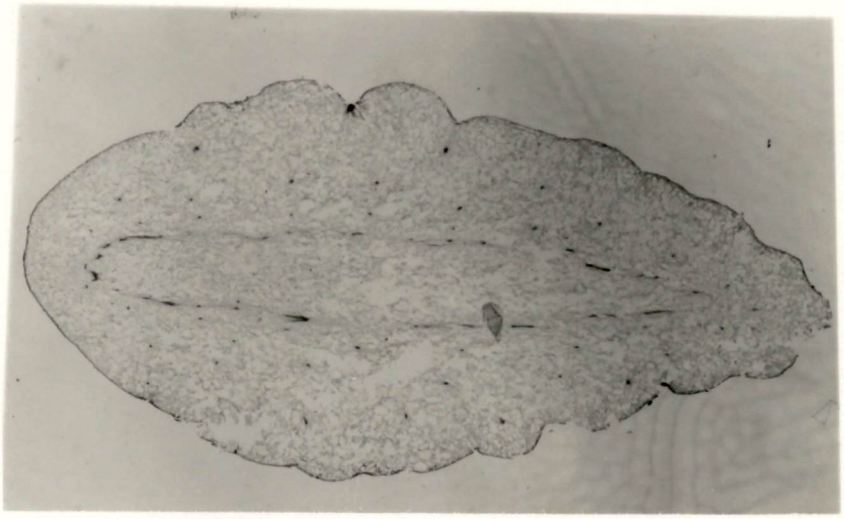
Plate XI. Tissue was previously frozen in the air-blast freezer and then freeze-dried. Photomicrographs were enlarged to 5X magnification. Epidermal and vascular tissues appear as dark areas. The dark spots are severed vascular bundles which ramified the outer tissues.

Plate XII. The tissue was frozen in an air-blast freezer before freeze-drying. Prints were enlarged to 52X magnification. Cortical tissue is located in the lower part of both prints. The cellular identity of the tissue was damaged. Structural integrity was distorted.

Plate XIII. Berries were prepared by a commercial firm. In the prints, enlarged to 5X magnification, the fruit tissues stained similar to those tissues in fresh berries. The overall appearance is similar to that observed in F-D berries frozen in an air-blast freezer (Plate XI).



a. Transverse section

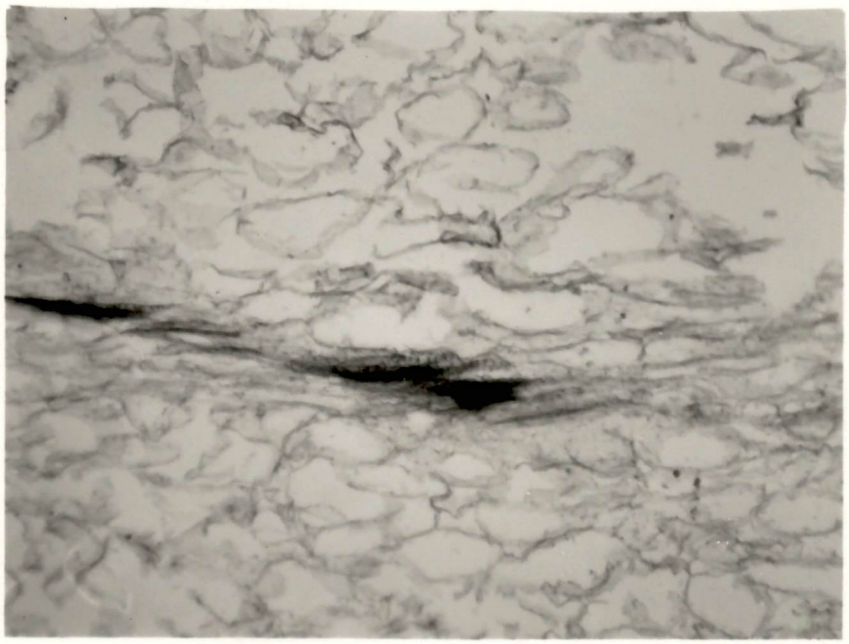


b. Longitudinal section

PLATE XI

PHOTOMICROGRAPHS OF FREEZE-DRIED STRAWBERRY TISSUE (FROZEN IN AIR-BLAST FREEZER) SHOWING THE WHOLE SECTION--PARAFFIN EMBEDDED. 5X.

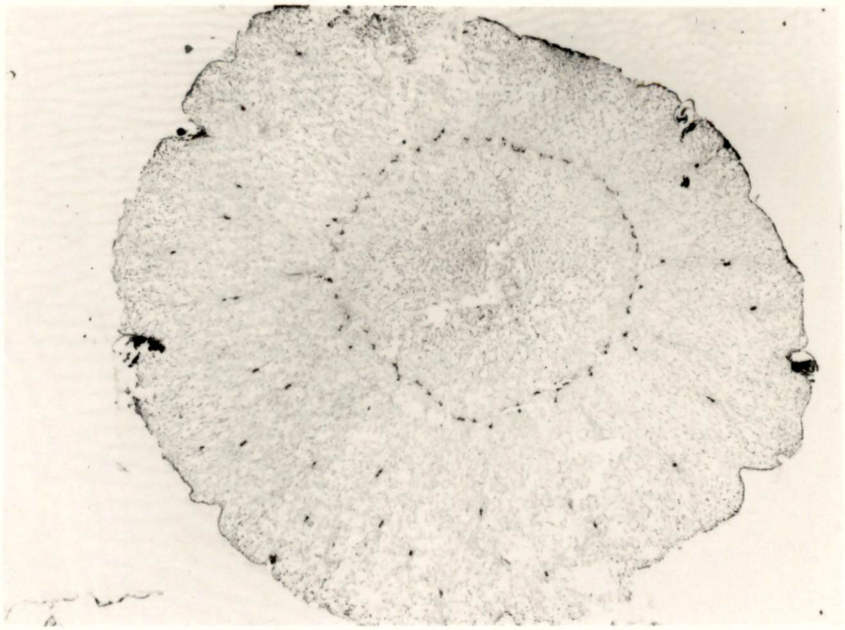
a. Transverse section



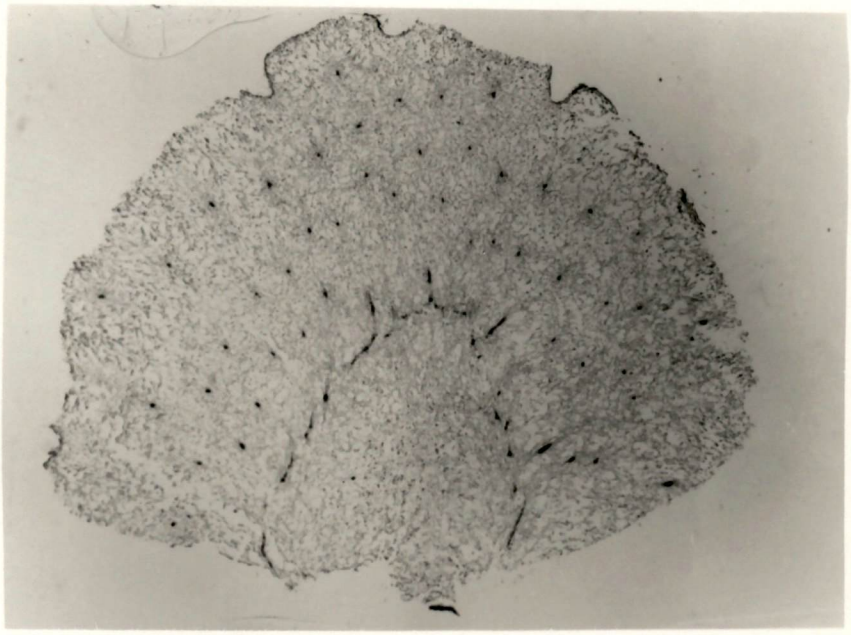
b. Longitudinal section

PLATE XII

PHOTOMICROGRAPHS OF FREEZE-DRIED STRAWBERRY TISSUE (FROZEN IN AIR-BLAST FREEZER) SHOWING CORTEX, VASCULAR BUNDLES AND PITH--PARAFFIN EMBEDDED. 52X.



a. Transverse section



b. Longitudinal section

PLATE XIII

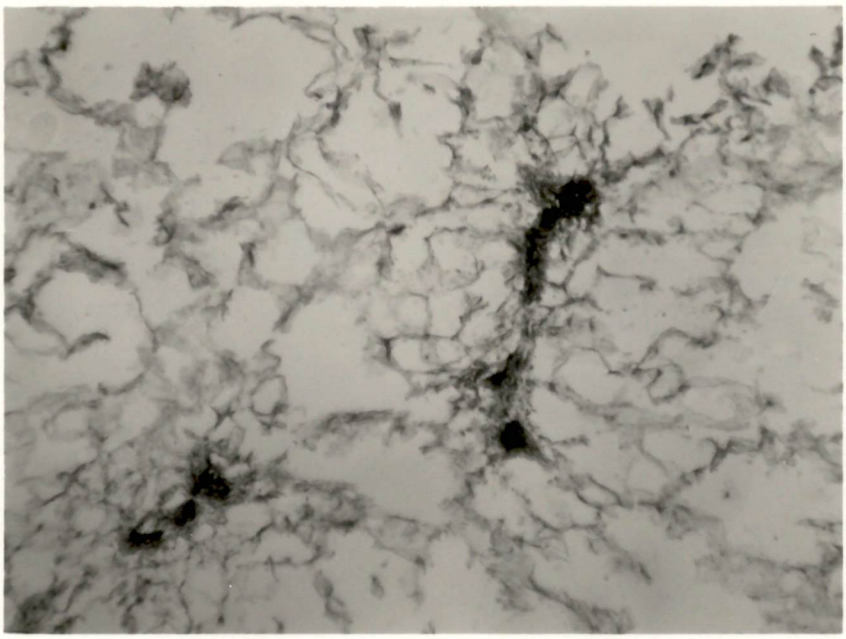
PHOTOMICROGRAPHS OF COMMERCIAL PREPARED FREEZE-DRIED STRAWBERRY TISSUE SHOWING THE WHOLE SECTION--PARAFFIN EMBEDDED. 5X.

Plate XIV. Photomicrographs were enlarged to 52X magnification. The berries were prepared by a commercial method. Cortical tissue is located in the lower right part of Plate XIV(a) and in the lower left part in Plate XIV(b). Cellular identity and structural integrity were adversely affected.

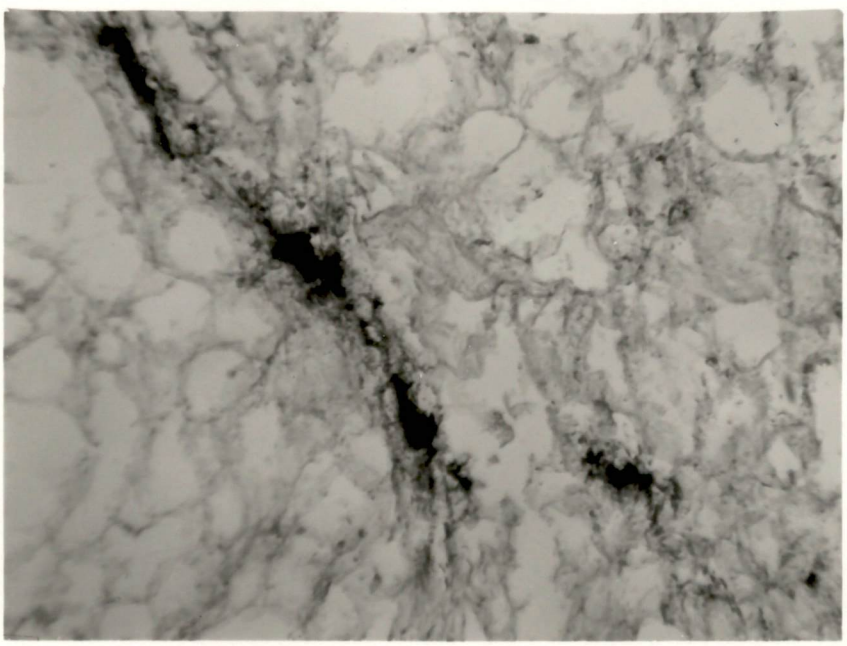
II. PENETRATION AND DISTRIBUTION OF DIFFERENT RADIOACTIVE SUBSTANCES IN FREEZE-DRIED STRAWBERRIES DURING REHYDRATION

Freeze-Dried Berries Previously Frozen in Freon 12

Figure 1. The control autoradiogram shows that no artifacts are produced by the specimen per se. This observation indicates that the darkened areas present in the other autoradiograms were produced by emanations of the radioactive substances. Figure 1(a) and (c) shows the movement of $NaCl^{36}$ into the fruit which was cut into halves on a transverse plane. Figure 1(a) indicates that the $NaCl^{36}$ entered the tissue via vascular tubes and voids. Figure 1(c) shows that the radioisotope was essentially evenly distributed except in the immature tissue, represented by white area. Movement



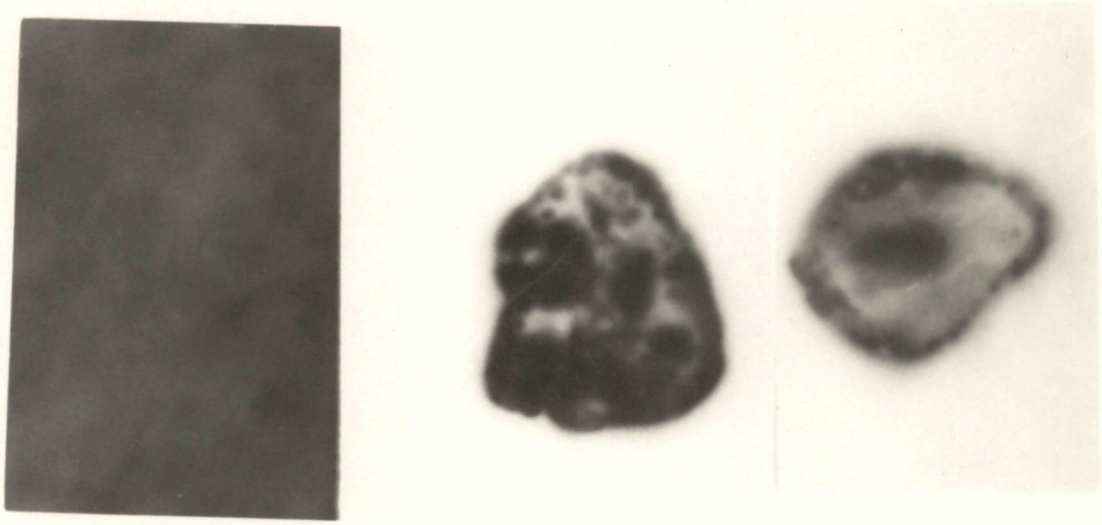
a. Transverse section



b. Longitudinal section

PLATE XIV

PHOTOMICROGRAPHS OF COMMERCIAL PREPARED FREEZE-DRIED STRAWBERRY TISSUE SHOWING CORTEX, VASCULAR BUNDLES AND PITH--PARAFFIN EMBEDDED. 52X.



Control

a. Transverse section

b. Longitudinal section

c. Transverse-longitudinal section

d. Longitudinal-longitudinal section

FIGURE 1

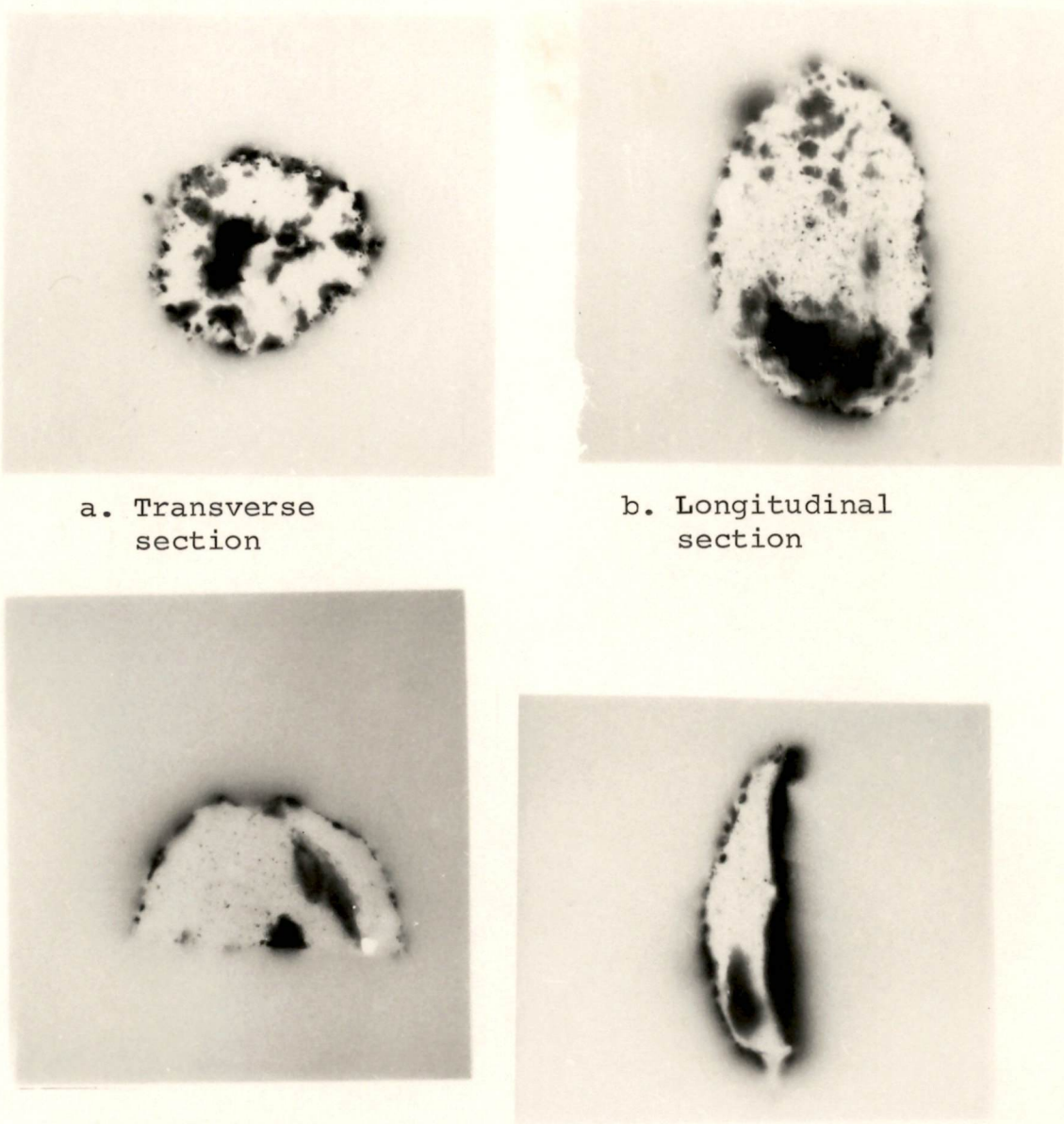
AUTORADIOGRAMS OF FREEZE-DRIED STRAWBERRY TISSUE (FROZEN IN FREON 12) REHYDRATED WITH 10 PER CENT NaCl SOLUTION CONTAINING 4 MICROCURIES OF $NaCl^{36}$ PER 100 MILLILITERS

of $NaCl^{36}$ in berries cut on a longitudinal plane was different from that in berries cut transversely. $NaCl^{36}$ was concentrated in the epidermis and pith of longitudinally cut berries. Penetration of the radioisotope was restricted in radial movement. Fruit sliced on a transverse plane showed a relatively deeper penetration of the salt.

Figure 2. Autoradiograms (a) and (c) show that $Ca^{45}Cl_2$ entered the tissue only through vascular vessels and voids. The radioactive substances penetrated the tissue only slightly. Relatively high activity of $Ca^{45}Cl_2$ is concentrated in the achene recessions. Longitudinally cut berries present relatively high radioisotope concentration in the pith. Little or no $Ca^{45}Cl_2$ penetrated the tissue in a radial direction.

Figure 3. The photoprint indicates that C^{14}- citric acid did not penetrate the intact tissue. Radioactive citric acid was present only at the epidermis and where openings into the tissue occurred.

Figure 4. Results presented in the photoprint indicate that C^{14}- sucrose entered the tissue only through



a. Transverse section

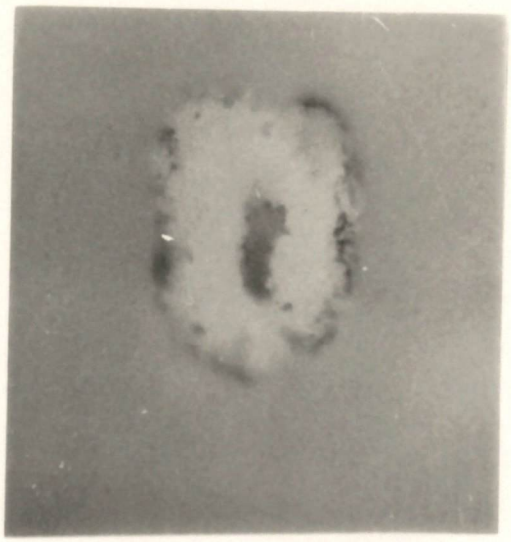
b. Longitudinal section

c. Transverse-longitudinal section

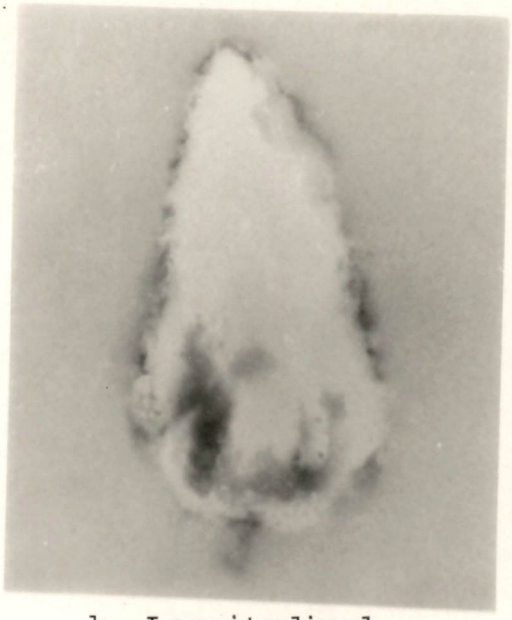
d. Longitudinal-longitudinal section

FIGURE 2

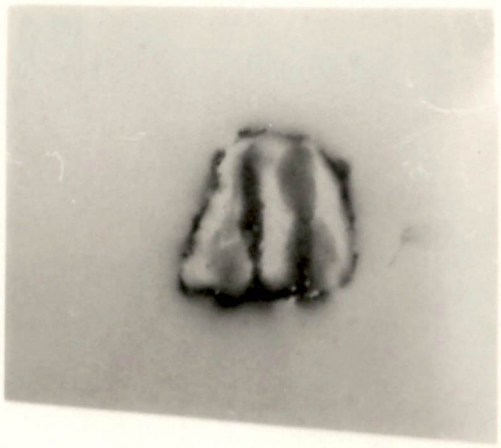
AUTORADIOGRAMS OF FREEZE-DRIED STRAWBERRY TISSUE (FROZEN IN FREON 12) REHYDRATED WITH 0.5 PER CENT $CaCl_2$ SOLUTION CONTAINING 4 MICROCURIES OF $Ca^{45}Cl_2$ PER 100 MILLILITERS



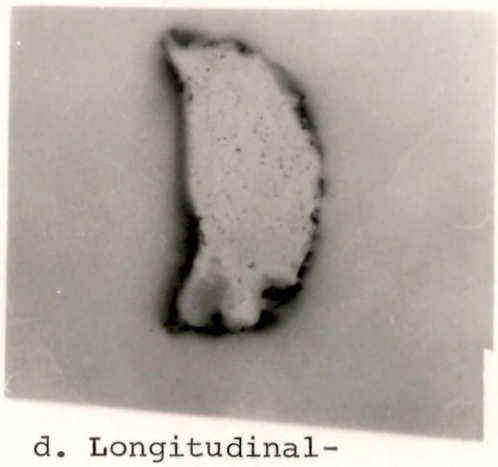
a. Transverse section



b. Longitudinal section



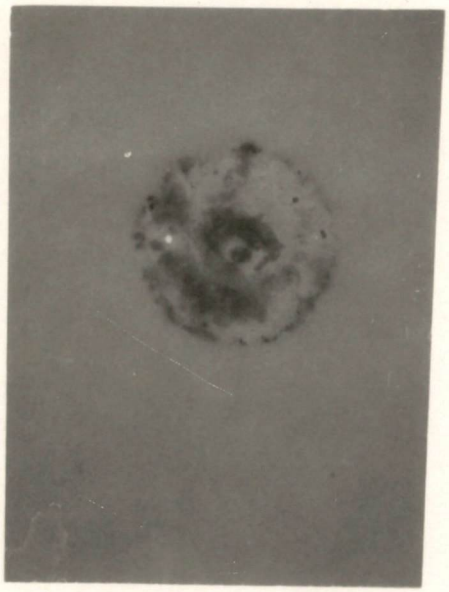
c. Transverse-longitudinal section



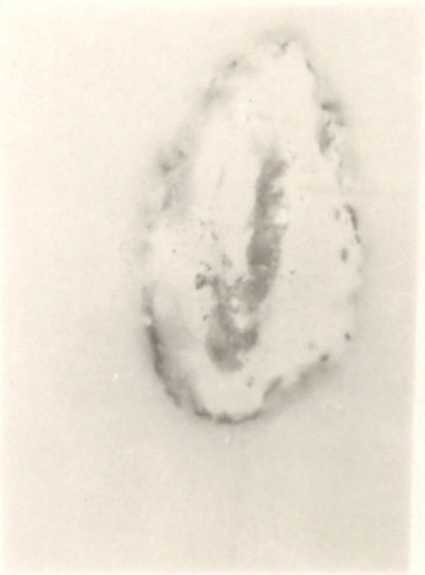
d. Longitudinal-longitudinal section

FIGURE 3

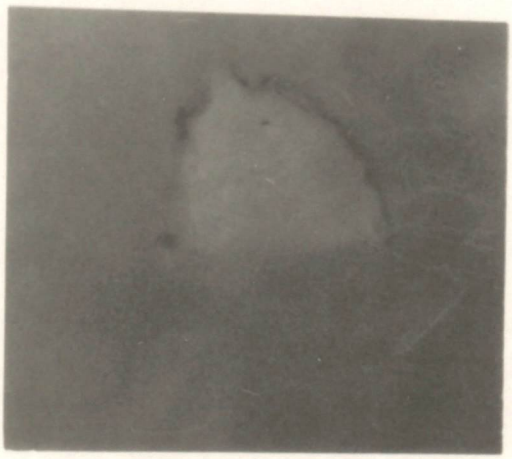
AUTORADIOGRAMS OF FREEZE-DRIED STRAWBERRY TISSUE (FROZEN IN FREON 12) REHYDRATED WITH 2 PER CENT CITRIC ACID SOLUTION CONTAINING 4 MICROCURIES OF C^{14}- CITRIC ACID PER 100 MILLILITERS



a. Transverse section



b. Longitudinal section



c. Transverse-longitudinal section

d. Longitudinal-longitudinal section

FIGURE 4

AUTORADIOGRAMS OF FREEZE-DRIED STRAWBERRY TISSUE (FROZEN IN FREON 12) REHYDRATED WITH 15 PER CENT SUCROSE SOLUTION CONTAINING 4 MICROCURIES OF C^{14}-SUCROSE PER 100 MILLILITERS

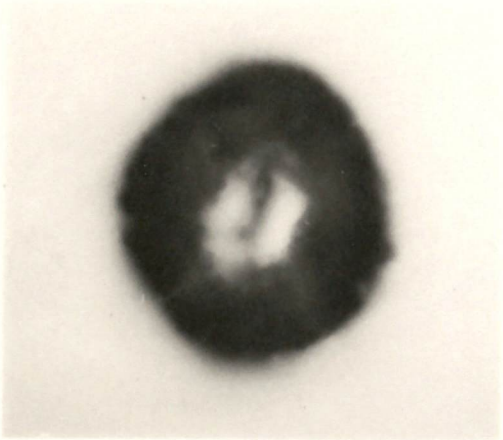
openings in the berries. Essentially no radioactive sucrose passed across the intact tissue. The epidermis and pith shows the greatest concentration of C^{14}- sucrose.

Freeze-Dried Berries Previously Frozen in an Air-Blast Freezer

Figure 5. An essentially even distribution of $NaCl^{36}$ occurred in the entire berry sections with one exception. In Figure 5(b) the light area resulted from the inability of the radioisotope to penetrate the immature tissue. In Figure 5(a) and (c) the light areas indicate voids present in the berries.

Figure 6. Distribution of $Ca^{45}Cl_2$ in the tissue was not uniform. The lightest areas indicate immature tissue; the gray portion represents tissue which appeared to be more mature than in the light region. There is a gradient of decreasing radioisotope concentration from the epidermis to the pith.

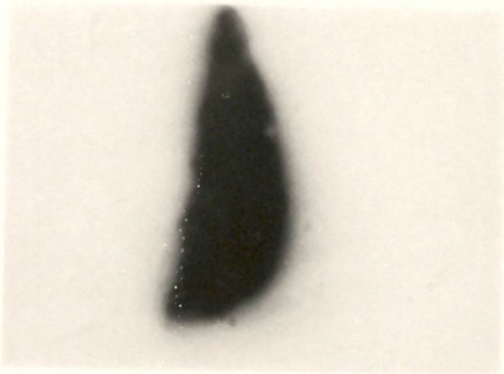
Figure 7. C^{14}- citric acid shows essentially an even distribution in the tissue considered mature. Movement of the radioisotope into relatively immature tissue was



a. Transverse section

b. Longitudinal section

c. Transverse-longitudinal section

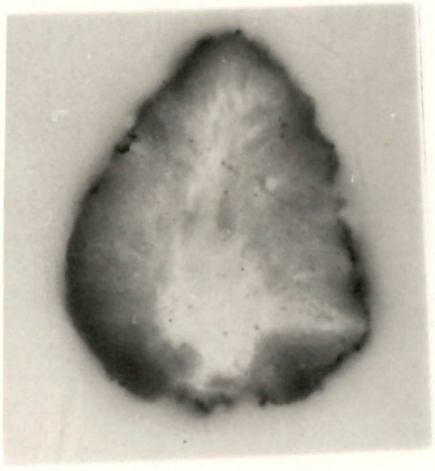


d. Longitudinal-longitudinal section

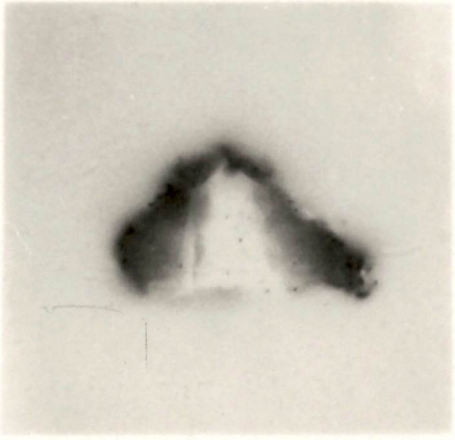
FIGURE 5

AUTORADIOGRAMS OF FREEZE-DRIED STRAWBERRY TISSUE (FROZEN IN AIR-BLAST FREEZER) REHYDRATED WITH 10 PER CENT NaCl SOLUTION CONTAINING 4 MICROCURIES OF $NaCl^{36}$ PER 100 MILLILITERS

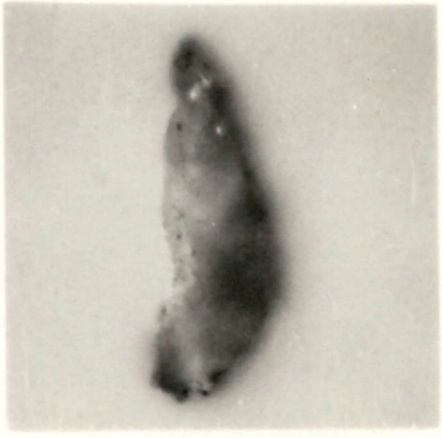
a. Transverse section



b. Longitudinal section



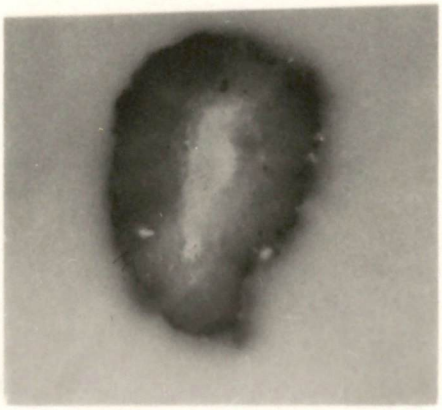
c. Transverse-longitudinal section



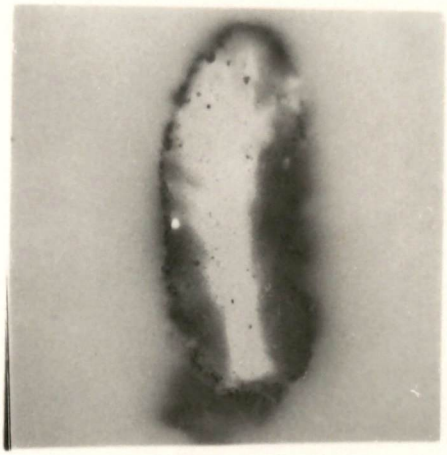
d. Longitudinal-longitudinal section

FIGURE 6

AUTORADIOGRAMS OF FREEZE-DRIED STRAWBERRY TISSUE (FROZEN IN AIR-BLAST FREEZER) REHYDRATED WITH 0.5 PER CENT $Ca^{45}Cl_2$ SOLUTION CONTAINING 4 MICROCURIES OF $Ca^{45}Cl_2$ PER 100 MILLILITERS



a. Transverse section



b. Longitudinal section

c. Transverse-longitudinal section

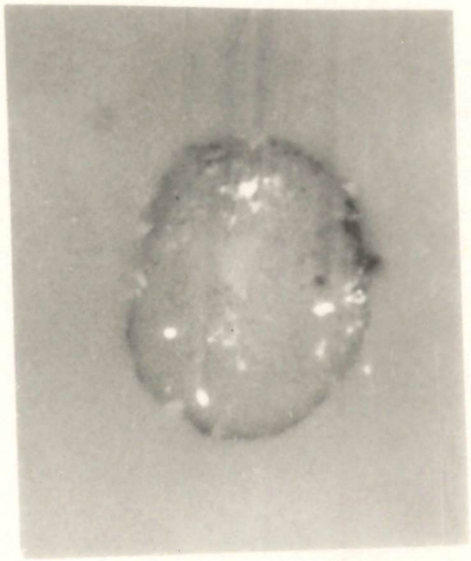
d. Longitudinal-longitudinal section

FIGURE 7

AUTORADIOGRAMS OF FREEZE-DRIED STRAWBERRY TISSUE (FROZEN IN AIR-BLAST FREEZER) REHYDRATED WITH 2 PER CENT CITRIC ACID SOLUTION CONTAINING 4 MICROCURIES OF C^{14}- CITRIC ACID PER 100 MILLILITERS

practically nil. The dark spots in Figure 7(b) and (d) indicate a concentration of radioactive citric acid in the vascular bundles.

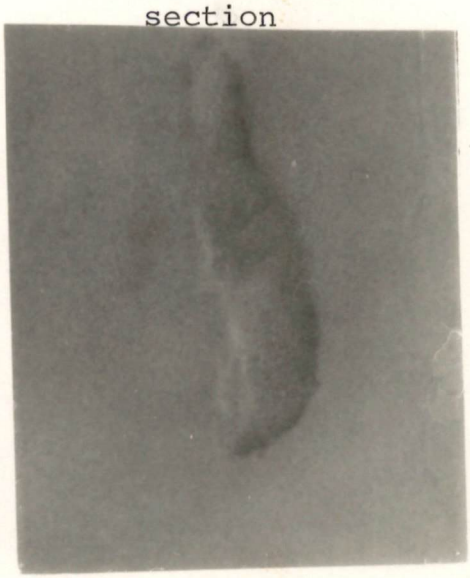
Figure 8. The highest concentration of C^{14}- sucrose present was observed at the outer periphery of the berry. The radioisotope penetrated the mature tissue uniformly; it did not penetrate effectively the immature areas.



a. Transverse section

b. Longitudinal section

c. Transverse-longitudinal section



d. Longitudinal-longitudinal section

FIGURE 8

AUTORADIOGRAMS OF FREEZE-DRIED STRAWBERRY TISSUE (FROZEN IN AIR-BLAST FREEZER) REHYDRATED WITH 15 PER CENT SUCROSE SOLUTION CONTAINING 4 MICROCURIES OF C^{14}- SUCROSE PER 100 MILLILITERS

CHAPTER V

DISCUSSION

The literature presents little or no information concerning the histology of the strawberry fruit. Knowledge of the cellular structure is believed essential for advancement of technology concerning strawberry processing. Histological studies have been conducted on numerous plant materials, but such observations on the strawberry are limited. The reason for inadequate histological investigation of the strawberry is due primarily to the difficulty encountered in conducting such a study. The strawberry is composed of delicate tissues; therefore, embedding and microtoming of such materials present numerous problems not always encountered with certain more rigid structures.

One of the most recent processing methods utilized to preserve strawberries is freeze-drying. Since freeze-drying of strawberries is a relatively new innovation, the effect of this process on the fruit tissue is not well known. Similarly, insufficient information is available concerning rehydration of F-D strawberries.

A histological study was conducted to investigate the cellular structure of fresh strawberries and to determine the effect of freeze-drying on the fruit. In addition, radioactive substances were employed during rehydration to ascertain the penetrability and distribution of different materials in F-D strawberries.

Before a routine histological investigation of the strawberry fruit could be initiated, a preliminary study was conducted to determine the technique most satisfactory for preparing the tissue for sectioning. The berries were embedded with three materials, carbowax, paraffin, and celloidin. The carbowax method proved unsuccessful and was discontinued. The reason for discontinuance was due to inadequate facilities for controlling humidity and temperature which is necessary for the utilization of carbowax.

It was found that fresh berries could be embedded satisfactorily with both paraffin and celloidin. Only paraffin was acceptable as an embedding medium for F-D berries.

For the two histological methods employed, FPA solution was an acceptable fixative for strawberry fruit. It produces less shrinkage of fresh plant tissue than other fixatives (6). Craf's fluid (10) was tried without success.

Tissue treated with this substance was too brittle to cut. The fruit was sliced to allow the fixative fluid to penetrate thoroughly in a relatively short period of time. The achenes were removed to avoid breakage of the specimen during microtoming.

The paraffin-embedding method was applied to fresh berries with a fair degree of success, but it caused the parenchyma cells to become distorted. Because of the high water content of strawberries, the melted paraffin "cooked" the parenchyma cells. The walls of parenchyma cells, being relatively thin, are easily broken. The walls of epidermal and hypodermal cells, and vascular vessels are tougher and thicker than parenchyma cell walls. For this reason these cells apparently maintained their original forms during the paraffin-embedding process. To embed strawberries successfully with paraffin, the procedure must be conducted under a high vacuum (ca. 28 in. Hg).

The paraffin technique was the only method utilized which was satisfactory for embedding F-D berries. The F-D berry tissue will withstand a longer period of infiltration with melted paraffin under high vacuum than fresh tissue, because the cell walls of F-D berry tissue are relatively

tougher. This phenomenon of F-D berries might be due to the comparatively lower moisture content.

The celloidin-embedding method was satisfactory for preparing fresh strawberries. Because no heat was applied in this method, the cell walls apparently were not altered. F-D berries could not be satisfactorily impregnated with celloidin because vacuum or pressure could not be applied to the system. Vacuum removes the solvents (ether and ethyl alcohol) from the celloidin medium. The application of pressure prevents uniform penetration of the celloidin medium due to occluded gases in the tissue.

Both paraffin and celloidin techniques have unique advantages and disadvantages for embedding strawberry fruit. The paraffin method is relatively simple in application; but the applied heat distorted the parenchyma cells of the fruit. The celloidin method allowed the cells to retain their original form; however, the specimen sectioned was relatively thicker than the paraffin-embedded specimen and the time required for embedding is much longer. The application of the celloidin technique is also complicated.

The receptacle, the edible part of strawberry fruit, is composed of five different tissues. The epidermis, with

cells often elongated and forming a continuous layer, exhibits a protective function. The epidermal cells are smaller than the other cells and they are somewhat flattened. The cell walls, being comparatively thick and more lignified, were readily stained. Beneath the epidermis exists hypodermal cells consisting of one or two layers. According to Winton (42) parenchyma cells originate from the meristematic cells of the hypodermal tissue.

The vascular tissue is comprised of phloem and xylem vessels. The xylem is composed of tubes having annular or spiral reinforcement material. Phloem elements could not be identified in the photomicrographs.

Cortical cells at maturation still possessed a nucleus; therefore, they were functional throughout the period of growth. The pith cells did not have a nucleus; they may be considered "dead" cells.

F-D berries previously frozen in Freon 12 were more similar in appearance microscopically to the fresh fruit than F-D berries which were frozen at a slower rate. As a result of rapid freezing, the parenchyma cells showed little or no physical damage. Freezing at the more rapid rate produces relatively small ice crystals, which causes negligible

cellular damage. The cell walls were not ruptured, and they exhibited no apparent evidence of distortion.

The cell walls of fresh tissue embedded with celloidin appeared thicker than cell walls of paraffin-embedded berries. The apparent difference in wall thickness may be due to degradation of cell wall components by heat utilized in the paraffin method.

The parenchyma cells of F-D berries previously frozen in the air-blast freezer and F-D berries obtained from a commercial firm were ruptured by the relatively large ice crystals formed during slow freezing.

The freeze-drying process removed practically all the water from the berries. This result was determined by visual observation. The drying process causes the lattice water, or bound water, to become disorderly arranged and as a result may change the physical, chemical and physiological properties of the cell wall (22).

Autoradiograms were made to investigate the movability of different substances ($NaCl^{36}$, $Ca^{45}Cl_2$, C^{14}-citric acid, and C^{14}- sucrose) into F-D strawberries. The commerically prepared F-D strawberries were omitted because

they had been cut previously into small sections which were difficult to handle in the rehydration operation.

The semipermeability of the cell walls in F-D berries, frozen with Freon 12, was lost. Since the walls no longer exhibited a selective permeability, the radioisotope solutions penetrated into the tissue even though the cells were still intact.

Results obtained from the autoradiographical study depend on factors such as duration of rehydration, the period of time that the specimen and x-ray film are in contact, and the degree of maturation of strawberry fruit. Autoradiograms from the berries previously frozen in Freon 12 show that none of the radioisotopes were evenly distributed in the tissues. Of all the radioactive substances studied $NaCl^{36}$ showed the greatest penetrability; C^{14}- citric acid and C^{14}- sucrose, the least. The ability of these radioisotopes to move into the tissue might be due to certain characteristics of the substances. These characteristics may be the relative amount of water hydration, molecular weight of the substances, and the electrical charges on the substances. The pith had numerous large cavities present; as a result, a relatively large amount of the tracer diffused into the pith.

Since the vascular tissue is composed of conducting vessels, the radioactive solutions moved through these ducts readily. Whole strawberries sliced transversely allowed all tracers to penetrate deeper into the tissue than berries sliced longitudinally. In the transversely cut fruit the radioisotopes move readily into the tissue via the vascular vessels.

Autoradiograms from berries previously frozen in the air-blast freezer indicate that radioisotopes were more evenly distributed in the tissues with the exception of immature parts. The immature tissue apparently was not damaged by ice crystal formation to the extent that mature tissue was damaged. This assumption is based on the fact that mature tissue showed a relatively even distribution of radioisotopes; the immature tissue did not.

The ability of berries to rehydrate is dependent upon the rate at which the fruit was frozen prior to freeze-drying. Where the fruit was frozen in Freon 12, little or no distortion of the tissue occurred; this condition is not conducive to rapid rehydration. Conversely, slow freezing in the air-blast freezer produced considerable cellular and tissue damage. However, the rate of rehydration in this fruit is faster than that in the former.

The experiment was conducted at the University of Tennessee Agricultural Experiment Station Laboratory (U.S., A.E.C.-U.T. Laboratory) located at Oak Ridge, Tennessee. The background radioactivity present in the laboratory was relatively high; therefore, the background (area on the x-ray film not representing the image) of the autoradiogram was frequently dark. This condition sometimes made it difficult to ascertain whether the darkened areas indicating the image was produced by the isotopes under investigation or from background activity.

CHAPTER VI

SUMMARY

This study was conducted to investigate the histology of the fresh strawberry fruit and to ascertain possible damaging effects to the berries caused by freeze-drying. Radioisotopes were employed in the rehydrating medium to determine penetrability and distribution of different substances in F-D strawberries.

To study the histology of fresh berries the following materials and procedures were employed. Berries (Ozark Beauty variety) were fixed in FPA solution. The fixed berries were embedded with paraffin, and celloidin. Specimens for microscopic study were microtomed at 15μ thickness and stained with safranin-fast green dyes.

F-D berries utilized for microscopic study were prepared in the following manner. Fresh berries were frozen with Freon 12, and in an air-blast freezer. The frozen berries were freeze-dried overnight. Commercially prepared strawberries were included in the study. The F-D fruit was then embedded with paraffin and sectioned into specimens of

25, 30, and 35μ thick. Specimens were stained by the procedure utilized to stain fresh material.

F-D berries were rehydrated with media containing different radioactive substances ($NaCl^{36}$, $Ca^{45}Cl_2$, C^{14}-citric acid, and C^{14}- sucrose). The rehydrated fruit was refrozen in Freon 12 and freeze-dried again. From these berries, sections were prepared for autoradiographical study.

The following conclusions may be made from this study:

1. Specimens for histological study of the strawberry fruit can be prepared using paraffin and celloidin as the embedding media.

2. Histological observations indicate that the fruit is composed of five different kinds of tissue, epidermis, hypoderm, cortex, vascular vessels and pith.

3. Microscopic examination of the tissue indicates that the rate of freezing affects the cellular integrity of the berries. Relatively slow freezing (air-blast freezer) causes the cell wall to rupture; also, large voids are produced. A more rapid rate of freezing (Freon 12) prevents these detrimental effects.

4. Autoradiograms of F-D berries showed that the different radioactive substances did not penetrate the tissue to the same extent.

5. $NaCl^{36}$ showed the greatest penetrability; C^{14}-citric acid and C^{14}- sucrose, the least. $Ca^{45}Cl_2$ exhibited an intermediate penetrating ability.

6. Autoradiograms of F-D fruit indicate that the isotopes were more evenly distributed in the berries frozen slowly than in those frozen rapidly.

7. Whole F-D strawberries sliced transversely allowed all tracers to penetrate deeper into the fruit than berries sliced longitudinally. In the transversely cut fruit the radioisotopes moved readily into the tissue via the vascular vessels.

8. The cell walls of fresh tissue embedded with celloidin appeared thicker than cell walls of berries embedded with paraffin.

9. FPA solution was an acceptable fixative for strawberries; Craf's fluid was not satisfactory.

10. Embedding the tissue with paraffin can be facilitated by the application of high vacuum (ca. 28 in. Hg).

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APPENDIX

VITA

The author was born January 19, 1933, in Liaunig Province, Republic of China. He was enrolled at Taiwan Provincial College of Agriculture, Taiwan, Republic of China, from September, 1950, until he received a Bachelor of Science degree in Agricultural Chemistry in June 1954. He then worked for Union Industrial Research Institute in his country. He was enrolled in the Department of Food Technology, The University of Tennessee, and sponsored by Chinese National Council on Science Development, from September, 1964, until he completed the requirements for the Master of Science degree.