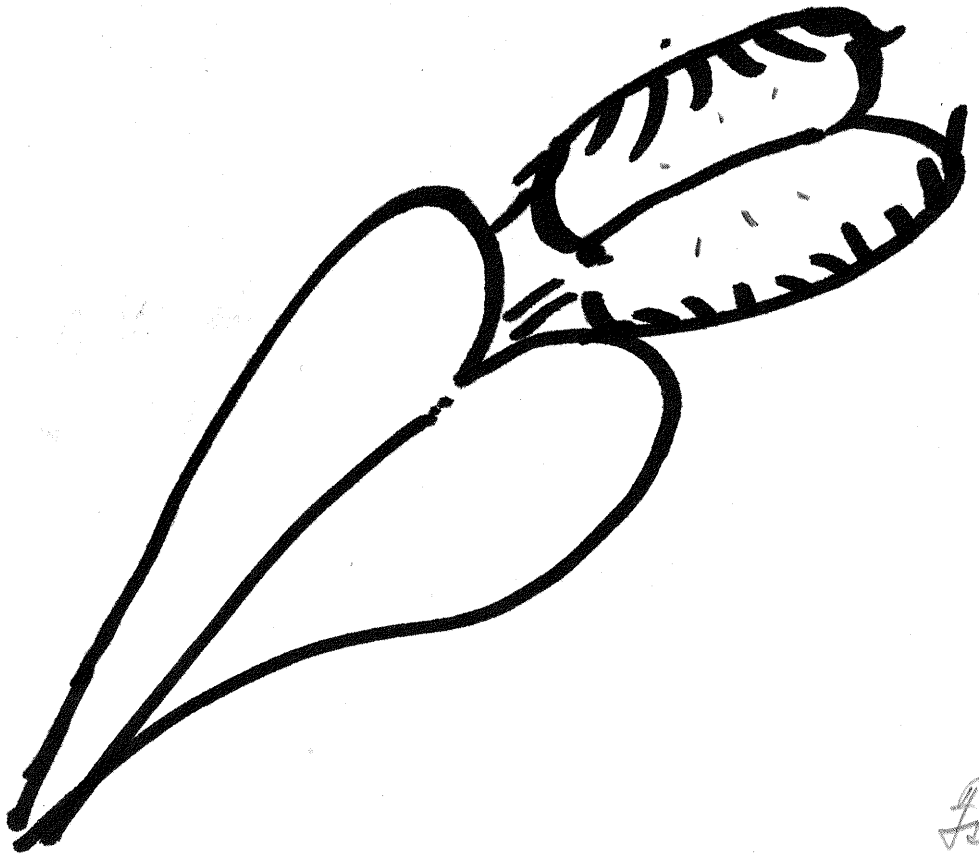
VFT



Francis Barany

BIOCHEMICAL AND BIOPHYSICAL PROPERTIES OF VENUS' FLYTRAPS

INTRODUCTION

The Venus' Flytrap (Dionaea muscipula) is probably the most interesting and certainly the fastest moving of all carnivorous plants. It is endemic to a small area of the United States, namely North and South Carolina. The plant is found in bogs, where there is a lack of nitrates. However, the multitude of insects infesting the bogs compensates for the deficiency of the soil.

I was interested in discovering what happens to captured insects; how the plant derives its nutrients; what are the basic differences between leaf and trap; when, why, and how the trap closes.

EXPERIMENTATION AND DISCUSSION

Isolation of Proteolytic Enzyme (VFT-ase) from Venus' Flytrps

Nature has bestowed upon Venus' Flytrap . . . the ability to perform enzymatic digestion of insects. I have isolated and purified a proteolytic enzyme from the Venus' Flytrap. Two different procedures, the trigger method and the extraction method were worked out as outlined in Scheme 1. The purification of the enzyme by Sephadex G-200 chromatography is shown in Graph 1. The amount of VFT-ase comprises only a small fraction of the total protein.

The purified enzyme was assayed in various media. The experiments demonstrated that salts are needed for activity. Ca^{2+} slightly increases activity, but a reducing agent is not required. VFT-ase is very stable. It retains activity after incubation at $37^{o}C$ for several days. The pH optimum of the enzyme is 5, as shown in Graph 2.

Gel-electrophoresis of the enzyme on polyacrylamide in the presence of sodium dodecyl sulfate at pH 7 showed one band with an approximate molecular weight of 40,000 daltons.

Nitrogen Metabolism

While other plants obtain their nitrogen from the soil, the Venus' Flytrap derives it from captured insects. I have studied the assimilation of nutrients into the plant.

Plants were fed, through the traps, with radioactive (^3H) amino acids and the incorporation was measured in different parts of the Venus' Flytrap as outlined in Scheme 2. Proteins in the traps were found to be heavily labeled, in contrast, only a small amount of radioactivity was present in the leaves, bulbs, and roots. The labeling of carbohydrates was negligible. These results indicate a drastic assimilation of nitrogen compounds into trap proteins while no transformation into carbohydrate constituents takes place. I performed additional experiments (e.g. effects of other nutrients or the effect of light), and they also suggest that the nitrogen and carbon metabolisms are separated.

Nucleic Acids and Proteins in Cell Components

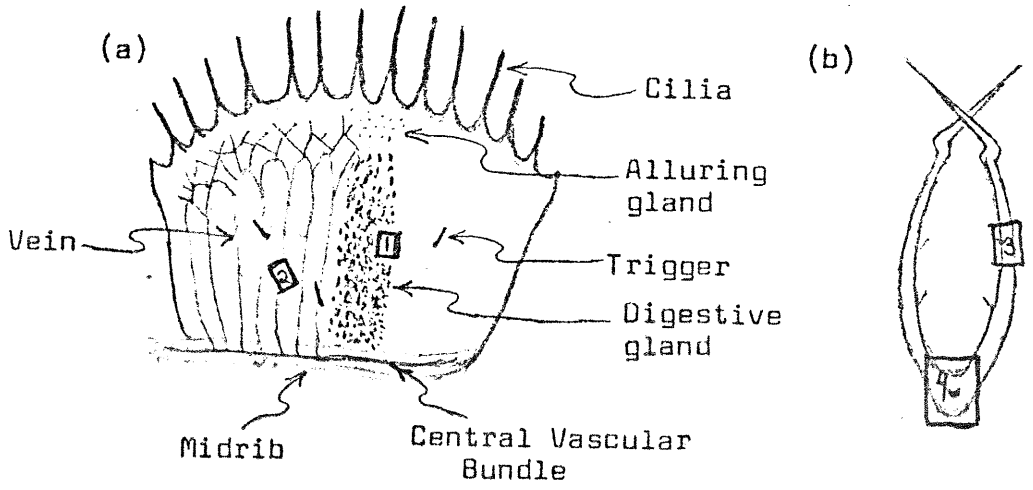
The leaf and trap of the Venus' Flytrap have different functions. I studied the nucleic acid and protein composition in the nuclear, mitochondrial-chloroplast and microsomal fractions of both leaf and trap. The cell components were fractionated according to standard procedure (7). The amount of DNA, RNA, and proteins per gram wet weight were determined respectively by the diphenylamine, orcinol, and

Lowry tests (2). The results are summarized in Table 1. These data point out differences in the various parts of the Venus' Flytrap. We may conclude that the leaf and trap differ greatly not only in function, but also in composition.

Histochemistry

To investigate various biophysical properties of the plant, such as its rapid movement, one must have an accurate knowledge of its structure.

The trap was fixed and embedded as explained in Scheme 3. Slides were stained with hematoxylin and eosin (4). Macroscopic views of the trap are shown below.

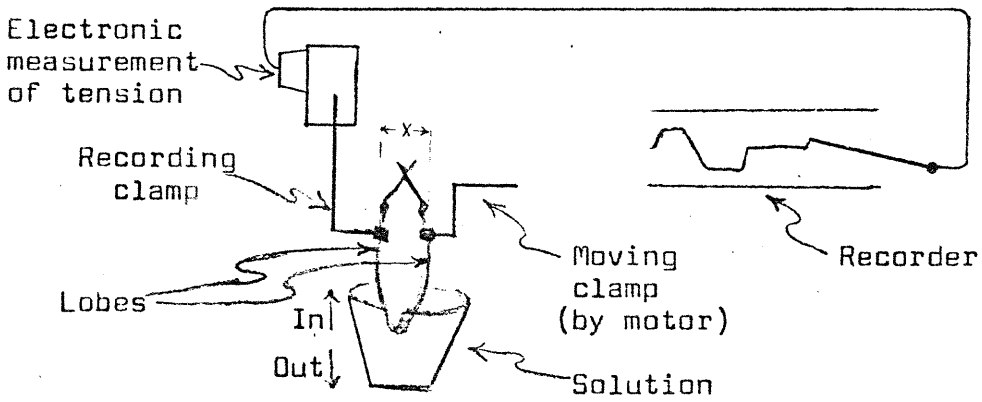


Cross sectional (a) and longitudinal (b) views of trap of Venus' Flytrap, identifying important structures.

Enlarged photographs of the digestive gland (1), vein (2), and longitudinal sections of the lobe (3) and midrib (4) are presented on Plates 1-4, respectively.

Tension

I have studied the tension produced by opening the closed traps. I designed a device which was attached to a polygraph, and enabled me to measure tension in whole traps or in trap-strips of 2mm width. The setup is schematically shown below:



The tension produced by a whole trap follows the equation $F=kx$, where F is the tension produced, k is a constant which depends on the size of the trap and the turgidity of the parenchyma, and x is the distance between the two clamps. Graph 3 shows this relationship.

The effects of various solutions on the tension are summarized in Table 2. All the effects were completely reversible. The data showed that removal of water from the trap by hypertonic solutions decreases tension while hypotonicity increases tension. The effect of ATP appears to be specific, since other anions elicited no change in tension.

Using radioactive ATP as a substrate, I tested the plant's ability to metabolize ATP. The results suggested that the Venus' Flytrap is capable of splitting ATP. This parallels a recent finding that there is a decrease in ATP content of the trap after closure (5).

Trap Closure

Perhaps the most fasinating aspect of the Venus' Flytrap is its rapid movement. The closure is induced by bending one trigger hair twice, or two different hairs once (6).

From the data in the literature and from my own results, I have postulated the following mechanism for the movement.

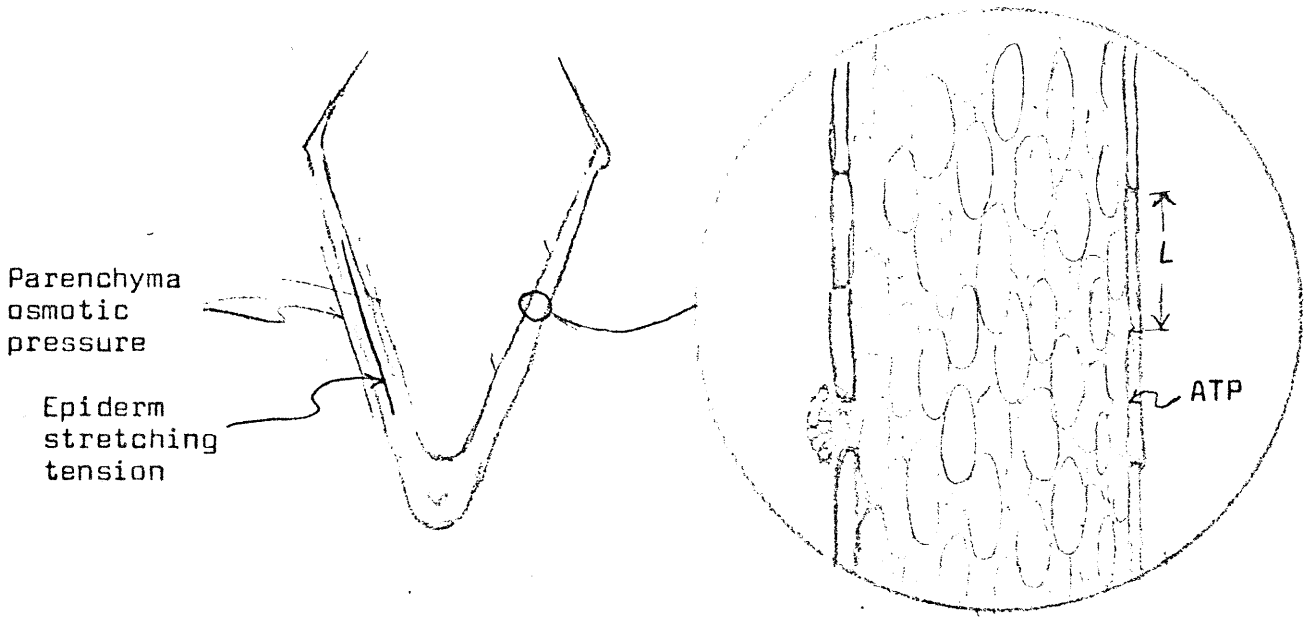
1) In the open condition the tensions in the trap are in equilibrium. The osmotic pressure of the parenchyma is opposed by the stretching of the inner and outer epiderms keeping the parenchyma in a confined volume.

2) Upon stimulation, this equilibrium is upset allowing the parenchyma volume to expand. The trap bends like a bimetallic strip, the outer epiderm stretching 6-7%, the inner scarcely at all. The shift of the equilibrium is correlated with the hydrolysis of ATP, catalyzed by an enzyme which is part of the cell membrane. This causes a change in the shape of the outer epiderm, which lowers the tension and allows further stretching. Consequently the parenchyma can expand.

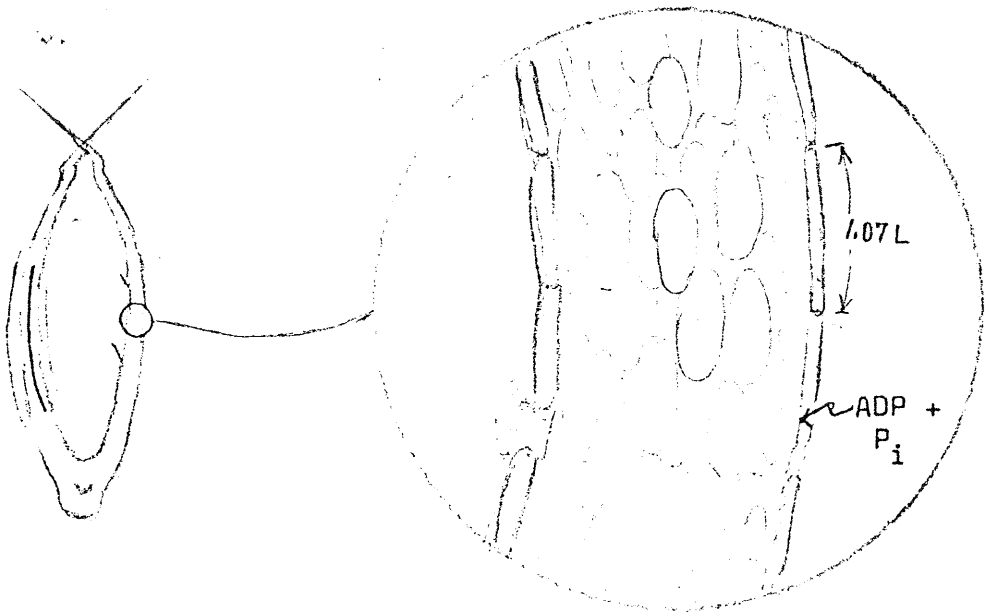
3) The lobes reopen by an increased growth of the inner epiderm, while the expansion of the outer epiderm is retained. This uneven growth resets the tension equilibrium.

The following drawing embodies the key features of my theory.

Longitudinal section of open trap of Venus' Flytrap showing tension equilibrium before closing. Enlargement shows outer epiderm with ATP.



Longitudinal section of closed trap. Enlargement shows the breakdown of ATP, which allows the outer epiderm to stretch by 7%.



SUMMARY

1. The proteolytic enzyme of the Venus' Flytrap, which digests captured insects, was isolated for the first time, purified and characterized.

2. It was demonstrated that the carbon (autotrophic) and nitrogen (heterotrophic) metabolisms are separated.

3. The leaves and traps exhibited different DNA, RNA, and protein composition in the nuclei, mitochondria, chloroplasts and microsomes.

4. Various important structures of the trap were analyzed using histochemical techniques.

5. Tension produced by a trap was measured and described by a linear equation.

6. The removal of water by hypertonic solutions decreased the tension in the trap, while hypotonicity increased the tension.

7. A mechanism for trap closure was proposed.

ACKNOWLEDGEMENT

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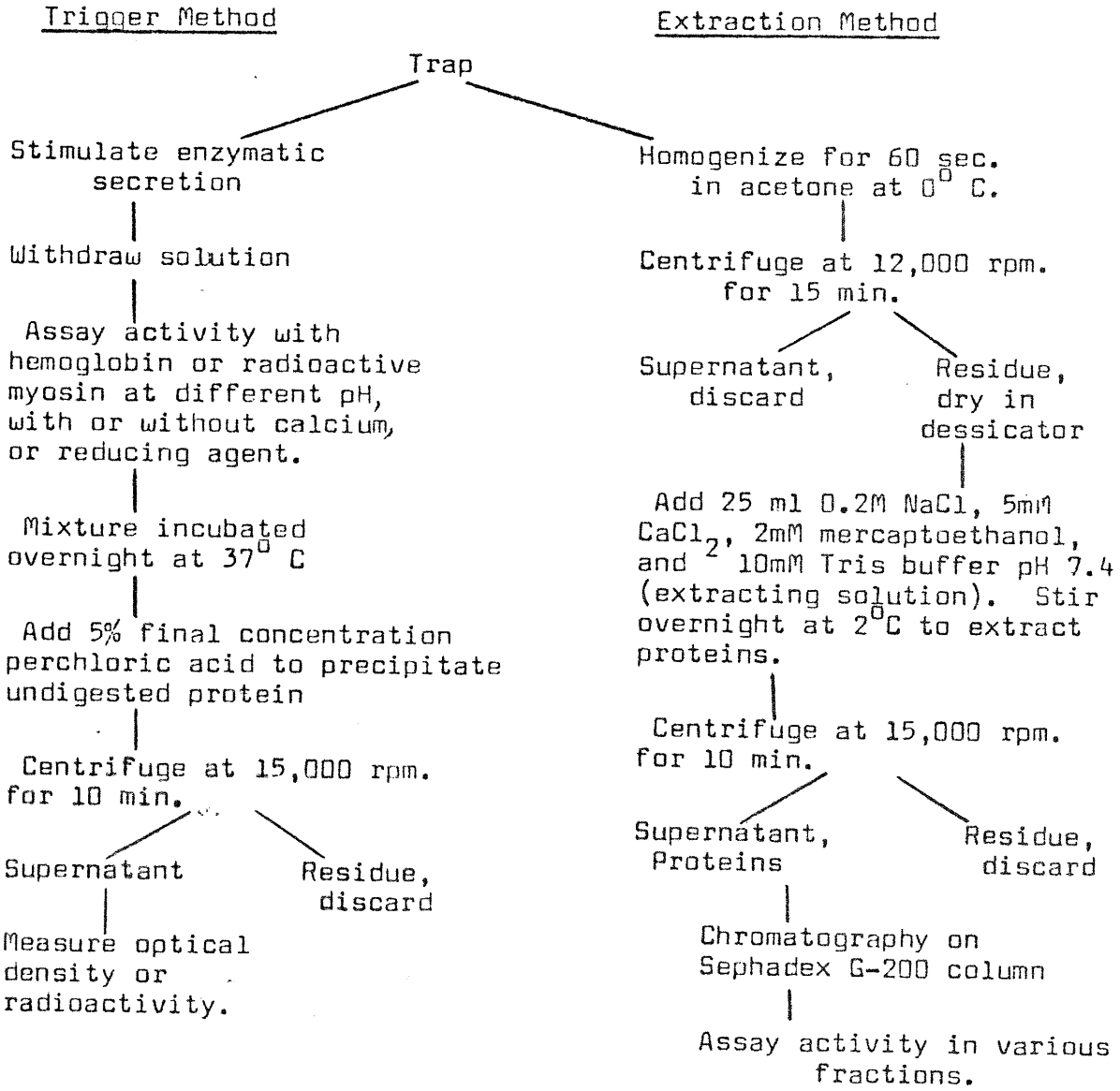
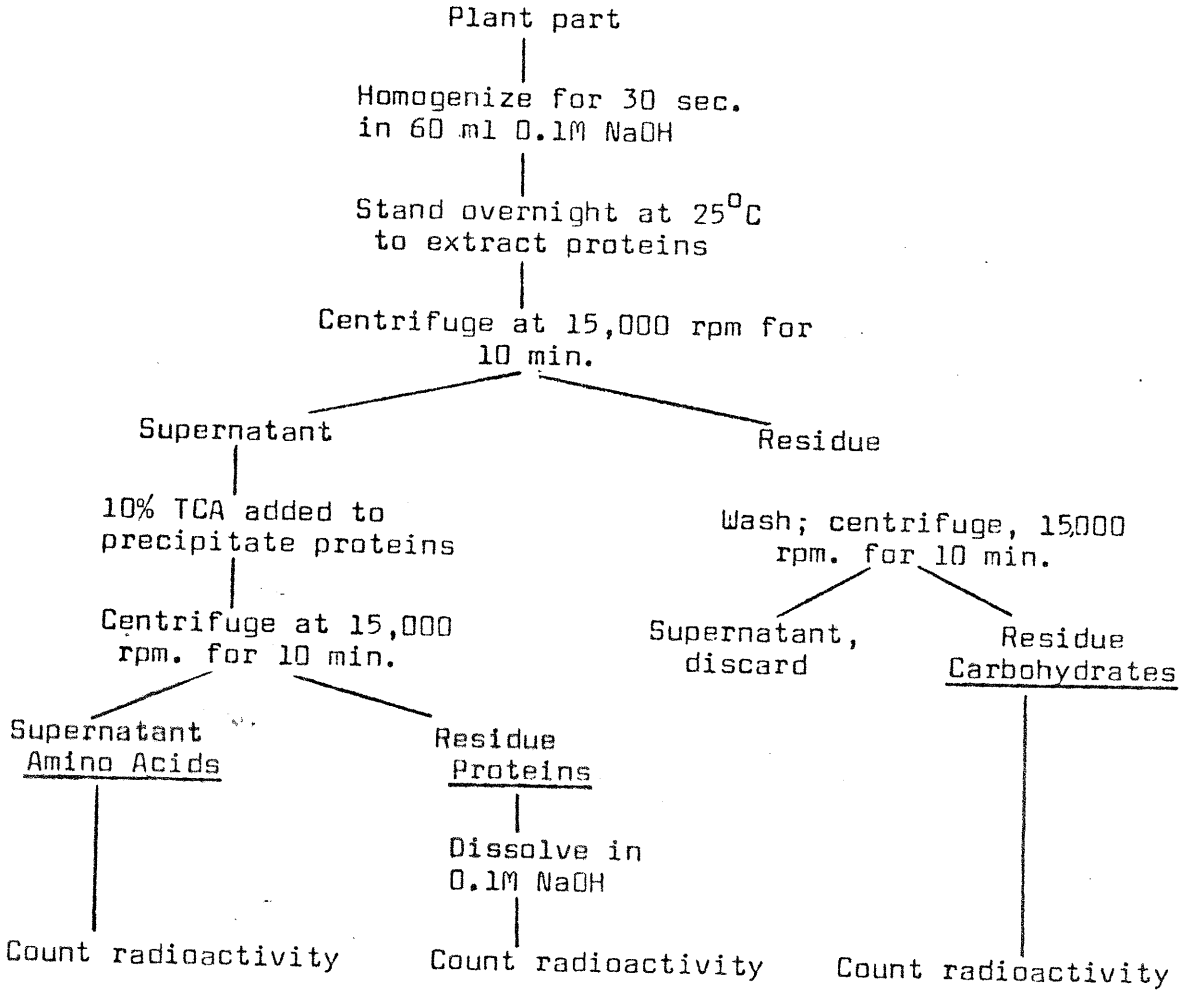
Scheme 1 Procedure for Isolation and Purification of the Enzyme from Venus' Flytraps



Scheme 2 Procedure for Monitoring the Incorporation of Radioactive Amino Acids in the Venus' Flytrap

Plant part: traps, leaves, bulbs, roots.



Scheme 3 Procedure of Fixing and Embeding Venus' Flytrap Tissue for Microscopic Studies

Tissue

↓

Embed in wax so that open traps retain open position

↓

Fix in 10% formalin for 1 day.
Remove wax, fix for two more days.

↓

Wash in running water for 4-5hr.

↓

Dehydrate for 1 hr. in each of the following:

30% alcohol
50%
70%
90%
95%
100%
100%
100%
xylene
xylene

↓

Place in melted paraffin and xylene for 1 hr. at 55°C. Melted paraffin, 2 changes, 1 hr. each.

↓

Pour melted paraffin into paper molds. Place tissue into mold and cool paraffin in water for several hrs.

↓

Remove paraffin block, and shape into smaller rectangle with tissue clearly visible. Mount on holder for cutting, and slice with microtome, thickness =12μ

↓

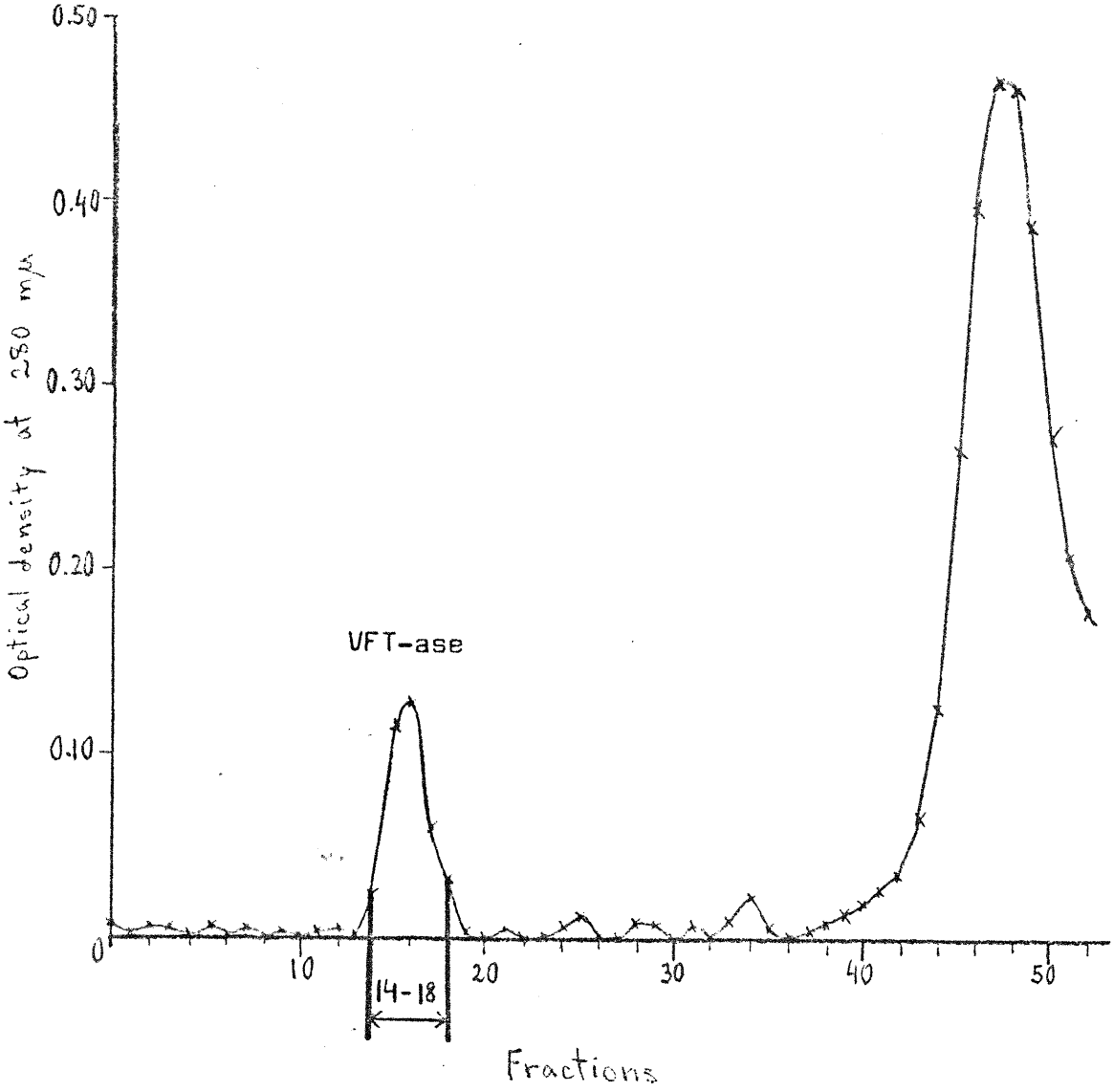
Expand sections to normal size in water.

↓

Mount sections on slides.

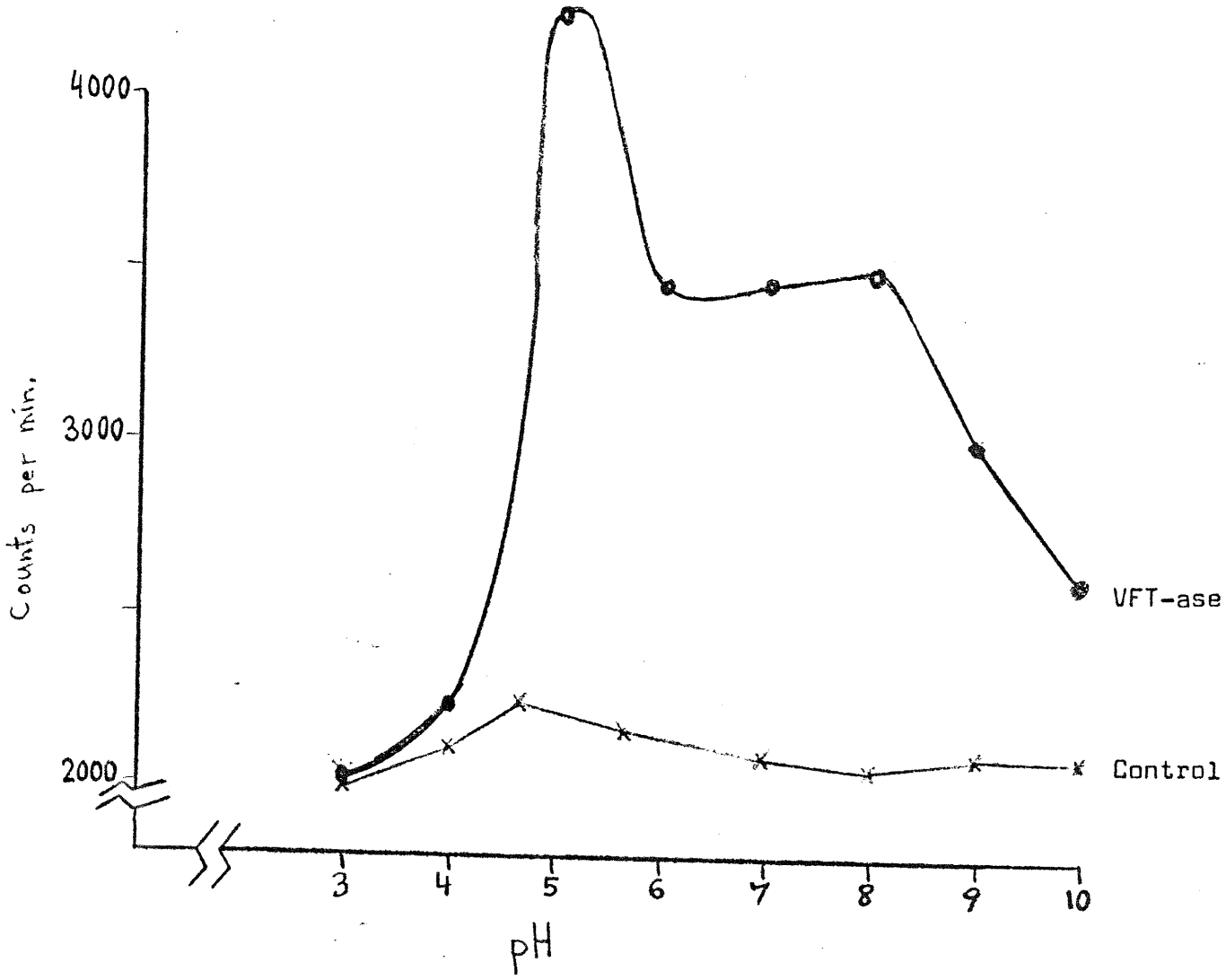
Graph 1 G-200 Sephadex Chromatography of the Extract from Acetone Dried Powder of Venus' Flytraps

(See Scheme 1, Extraction method)



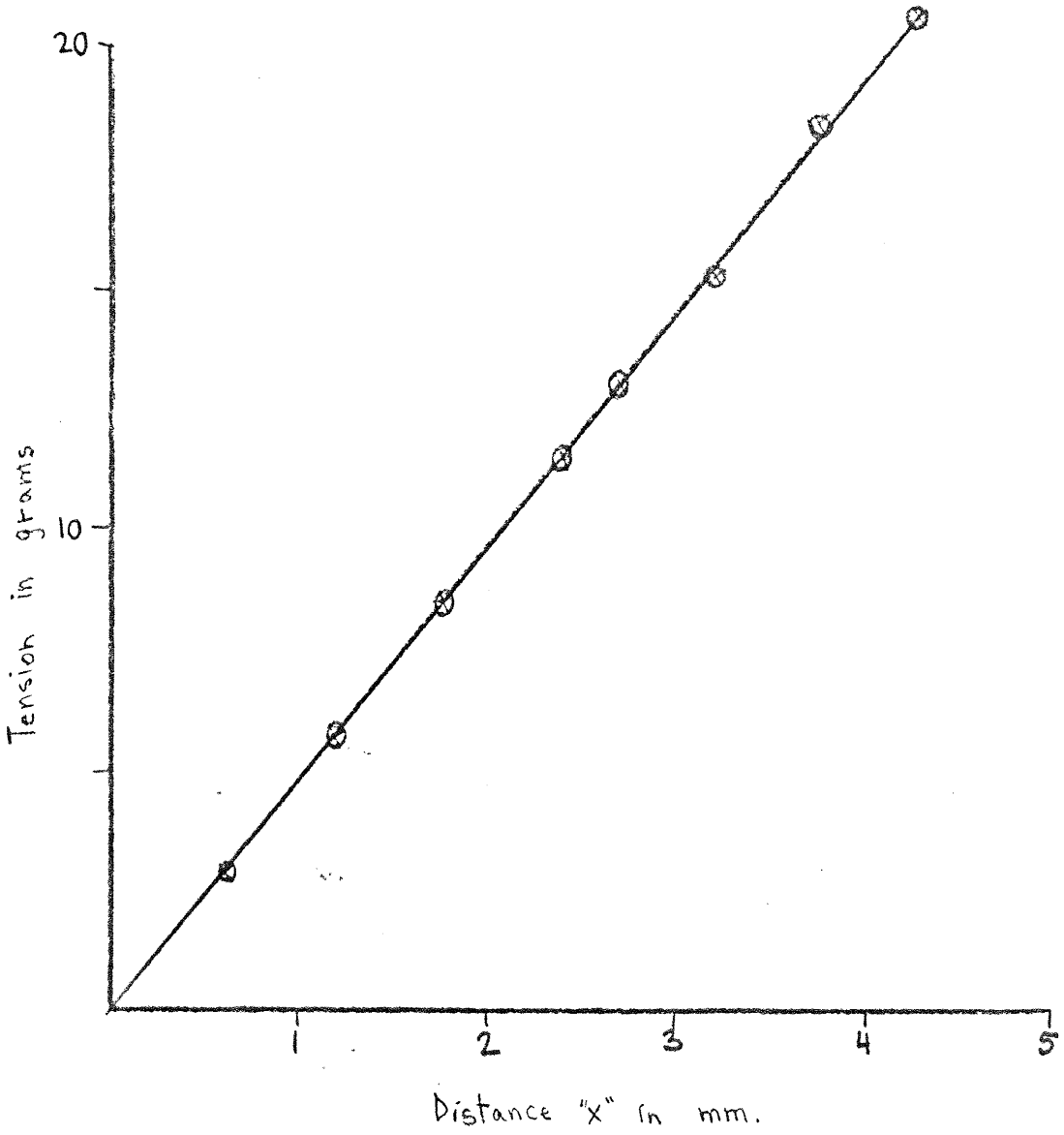
The amount of protein in each fraction is proportional to the optical density reading at a wavelength of 280mμ. The various fractions were assayed for activity. VFT-ase appears in fractions 14-18. The amount of proteolytic enzyme in the Venus' Flytrap comprises only a small fraction of the total protein as evidenced by the relative sizes of the peaks.

Graph 2 The Effect of pH on VFT-ase Activity



VFT-ase was isolated and assayed as outlined in Scheme 1. The pH of the VFT-ase solution, shown on the abscissa, was regulated by Tris-Acetate buffer. Radioactive myosin was used as a substrate, and the ordinate shows the counts liberated by digested myosin. Since myosin breaks down spontaneously during the overnight incubation at 37°C, myosin without VFT-ase was used as a control. The difference in the counts is proportional to the activity. The pH optimum of VFT-ase is 5.

Graph 3 Trap Tension as a Function of the Distance between the Two Lobes



When a closed trap is opened by a device (see picture on page 5) it produces tension (F) which is proportional to the distance between the two lobes (x). The proportionality constant (k) depends on the physical properties of the trap.

$$F=kx$$

In this experiment, k=50gm./cm.

Table 1 Nucleic Acid and Protein Composition in the Cell Components of the Venus' Flytrap

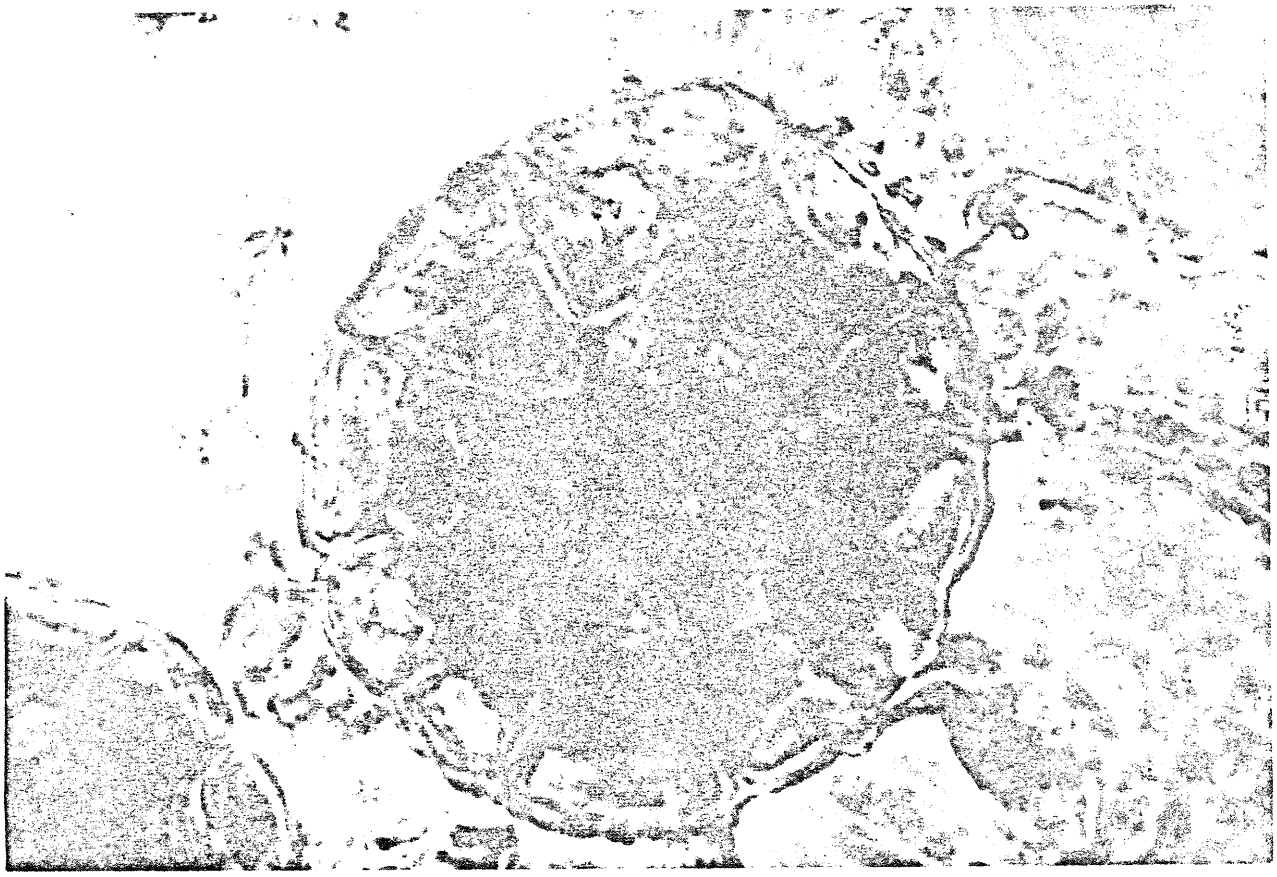
	DNA	RNA	Proteins
	Units in μg/gram wet tissue		
Traps			
Nuclei	470	-	3,150
Mitochondria-Chloroplasts	287	91	830
Microsomes	-	115	22
Leaves			
Nuclei	623	-	1,030
Mitochondria-Chloroplasts	420	242	1,430
Microsomes	-	338	129

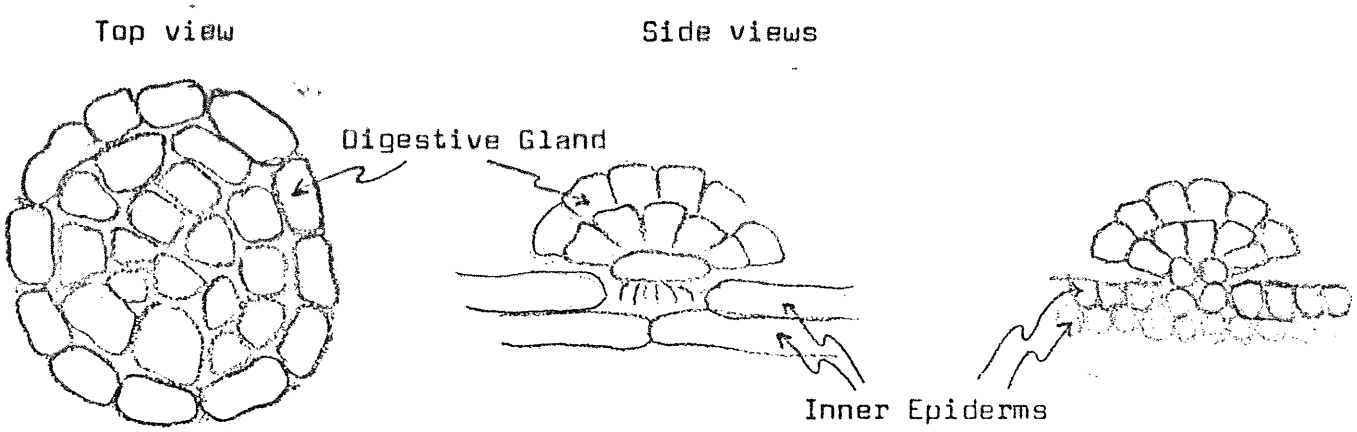
The Venus' Flytrap exhibits no DNA in the microsomes and no RNA in the nuclei. The energy transducers (mitochondria and chloroplasts) are almost twofold as abundant in the leaves as in the traps. The amount of nuclear proteins of the traps is strikingly higher than that of the leaves.

Table 2 Changes in Tension of Venus' Flytrap by various Substances

Substance	Nature of Substance	Effect on Tension
1.0M KCL	high salt, hypertonic solution	decrease
0.5M $NaHCO_3$	high salt, hypertonic solution	decrease
0.1M $CaCl_2$	high salt, hypertonic solution	decrease
0,1M $BaCl_2$	high salt, hypertonic solution	decrease
0.1M $CoCl_2$	high salt, hypertonic solution	decrease
1.0M urea	neutral, high molarity	decrease
10% glycerol	neutral, high molarity	decrease
0.1M KCl	low salt	no noticeable change
0.1M NaCl	low salt	no noticeable change
0.1M $NaHCO_3$	low salt	no noticeable change
0.1M Na-EDTA	anion (4^-), chelator	no noticeable change
0.1M NH_4-oxalate	anion (2^-), chelator	no noticeable change
1mM salyrgan	-SH reagent	no noticeable change
0.1% sodium dodecyl sulfate	detergent	no noticeable change
10-40% ethanol	lipid solvent	no noticeable change
H_2O	solvent	increase
Hoglands solution	physiological solution of normal plants	increase
0.05M ATP	substrate for ATPase	increase

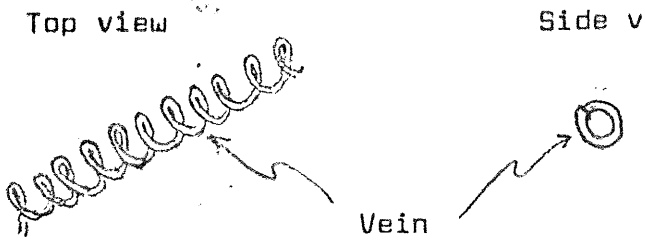
Plate 1





Microscopic cross section of a digestive gland of Dionaea muscipula showing oval shape. Hematoxylin and eosin stain, x 1,400 , phase contrast. The gland was displaced from the inner epiderm by a microtome. The gland secretes proteolytic enzymes which digest insects.

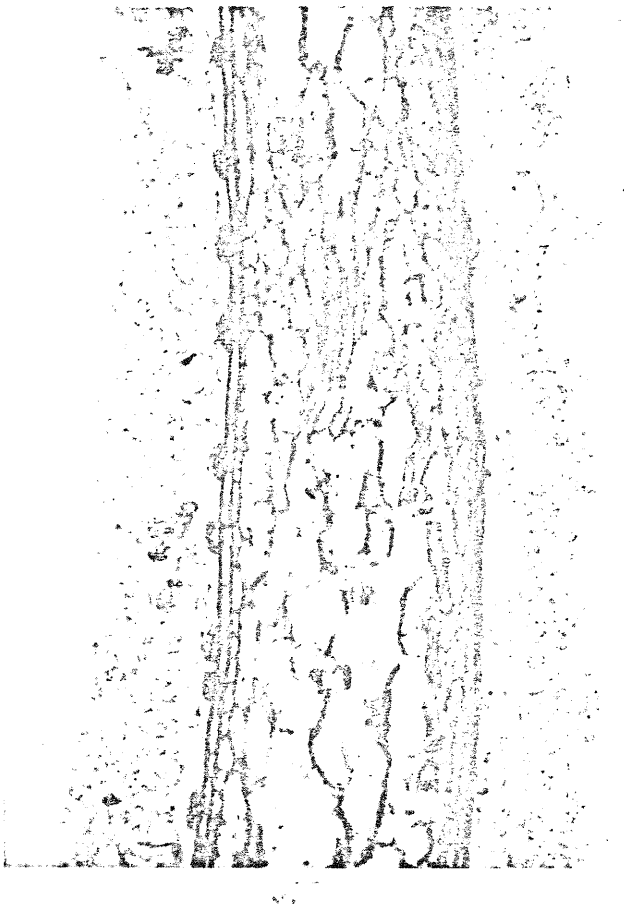
Plate 2

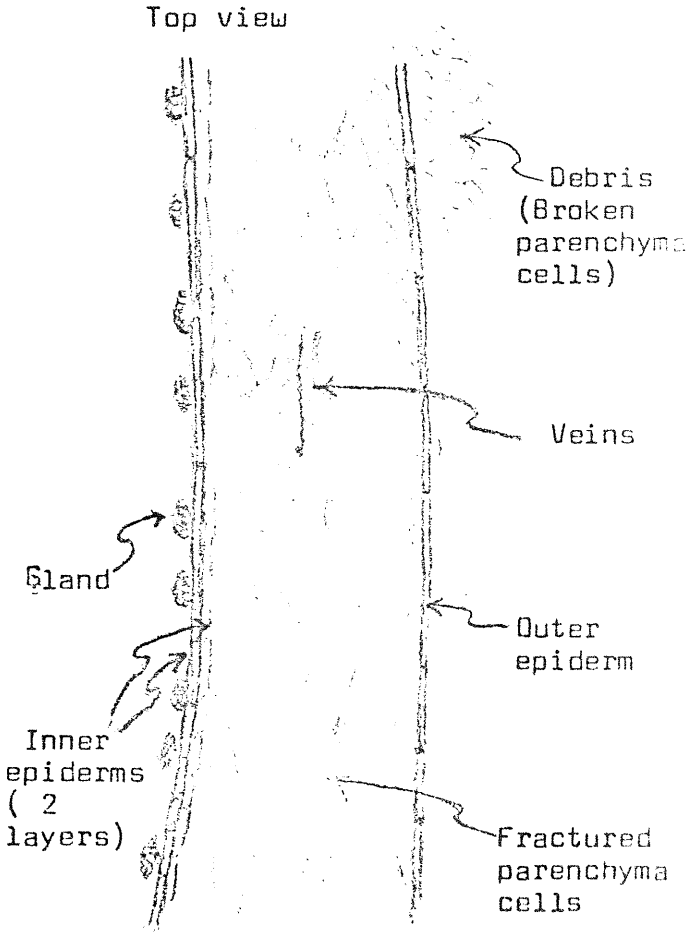


Microscopic cross section of a vein of Dionaea muscipula, displaying the spiral structure. Prepared from longitudinal cross section of fresh trap, x 1,250, phase contrast. The veins (vascular bunble) transport nutrients throughout the plant.

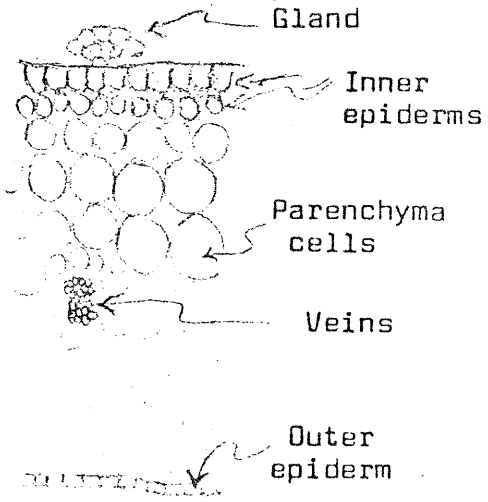
Plate 3

Longitudinal section of lobe of Dionaea muscipula showing various structures as well as the relative sizes of the cells. Hematoxylin and eosin stain x 50. Drawings show transverse and longitudinal sections of lobe, and identify the glands, veins, parenchyma cells, and inner and outer epiderms. Debris on outside are broken parenchyma cells.





Transverse section of lobe of Dionaea muscipula x 75



Longitudinal section of lobe of Dionaea muscipula x 75

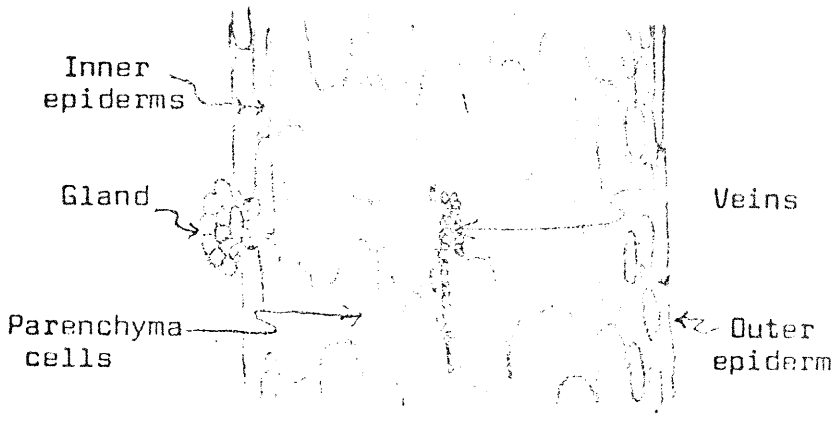


Plate 4

Longitudinal section of midrib (hinge) of Dionaea muscipula showing central vascular bundle and other structures. Hematoxylin and eosin stain x 75. Drawing shows top view and identifies structures.