

Developmental Plant Anatomy

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Overview

Plant anatomy is central to many botanical disciplines. Being able to recognize cells and tissues correctly, to know about their distribution and cell wall composition, their function in the whole plant is paramount to diverse disciplines. Unfortunately plant anatomy is often overlooked in favour of 'cooler' things like molecular biology or plant biochemistry – many botanists have no clue about plant cell structure and will pass themselves off as 'experts' in their particular fields, without really knowing the difference between something simple like mesophyll (specialised photosynthetic tissue in leaves) compared to chloroplast-containing outer cortical layers in young stems which are associated with chloroplasts; recognising the difference between protoxylem vessels and lignified fibres! At a mundane level, do you know the structural and functional difference between cortical stem parenchyma cells, and those associated with say, the vascular tissue? What about bundle sheaths? Can you recognise them? Most likely not, but you are not alone. By the time you have finished this course, you will hopefully not be in the same boat, and will be able to recognise cells by form and distribution, and more importantly, realise that position is important with respect to function as well. We have just this year, published Plant Anatomy an Applied Approach- (Cutler, Botha and Stevenson) the book includes a CD-ROM entitled '*The Virtual Plant*' which contains many useful exercises, including one on how to use a microscope correctly, and how to observe the kinds of specimens presented in this and the subsequent lab exercises. The Library has ordered several copies. *The Virtual Plant* is also located at <http://anubis.ru.ac.za/> - please make use of this (for now) free resource.

ASSIGNMENT 1. Introduction to microscopy and histochemistry

A. SOME SIMPLE MICROSCOPE EXERCISES.

OBSERVATION AND DRAWING POLLEN AND OTHER SMALL OBJECTS.

When you look at a section, the whole specimen will rarely lie in the same focal plane and therefore, re-focusing by means of the fine adjustment should be carried out, all the time that you are looking through the microscope's eyepiece. Severe eyestrain will result, if you attempt to "focus" with your eyes alone. Eye strain will also be avoided if you keep both eyes open at all times, whilst using the microscope. This will understandably, be a difficult exercise at first, but it is only a matter of practice. With your left eye at the eyepiece and your right eye looking at your laboratory book, draw one of the pollen grains, as seen under the high power (H P, usually the 40x objective lens). Your drawing should be at least 5cm in diameter.

What do you need to examine?

Hibiscus pollen

Stage Micrometer

Lab Specimens, stain freehand sections

You will need to select a specimen from those provided in the laboratory. Remove a piece of the material (stem will be provided) and place it in water in a Petri dish to keep moist). Sections need to be cut from this and individually stained using the three stains provided.

1. Pollen of *Hibiscus*.

Place a little pollen of the *Hibiscus* (or other flower, if *Hibiscus* is not available) on a microscope slide, add to this, a drop of water and place a coverslip over the drop of water. Put the slide on the microscope stage, after making sure that the **lowest power objective** is in position. Focus carefully, using the **coarse focusing control**, until the pollen grains are visible through the eyepiece. Make a drawing of a of a single pollen grain, draw it at about 3cm across. Keep the section, as you will need it to make measurements of the size of the pollen grains that you have seen.

2. Using a stage micrometer.

Making measurements of cell is important when studying cells, tissues and organs. Being able to determine size and magnification are thus paramount in this process. Imagine not being able to work out how small a cell really is or how large the microscope makes it! As a cell biologist you'd be up the creek. Measurement requires a micrometer, with which you can make a fairly accurate assessment of

size. In this exercise, you are provided with a simple micrometer made from a transmission electron microscope grid. This has been mounted on a glass microscope slide.

Each aperture in the grid measures 200x200 microns and the width of the copper wires is nominally 40 microns. Measure as accurately as possible, the diameter of the field of view of your microscope¹, using the low (LP) and HP objectives.

Complete the table of the various field of view parameters which you have calculated. Use this data to help measure your drawings and calculate the **scale of magnification**² i.e., how much larger are your drawings, than the **actual size** of the object itself? Please see <http://anubis.ru.ac.za/Main/ANATOMY/Practical%207.htm>

for details of the procedure.

Objective Magnification	Diameter of field of view	Area of field of view
4X		
10X		
40X		

When you make drawings with the aid of the microscope in future, get into the habit of indicating the **scale of magnification**.

Note that whatever the nominal magnification of the optical system may be, you will be able to use this table in two ways:

- To estimate the size of the microscopic objects that you look at during the practicals;
- To estimate the scale of magnification of your drawings.

Now, look again at the mount that you made of the pollen grains and estimate the size of these grains. Are they very similar in size? Measure a few, and then calculate the mean diameter of the pollen grains. Measure the pollen drawing and compare this with the actual size (in μm). Only by measuring the drawing and comparing this with the actual size of the object or specimen, using the data that you calculated in the table above, can you calculate the actual scale of magnification of your drawing. You should repeat this process until you can accomplish it easily.

3. Water droplets on a slide.

Place a small drop of water on a slide and carefully drop a coverslip onto it. Examine the trapped air bubbles under LP and HP. Note the difference in appearance when the bubbles are seen in surface view and in optical section, as the level of focus is altered. Note particularly, the apparent “**thick black wall**”, which is due to refraction of the light at the edge of the bubble. It is important that you learn to recognize air bubbles; otherwise it is certain that later in the course, you will be calling upon a demonstrator to help you identify rather strange “cells”.

IRRIGATION

This is another important technique. You will need to use this when you apply dyes and histochemicals to sections. Careful work is required. Take your pollen slide and place a small drop of iodine or iodine-potassium iodide at one edge of the coverslip. Use a piece of filter paper to draw the iodine solution at least halfway through the mount. Place your section back on the microscope stage and look to see what changes are taking place.

1 *Field of view*: The area that you can see when you look through the eyepiece. As magnification is increased, the field of view becomes smaller. You effectively see less, but at higher magnification.

2 *Scale of magnification*: Size that the object appears on paper (your drawing) divided by the actual size of the object, calculated from what you see using the microscope

Practice doing this several times, introducing more or less iodine. Take care NOT to move the coverslip which ensures that damage to the section is minimized. Examine the starch grains at the edge of the solution (why not in the strongest solution ?) and make a note of their appearance. Study the diagram below.

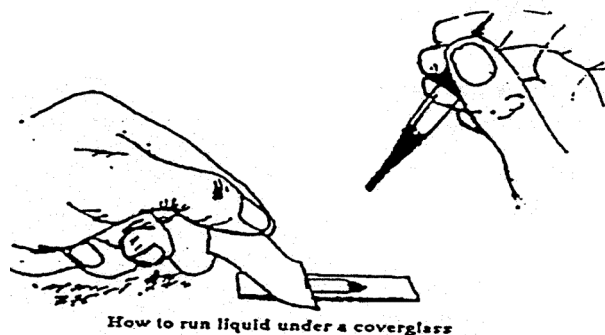


Figure 1. Shows the technique required to **irrigate** a section.

B. HISTOCHEMISTRY

Introduction

"There is only one reliable criterion by which we can determine whether the image that we see with the microscope is a good representation of what existed in life, and that criterion is comparison with living cells".³

The preservation of structural details of cells and tissues is clearly influenced by the condition of the material at the time of collection and by the subsequent preparative steps used for killing and fixing the material. In other words, if you wish to prepare sections in which the structural details are well preserved one should select healthy plant material. Exceptions to this rule apply only when the researcher is interested in observing the effects of disease, fungal infection, insect damage etc. on the normal structure of the plant material being examined.

Using Dyes

Dyes make structures within sections of plant material visible. The practise of **staining** is thus important and has been used for a very long time. Several dye substances will differentiate between primary and secondary wall structures, show the presence or absence of lignin within the walls, show the presence or absence of other specialized chemical substances within cell walls, and may stain nuclei, or chromosomes within living cells. These substances rely on the cationic or anionic nature of the substances within the cell walls, or within the cytoplasm of the cells themselves. By carefully selecting dyes and relatively simple chemical reactions, one may undertake histochemical analysis of the wall, and the cytoplasm. These simple tests are thus invaluable, if we are to determine the nature of living plant cells.

The histochemical tests described below are useful aids in determining cell wall structure in plants. It is important to note however, that these do not always work. A negative test generally shows the absence of a compound

BASIC HISTOCHEMICAL TESTS

3 Baker, J.R. 1966: *Cytological Technique* (5th Ed.) Methuen: London.

Protocol:

Select a clean, double-edged razor blade. Carefully rinse this in absolute alcohol and dry it. Cut a short piece of masking tape and cover the one edge of the blade with the tape – this will minimise the risk of cutting yourself. **WARNING!** Cut fingers are **EXTREMELY PAINFUL** Please be careful!

After having taken the necessary safety precautions, cut a number of sections of the material that you are going to use and place these in water, in a watch glass.

Sample some of the sections, to ensure that you have achieved the right plane of section! Try to cut thin sections—remember it is NOT NECESSARY to cut complete sections. Thin wedges, which contain all relevant cell types, are suitable for your study.

Add **only a small drop of stain** to the section. Excess liquid around the cover glass should be removed with a filter paper.

There should be **NO** liquid anywhere on the slide, other than **under** the coverglass!

or substance, but, where possible, more than one test should be used to confirm the result.

STAINING PROCEDURE:

Prepare free hand sections as described above – float into a watch glass in water.

Transfer sections onto a slide. Use a fine paintbrush to do this NOT forceps, pencils or fingers! Hard objects will destroy the fragile tissues.

Always add the dye/reactant to a section that is floating in a drop of water. Wait a minute or two, before applying a coverglass. This will allow for good diffusion into the specimen. DO NOT examine the specimen with a microscope, before you have placed a coverslip over the specimen, and have cleaned up the slide as described above.

Prepare sections for histochemical analysis.

(a) Phloroglucinol-HCl.

Lignin is a very common constituent in the secondary wall of plant cells; e.g., the walls of xylem elements and sclerenchyma tissue. The cinnamaldehyde end groups of lignin appear to react with phloroglucinol-HCl to give a red-violet colour (Gahan 1974)⁴. Although the reaction is not very sensitive, because of the ease of staining, this procedure is still often used as one of the tests for the presence of lignin in plant cell wall.

Mount some sections in phloroglucinol (1% in 95% alcohol). Now run in a drop of 25% HCl at the side of the coverslip. lignified walls will turn pink. Carried out in this way the test is sensitive, but note that it is also positive for hemicellulose pentosans. Phloroglucinol can be examined using a fluorescence microscope as well, as the dye selectively quenches (suppresses) the fluorescence associated with lignin, allowing other chemical constituents to be visualised.⁵ NOTE This solution is potentially dangerous, and you should handle it with care.

(b) Iodine – potassium iodide

The iodine-potassium iodide (IKI) stain is specific for starch. According to Yeung (1998)⁶ the basis of the reaction is the accumulation of iodine in the centre of the helical starch molecule. The length of the starch molecule determines the colour of the reaction - the shorter the molecule, the more red the colour; the longer the molecule, the more blue the colour.

4 Gahan, P. B. 1984. Plant histochemistry and cytochemistry - an introduction. Academic Press, London, 301 pages. [ISBN 0-12-273270-7].

5 Biggs AR. Detection of impervious tissue in tree bark with selective histochemistry and fluorescence microscopy. Stain Technol. 1985 Sep; 60(5):299-304.

6 Yeung, E. C. 1998. A beginner's guide to the study of plant structure. Pages 125-141, in Tested studies for laboratory teaching, Volume 19. (S. J. Karcher, Editor). Proceedings of the 19th Workshop/Conference of the Association for Biology Laboratory Education ([ABLE](#)), 365 pages.

(c) Fabil.

Fabil is a useful stain, which allows us to separate primary from secondary walls quite easily. **Primary cell walls** (cellulosic) will stain in the range pale blue to dark blue (starch grains will also stain, as the stain contains iodine) **Secondary cell walls** (containing lignin) will stain a red to deep red-purple colour.

CORE ASSIGNMENT

1. Calculate the field of view of your microscope for 4, 10 and 40X objectives.
2. Draw pollen grains, calculate their real dimensions, and also calculate the magnification (scale of magnification) of your drawing. Remember, all drawings should include a scale of magnification.
3. Make a **plan diagram** (see page 28) to show the distribution of specific cell wall components identified through the histochemical tests that you have carried out. Also make notes on the colours of cell walls and tissues.
4. Write a **short review** of the histochemistry of cell walls that you have seen. This should not be more than TWO typed pages (at 1.5 line spacing).

Marks: 1 = [5] 2 = [5] 3 = [25] 4 = [20]

Assignment 2. Introduction to root, stem and leaf anatomy

INTRODUCTION

Vascular plants consist of three distinct organs – roots, stems and leaves. These organs function in unity to ensure that anchored roots can supply water and nutrient via the roots, through stems, to leaves. Transport of water and assimilated carbon takes place from source to sink – (roots are sources of water and nutrients; sinks for carbohydrate); leaves are water 'sinks' and, depending on the state of development, can either be assimilate sinks or sources.

The principal function for the stem, is to provide a stable linking platform, from which lateral appendages emerge – be these lateral roots basipetally, or aerial branches or leaves. The vascular system is present in all three zones, but as we will see, takes on somewhat different structure depending on location. Roots, for example, do not have vascular bundles, instead the vascular supply consists of a number of **strands of xylem** and **strands of phloem**. In most roots, **these alternate** and the first-formed (protoxylem) xylem, is exarch to the later-formed metaxylem. In stems, this is reversed, metaxylem is exarch, protoxylem is endarch. In leaves xylem is usually adaxial with respect to the phloem. In contrast to generalized root anatomy, the vascular supply in stems and leaves is contained within vascular bundles.

In this exercise, we will examine a limited number of examples to illustrate the basic anatomy of root, stem and leaf.. It is highly recommended that you explore *The Virtual Plant* <http://anubis.ru.ac.za/index.html>

for more information. **Note:** this will not be available outside of the RU web, to comply with Wiley-Blackwell's Publishers requirements).

Inasmuch as the words 'generalized' and 'typical' are used in this prac manual, be aware that there is really no such thing as a 'generalized' structure, or even a 'typical' one! There is in reality, a huge range in form and structure.

THE PRINCIPLE OF TISSUE SYSTEMS

One of the most useful schemes developed for understanding general topographical anatomy, was devised by the German Botanist, Julius von Sachs (1875). His classification system has a great deal of merit - firstly, because it was relatively simple, and second, due to its wide applicability to the juvenile and young leaf, stem and root.

According to von Sachs' scheme, there are three principle tissue systems:

- (1) The **epidermis and cork layers** which comprise the **dermal** (protective) **system**,
- (2) The **conducting strands of xylem and phloem**, which make up the vascular system, and
- (3) The remaining **non protective and non-conductive tissues**, which make up the fundamental or ground system.

A. AN OVERVIEW OF ROOT STRUCTURE

Roots serve two important functions - the first is purely mechanical, as they are responsible for anchoring the plant firmly within the ground. In epiphytes, the roots also serve to anchor the plant to others. The second important function is ensuring that an adequate supply of water and nutrient reaches the aerial parts of the plant, via the xylem. The roots also contain phloem tissue which is the pathway through which assimilated carbohydrate moves from the points of synthesis (source) to the sites of storage (sinks).

Roots normally contain an endodermis. The endodermal cells often become lignified, and have conspicuously thickened radial and inner tangential walls. Some cells remain thin-walled. These are called passage cells, which, as their name implies, allows the passage of water and nutrient from the cortex (above) to the xylem within the stele. Passage cells are also the route taken by carbohydrate to the living cells within the cortex, from the stele.

Root structure is generally poorly studied in comparison with that of the stem or leaf of the same plant. All roots are composed of the same **tissue systems** as are found in the stem and leaf; namely **dermal**, the **fundamental** or **ground**, and the **vascular** systems. Roots are divided into a **cortex** or **outer region**, and a **stele**, or inner region, which contains the **vascular** system. The images to the right show some common **cortical** root variations. Nearly all roots have **root hairs** (C) which grow nearer the growing tips of the young roots.

The dermal system is typified by the **epidermis**. This structure may be modified dependent upon environment and particular adaptations that may take place during the life of the root system. Root hairs (C) For example an **hypodermis** may be present beneath the epidermis. This layer is in effect, a mirror image of the epidermis, and serves principally, as an additional protective layer. **Aerial roots** undergo considerable modification to allow uptake of water through a specialized compact multicellular layer, called the **velamen** (E), as shown in many orchids.

The **cortex** too, is highly variable, and this is often dependent upon the environment in which the roots are anchored. For example, in **hydrophytes** or in plants growing under damp or boggy conditions, as in the cortex of the rush (A-C) often contains and extensive intercellular space system, (lacunate, B) to accommodate **gas exchange**. In contrast to the rush, the orchid contains a solid cortex (F).

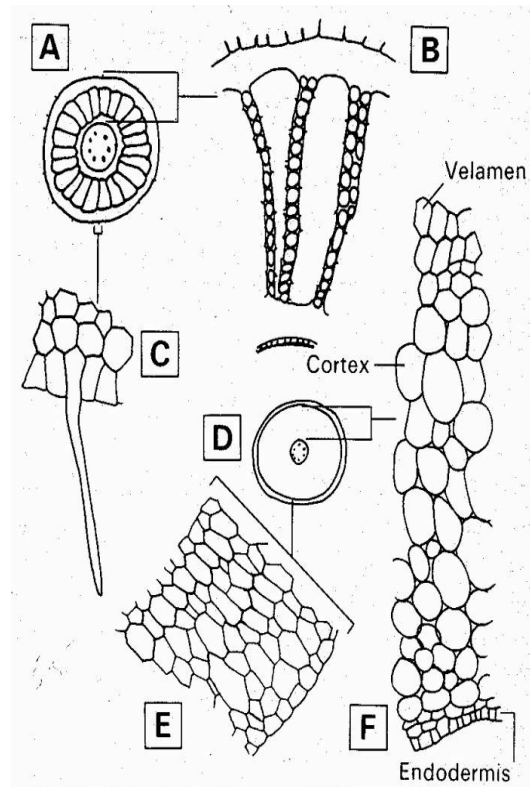


Fig. 2. Roots in T.S. A-C *Juncus acutiflorus*, A, diagram; B, lacunate cortex, C, root hair, D-F *Cattleya granulosa*, D, diagram; E, velamen F, 'solid' cortex. From: Cutler Botha and Stevenson: Applied Plant Anatomy.

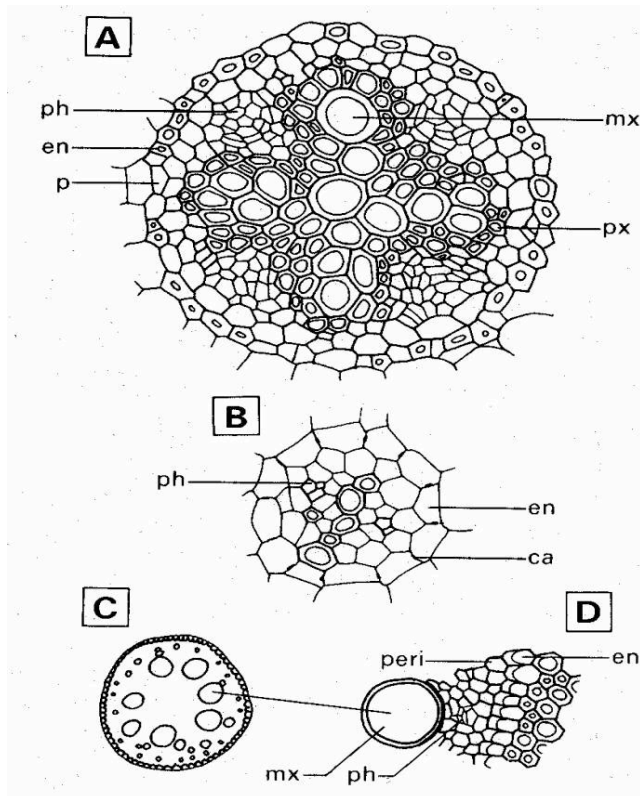


Fig. 3. Some root vascular systems.. A, *Ranunculus acris* **tetrarch** root. B, *Echinodorus cordifolius* **diarch** root. C, D, *Juncus acutiflorus* **polyarch** root. **ca**, Casparian strip; **en**, endodermis; **mx**, metaxylem; **p**, passage cell; **peri**, pericycle; **ph**, phloem.

The cortex has two recognizable **boundaries**. Both serve important physiological functions associated with the uptake and transfer of water and dissolved inorganic and organic molecules. Whilst the **epidermal layers** are chiefly concerned with uptake, it is the innermost boundary, the **endodermis**, which is perhaps the more important layer. The endodermis is often striking, due to its **thickening** as well as the presence of **Casparian strips**. The combination of these two specific modifications, form effective barriers to unregulated transport.



The endodermis of the *Iris* root forms a near-impenetrable layer, with transport across this interface, regulated by specialized thin-walled **passage cells** (shown above) which, as their name implies, allow symplasmic as well as apoplasmic transport from the cortex to the stele, and vice versa.

From: Cutler Botha and Stevenson: Applied Plant Anatomy

Practical examples

1. *Ranunculus* (buttercup, dicotyledon)
2. Young and old *Helianthus* roots (dicot, secondary thickening)
3. *Zea mays* (monocotyledonous).

Make simple sketch plans to show the relationship of the cortex to the stele. Show the distribution of the vascular tissue. Are there vascular bundles present? Pay particular attention to, and note the five key zones listed above.

Examine the specimens listed above, and identify:-

The epidermis;

The cortex;

The endodermis;

The pericycle;

The vascular cylinder.

Pay particular attention to the distribution and arrangement of the vascular tissues. [30].

B. AN OVERVIEW OF STEM STRUCTURE

Because of their association with leaves, stems are much more complex structurally than are the corresponding roots of the same plant. Unlike roots, shoots are composed of nodes and internodes. One or more than one leaf may be attached to nodes. The stem assumes many forms and sizes and may be of variable length as well. Nodal anatomy is more complex than that of the internodal regions, as here, one or more strands of vascular tissue diverge from the axis of the stem, extending and connecting to the vascular system within the leaf. The point of emergence from the stem, results in the formation of one or more leaf gaps. Stems have several important functions. Firstly, support. They have to support the total aerial mass of the plant ensuring exposure to sunlight and ensure that the plant remains competitive within its ecological niche.

The vascular bundles can take up a variety of arrangements. In dicotyledons they usually occupy one ring, just to the inner side of the cortex. This is described as being eustelic. In monocotyledons they may form one ring, or may appear to be scattered in several to many rings, or lie without apparent order in the central ground tissue. This is described as being atactostelic. The possession of several rings of vascular bundles is not the prerogative of monocotyledons. Several families of climbers amongst the dicotyledons have several rings of vascular tissue. When vascular bundles are not scattered, the centre of the young stem is usually parenchymatous, and may become lignified in mature stems.

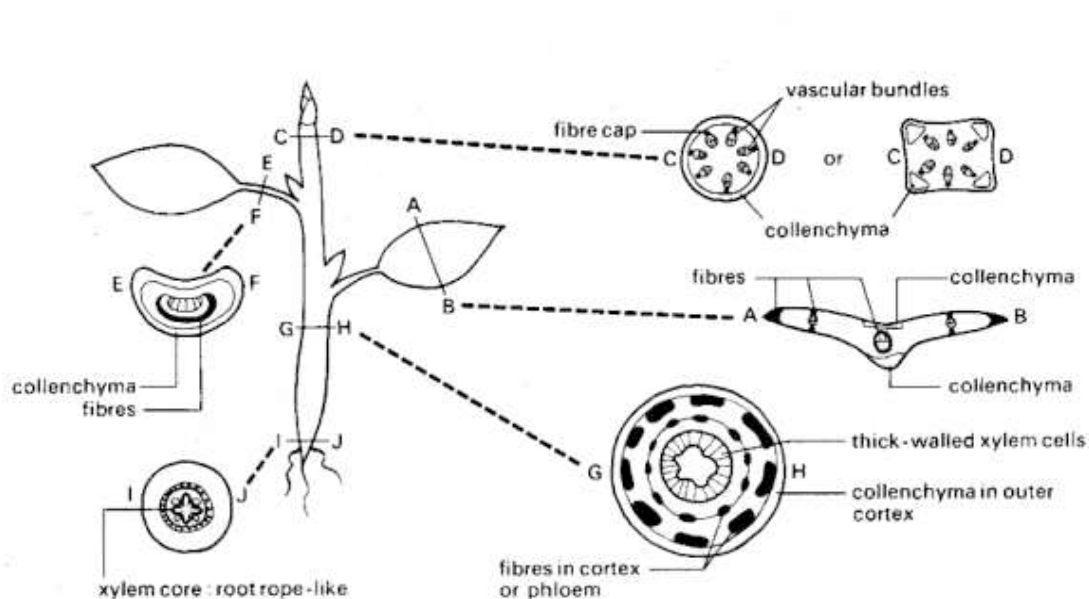


Fig. 4 illustrates some mechanical systems in dicotyledons. A schematic plant with position of sections indicated. Liquid pressure occurs in turgid cells through the plant. Collenchyma is often conspicuous in actively extending regions and petioles. Sclerenchyma fibres are most abundant in parts that have ceased main extension growth. Xylem elements with thick walls have some mechanical function in young plants and give a great deal of support in most secondarily thickened plants.

The cross-section of a primary stem may have a more or less angular to circular outline. However, it can take on one of a wide range of forms, some of which assist in the identification of a family, as in Labiateae, where the section is square or may help to distinguish genera for example, in the sedges, *Carex* species have stems with a triangular section. Often the outline is

modified near to nodes or in regions of leaf insertion. Sometimes a wing or ridge of tissue in line with a petiole may continue down the internode as in, for example, *Lathyrus*. In general, the outline of the section taken in the middle of an internode would be described for comparative purposes.

Many stems have all or most of the following tissues, working from the outside inwards: epidermis, hypodermis, cortex (with both collenchyma and chlorenchyma, or either), an endodermoid layer (or a well-defined starch sheath), vascular bundles in one or more rings, or apparently scattered, and a central ground tissue or pith. Sometimes a pericycle can be distinguished, but this is normally regarded as part of the phloem. A true endodermis with Casparian strips is rarely present.

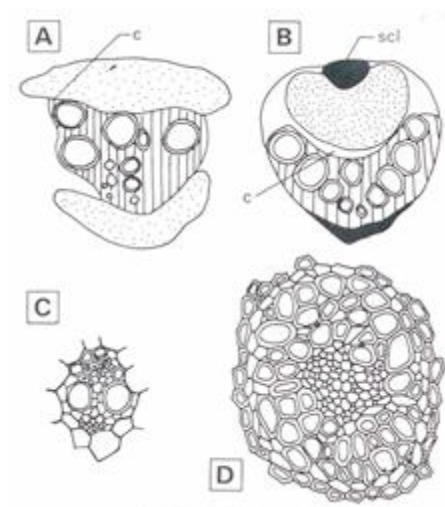


Fig. 5. Vascular bundle types from stems. A, *Cucurbita pepo*, diagram of bicollateral bundle, x 15. B, *Piper nigrum*, diagram of collateral bundle; cambium remains fascicular, x 15. C, *Chondropetalum marlothii*, detailed drawing of collateral bundle, lacking cambium, x 110. D, *Juncus acutus* detailed drawing of amphivasal bundle, x 220. c, cambium; scl, sclerenchyma.

From: Cutler Botha and Stevenson: Applied Plant Anatomy

Practical examples of stem specimens

Pelargonium, *Zea mays*, pine.

Examine all three specimens, and identify the following zones, making sure that you can identify cell and tissue types making up these regions of the stem.

The epidermis;

The cortex – if subdivided, then identify outer and inner cortex and composition;

The starch sheath;

The vascular tissues – what is the relationship of vascular tissue to the cortex and the starch sheath?

The stele.

1. *Pelargonium* is an example of a herbaceous plant in which secondary growth occurs within the stem and is thus representative of species that undergo secondary growth. It makes an ideal study plant, as many features such as the development of a periderm, perivascular fibers and interfascicular as well as fascicular cambial activity can be demonstrated using *Pelargonium* species. The micrograph to the left shows a cross section of a stem which has completed its primary growth phase, and is at the onset of secondary development.

2. *Zea mays* is an important crop plant, and its structure has been well studied. Maize is a monocotyledonous plant, and resembles other grasses in the arrangement of tissues in the stem leaf and root. The stems of monocotyledons generally have a single ring of vascular bundles immediately beneath the epidermis, and internal to this a system of vascular bundles that are scattered throughout the pith. The peripheral vascular bundles are those that immediately join the leaf traces, and, as such any differences in the structure of the superficial vascular bundles and the deeper-seated bundles, might reflect some of the known structural components associate with the leaf vascular bundles.

3. Pine. Gymnosperms are an important study group, as they are of great economic importance. Structurally, Gymnosperm stems differ from the dicotyledonous and monocotyledonous examples shown earlier, in that the vascular tissues are more primitive. The phloem for example, lacks sieve tubes and companion cells, where the conduction of carbohydrate is managed by sieve cells. The sieve cells are accompanied by albuminous cells and associated parenchyma cells. In contrast to the dicotyledons, gymnosperm wood contains only tracheids, and no vessels. All Gymnosperms contain resins of some kind, which is transported in **resin ducts** or *resin canals*, which are usually associated with the cortical tissues in the stem.

Core assignment - stems

1. Draw LP diagrams of each of the specimens and identify and label all tissue zones visible. Comment on the distribution of mechanical supporting tissues, as well as of the vascular tissues, pay particular attention to the **differences** that you can see between the specimens. Note, you only have to draw HALF the specimen [15].

2. Using the diagrams that you have drawn, write an overview of stem structure based upon the three examples that you have examined. Comment on the arrangement of cortex and stele and the distribution of supporting and conducting tissues. One page, no more than 500 words, [10]

C. AN OVERVIEW OF LEAF STRUCTURE

Leaves can therefore show a surprisingly wide range of form when it is considered that in the majority of plants they perform three basic physiological functions involving;

the manufacture of food materials through the process of photosynthesis,

the transport of assimilated material and,

the evaporation of water, a process that drives the transpiration stream, and concomitantly, aids cooling of the leaf in hot conditions, whilst available transpirational water is not limiting.

Each of these functions is either initiated or takes place directly in the mesophyll leaves.

Total absorbing surface, both for gas exchange and sunlight utilization, Permeability of the epidermis to gases, The extent of the total intercellular space, The nature and distribution of the vascular tissues and climate and habitat

Leaves have vastly differing internal structure and this is demonstrated by the mesophyll cells which are arranged in **different patterns** and locations, which may be ascribed directly to the functional processes of the photosynthetic cycle occurring within the leaf. Leaves of dicotyledonous plants differ greatly from those of monocotyledonous plants and to those of gymnosperms. Of course, there is some degree of intergradation, but generally, it is possible to separate these leaves, using some basic diagnostic criteria. Dicotyledons generally have a mesophyll which is composed of two differing photosynthetic cell types – palisade and spongy mesophyll cells. Leaves may be isolateral, isobilateral, dorsiventral or even needle-like in cross-section. Whatever the leaf shape, chloroplasts are concentrated within the cytoplasmic matrix of these cells and, for the most part, the majority of the chloroplasts are to be found in the upper palisade mesophyll cells. Mitochondrial populations in these obviously-photosynthetic cells may be high as well.

TRANSLOCATION in plants involves movement of water and dissolved inorganic nutrients through the xylem from roots to the aerial parts of the plant, and the transport of photoassimilated material from sites of synthesis (source) to sites of utilization (sink) via the phloem.

This transport occurs in a vein network, termed the **major** and minor vein network. In mature leaves the minor veins are involved in the uptake of assimilate from the mesophyll and transport of these assimilates towards the larger veins, then the major veins, out of the leaf, and into the stem vascular system. Water transport essentially follows the reverse pathway.

The xylem is responsible for apoplastic transport in vascular plants, which is not limited totally to water transport, but in addition, the transport of various macro and micronutrients, amino acids and other important inorganic substances, from the roots to the stem and ultimately, the leaf via the apoplastic continuum.

The phloem is responsible for the transport of the major proportion of soluble carbohydrate as well as other essential products. The phloem forms the major long-distance symplasmic transport pathway in all vascular plants. Translocation usually takes place from a site of synthesis of assimilated material (called a source) to a site or sites of utilization (called sinks). The assimilated material is translocated in a water-based medium, which emphasizes the essential inter-relationship between the xylem and phloem, more particularly so in the leaf where most of the phloem loading takes place in mature plants. In many species, the companion cells are highly modified, with a very high frequency of plasmodesmata between the bundle sheath cells and the intermediary cells. The intermediary cells are distinctive in that they often contain numerous small vacuoles, and a large population of mitochondria. These plants synthesize sucrose during photosynthesis, and the sucrose is transported to the intermediary cells, via the numerous functional plasmodesmata. Once in the intermediary cells the sucrose is converted to larger raffinose-family oligosaccharides (RFOs), which cannot leak back out from the intermediary cell, and are trapped, increasing the osmotic potential, and causing the larger polymer to move into the sieve tubes, and thus transported to other regions of the plant via the phloem.

The three images below, illustrate aspects of structure of leaves that are common to groups of plants that live partly submerged, with floating leaves (left), on land under conditions of adequate rainfall (middle), and high stress (right). The water Lilly (*Nymphaea*) leaf shows many features common to plants with floating leaves. These plants are called hydrophytes. The most prominent here, are the large intercellular cavities that maintain buoyancy and facilitate good aeration. The *Ligustrum* leaf shows features of the majority of land plants, living in regions of adequate rainfall- perhaps high sunlight stress during summer (hence the red stained cells in the mesophyll, which contain tannins). This mesophyte has good aeration through a large body of interconnected intercellular spaces. The image to the right is a detail of the leaf of *Hakea* which is a typical fynbos plant. It survives harsh dry summers (high light intensity, high temperatures, low humidity) without rainfall, so the leaf is engineered to withstand high water stress, and it therefore contains a great deal of mechanical supporting tissue, to prevent leaf collapse during extreme dry conditions.

Practical examples

Examine the following specimens and identify:-

The epidermis

The mesophyll

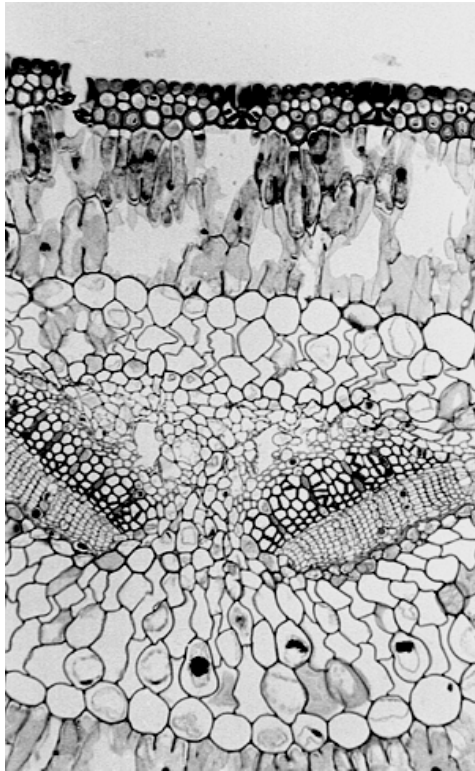
The vascular tissues:-

Specimens

The pine leaf TS

The *Zea mays* leaf TS

The *Nymphaea* (water Lilly) leaf TS.

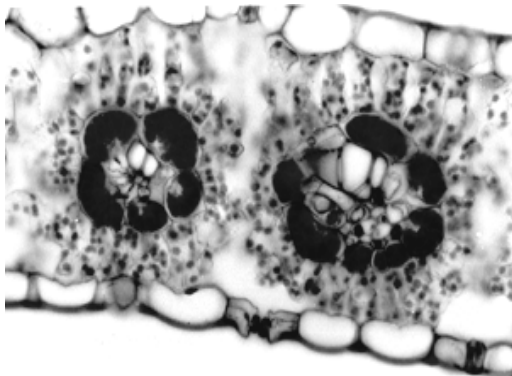


The pine leaf This is an example of a Conifer (Gymnosperm) leaf. Conifer leaves vary from needle-like to rather flattened structures. The pine leaf, is of course, an example of a needle-like leaf.

1. **Epidermis.** Epidermal cells with thick walls and cuticle. The guard cells are sunken and attached to prominent subsidiary cells.

2. **Mesophyll.** The outer cells of the ground tissue are differentiated as a hypodermis and are sclerenchymatous. Many of the remaining cells of the ground tissue have internal ridges projecting into the cell lumina. This is plicate mesophyll. In transverse sections the mesophyll appears compact but longitudinal sections would reveal prominent intercellular spaces. Most pine leaves contain two or more resin ducts, but the leaf you are examining contains several such ducts.

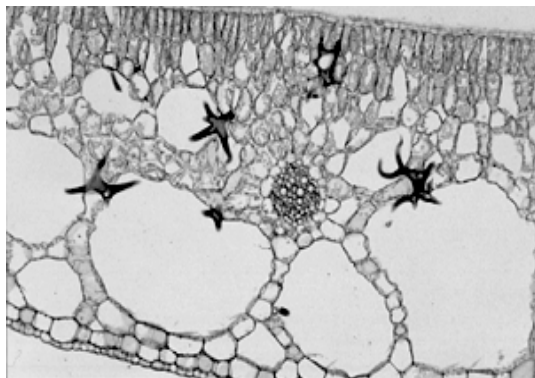
3. **Vascular tissue.** This pine leaf contains two vascular bundles, which are surrounded by transfusion tissue. The latter is composed of tracheids and parenchyma cells. The transfusion tissue is surrounded, in turn, by a conspicuous endodermis, the innermost layer of ground tissue. The endodermal cells may have somewhat thickened walls, especially the outer tangential wall, which contains conspicuous simple pits. At one time these endodermal cells may contain a well-developed Casparian strip. Each vascular bundle contains a vascular cambium. Some secondary xylem and secondary phloem has been laid down.



Zea mays. Zea is an example of a C_4 plant. Several anatomical features are commonly used to distinguish C_4 from C_3 plants. The most notable of these is the presence of a bundle-sheath surrounding the veins in the leaf which usually contains large, conspicuous chloroplasts. Another important feature of C_4 plants is the close spacing of the longitudinal vascular bundles.

Note that the mesophyll radiates from the bundle sheath - it looks **wreath-like**, hence its German-origin name, the **Kranz mesophyll**. This Kranz mesophyll layer contains numerous plasmodesmata through which intercellular transport from the mesophyll as well as from the mesophyll to the bundle sheath, can take place. These structures are minute -- ranging in size from 50 - 100 nm in total diameter! A diagnostic feature which can be used to separate monocot from dicot foliage leaf, is the fact that when monocot leaves are viewed in transverse section (i.e. such as the section that you can see in the sections presented in this exercise) **all the veins are cut in transverse plane**, whereas typical transverse sections of dicots will have veins which are mostly cut obliquely. Can you work out the reason why this should be so?

Most monocot leaves have groups of large, swollen epidermal cells, interspersed with smaller epidermal cells. These large cells are called **bulliform cells**. These bulliform cells have a significant role in the life of the plant, in that in times of water stress, the leaf is able to roll up, due to the rapid loss of water from the cytoplasm of the bulliform cells. This results in a smaller portion of the leaf being exposed to the atmosphere and as a result, a lower rate of water loss due to transpiration or evaporation.



The floating leaf of the Water lily is an excellent example of a **hydromorphic leaf**

Examine the detail image and locate the following:

1. Upper and lower epidermis. Do both lack a cuticle? All of the guard cells occur in the upper epidermis. Why is this so? The lower epidermis contains many small mucilage-secreting cells.

2. Mesophyll. Most of the ground tissue of this leaf is highly lacunose, that is, it has a large volume of intercellular space. The most compact mesophyll tissue is the palisade which consists of 2-3 layers of cells and comprises only about a quarter of the total thickness of the leaf. The spongy tissue is made up mostly of chains of parenchyma cells which form a network enclosing the large intercellular spaces. Note that where the chains meet, the parenchyma cells are branched. Note also that the large branched sclereids (trichosclereids), with arms and processes extending into the air chambers of the mesophyll. Small angular crystals are embedded in the walls of the sclereids.

3. Vascular tissue. The spongy parenchyma cells form a compact tissue and sheath around the vascular tissues in the region of the veins.

4. Note the large proportion of phloem to xylem in the veins of this hydromorphic leaf. Why do you think this is so?

Core assignment leaves

1. Make LP diagrams (see page 28) that identify:-

The epidermis

The mesophyll

The vascular tissues

Mechanical tissues. **[15]**

2. Write a short account (1 but no more than two A4 pages maximum, 1.5 line spaced) in which you attempt to **interpret the trends** which may be evident in the specimens that you have been given to examine. **Hint:** Look at the differences in distribution of vascular tissues; look also at presence or absence of vessels and sieve tubes; Distribution of supporting tissues; photosynthetic tissues and other features that distinguish root from stem, from leaf. Include the write-up with the assignment, and cross-reference the write up to the drawings that you have made during the assignment. **[10]**

Assignment 3. Organization and structure of the vascular system in leaves

In this assignment we will revisit the leaf, this time focussing more attention on their vascular anatomy. As with the other assignments, there is time only to show you a few examples which illustrate the basic differences between the gymnosperm, and angiosperm leaf (dicotyledon and monocotyledon). There are key differences between all of them, and you are encouraged to look at **The Virtual Plant** for more useful information in the section which deals with the classification of plant parts. Follow the links from <http://anubis.ru.ac.za/index.html> and specifically you should look at the introduction at, <http://anubis.ru.ac.za/Main/ANATOMY/vascular.htm>

Specimens:

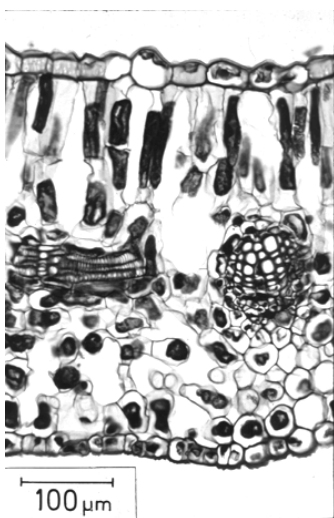
1. *Ligustrum* leaf TS (mesophyte, C₃ dicot)
2. *Pinus* leaf TS (gymnosperm)
3. *Nerium oleander* leaf TS (xerophyte, C₃ dicot)
4. *Amaranthus* TS leaf (C₄ weedy dicot)
5. *Nymphaea* TS leaf (hydrophyte, C₃ dicot)
6. *Zea mays* TS leaf (C₄ monocot)

When studying the anatomy of various organs, you should keep in mind the **functions** that these organs perform. This is particularly important in the case of the leaf. An understanding of leaf anatomy is impossible unless correlated with some knowledge of leaf function. For example the main functions of the leaf are photosynthesis and transpiration, both of which involve gaseous exchange between living cells and the atmosphere. Consequently it is necessary to consider such features as :-

- Total absorbing surface, both for gas exchange and sunlight,
- Permeability of the epidermis to gases,
- The extent of intercellular spaces, and
- The nature and distribution of the vascular tissues influences transpiration, water distribution, assimilate uptake.

Our focus in this practical is the vascular tissue – and specifically, the arrangement of vascular bundles, their distribution through the midrib as well as the lamina of the leaf, and the various sizes of **Our focus** in this practical is the vascular tissue – and specifically, the arrangement of vascular bundles, their distribution through the midrib as well as the lamina of the leaf, and the various sizes of **major** and **minor** veins.

Ligustrum (Privet) leaf



Examine the prepared slide of a TS of this leaf. First orientate the section to be sure the upper surface of the leaf is at the top of the slide (the xylem towards the top of the slide (i.e., away from you)), as seen through the microscope. **Make a LP diagram** of a representative part of the section, including the ad- and abaxial epidermis and a **HP drawing** of part of the lamina, including a vein and two or more stomata (if these are visible). Note the following:

The midrib contains a single, large **collateral** vein. Here and in the lamina, the upper epidermis has a thick cuticle. The large vein is completely surrounded by parenchyma cells, which form a bundle sheath. A well-developed cambium separates the upper (adaxial) xylem from the lower (abaxial)

phloem. Most of the xylem and phloem is primary. Some of the parenchyma cells above the lower epidermis are collenchymatous and thickened.

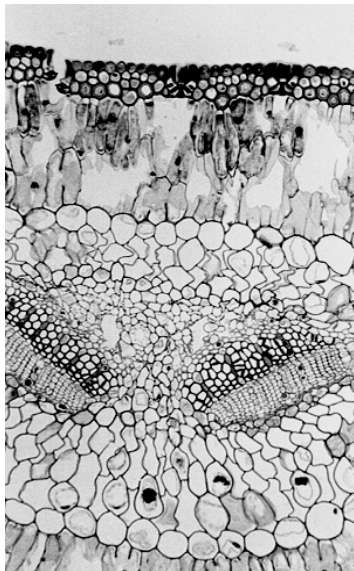
The Lamina consists of-

Upper and Lower Epidermis. Note cuticle and distribution as well as the appearance of guard cells. The guard cells have conspicuous outer ledges composed of cutin. Trichomes occur on both upper and lower epidermis.

Mesophyll. Distinguish between **palisade** and **spongy mesophyll**: numbers of layers in each tissue, form of cells and size of intercellular spaces, manner of connection, if any, of cells to each other, content of cells, arrangement of chloroplasts in the two kinds of mesophyll cells, and relation between stomata and intercellular spaces (any sub-stomatal chambers) in the mesophyll.

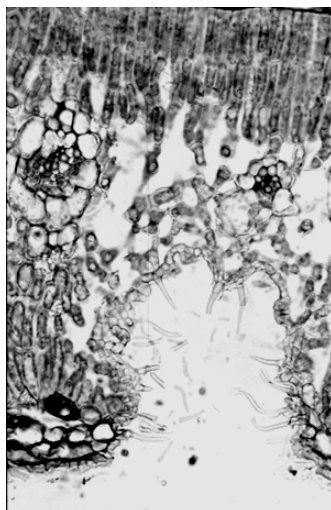
Vascular Tissue. You have already examined the large vein or bundle of the midrib. The smaller bundles are embedded in the mesophyll, but each is bounded by a **bundle sheath**. Note the distribution of the chloroplasts in the sheath cells, the position of the xylem and phloem within the bundle. Some of the bundles are seen in TS, some in LS, and some are oblique - why?

Pine leaf



Vascular tissue. This pine leaf contains two vascular bundles, which are surrounded by a **transfusion tissue**. The latter is composed of tracheids and parenchyma cells. The transfusion tissue is surrounded, in turn, by a conspicuous endodermis, the innermost layer of ground tissue. The **endodermal cells** in your slide have somewhat thickened walls, especially the outer tangential wall, which contains conspicuous simple pits. At one time these endodermal cells contained a well-developed **Casparian strip**. Each vascular bundle contains a **vascular cambium**. Some secondary xylem and secondary phloem has been laid down.

Nerium oleander leaf



This is an example of a **xeromorphic leaf**. Examine the prepared slide of a TS of the oleander leaf. Under LP note the many invaginations of the lower epidermis. These are stomatal cavities, which contain many trichomes as well as all of the stomata. Note the distribution and size of the veins.

Now use the HP objective, and examine it in more detail.

Note the following:

Upper and lower epidermis with thick cuticle. As mentioned, the stomata are restricted to the stomatal crypts of the lower epidermis. These invaginations with their numerous trichomes, probably greatly reduce the loss of water vapour from the leaf.

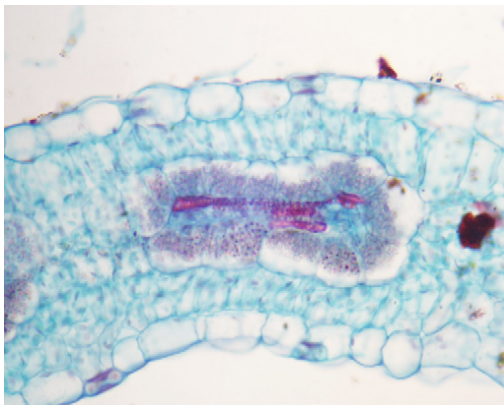
Mesophyll. The mesophyll or ground tissue, of the oleander leaf is differentiated.

Hypodermis beneath both upper and lower epidermies. This tissue is devoid of chloroplasts.

Beneath each hypodermis are **2-3 layers of palisade parenchyma** with **spongy parenchyma** in the centre of the leaf. This leaf is **isolateral**. Very large intercellular spaces occur among the spongy parenchyma. Notice the crystals (druses) in some of the mesophyll cells.

Vascular tissue. Examine the large vascular bundle of the midrib. This is a **bicollateral bundle**. A vascular cambium has developed on the lower side of the bundle and has produced some secondary xylem, but little if any, secondary phloem. The smaller vascular bundles or veins of the leaf (that is, those found in the lamella) are collateral. Note the conspicuous, well-developed parenchymatous bundle sheaths surrounding the vascular tissues. Some of the bundles contain bundle sheath extensions.

Amaranthus leaf



Amaranthus is a C_4 plant. Note the conspicuous, large **chloroplasts** surrounding the vascular tissue in this vein.

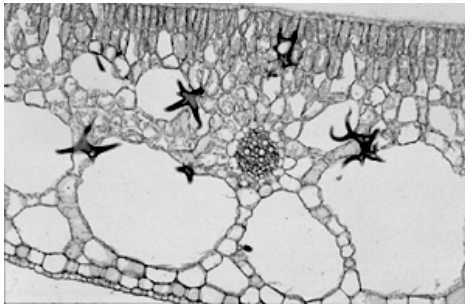
Examine this slide and note the following:-

Epidermis. Both upper and lower with cuticle and relatively thick tangential walls. Stomata are easy to find in both epidermies. Note the prominent inner and outer ledges of the guard cells.

Mesophyll. Not differentiated into palisade and spongy parenchyma, but with small cells with small intercellular spaces among them below either epidermis. The mesophyll cells in between are radiate, larger and contain well-developed, sizeable intercellular spaces. Note the distribution and arrangement of chloroplasts. This is typical of a C_4 dicotyledonous plant - it is an example of a C_4 photosynthetic specimen (*Z. mays* is also an example of a C_4 plant, but is monocotyledonous).

Vascular bundles. Very uniform in size and arrangement. Prominent bundle sheath composed mostly of parenchyma cells, some of which contain crystals (raphides) cut in transsection. Some sheath cells have thin secondary walls. The bundle sheath cells may contain very prominent chloroplasts. If so, then the specimen is a C_4 photosynthetic dicotyledon. Distinguish between the protoxylem and metaxylem, protophloem and metaphloem. *Many of the sieve elements of the metaphloem contain small, triangular-shaped companion cells.*

***Nymphaea* leaf**



The floating leaf of the water lily is an excellent example of a hydromorphic leaf. Examine the prepared slide of a TS of this leaf and note the following:

Make an LP diagram of part of the lamina and an HP drawing to show Part of the network of parenchyma cells with its included intercellular spaces and at least one sclereid.

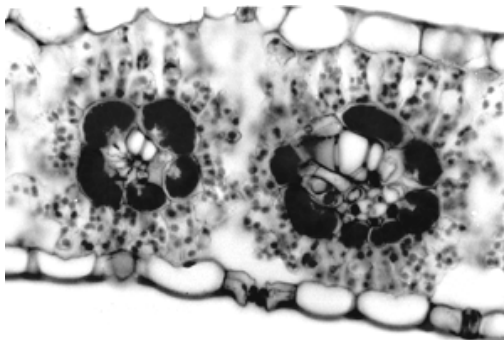
Note: Upper and lower epidermis lack a cuticle. All of the guard cells occur in the upper epidermis. Why? The lower epidermis contains many small mucilage-secreting cells.

Mesophyll. Most of the ground tissue of this leaf is highly lacunose, that is, it has a large volume of intercellular space. The most compact mesophyll tissue is the palisade, which consists of 2-3 layers of cells and comprises only about a quarter of the total thickness of the leaf. The spongy tissue is made up mostly of chains of parenchyma cells that form a network enclosing the large intercellular spaces. Note that where the chains meet, the parenchyma cells are branched. Note also that the large branched sclereids (trichosclereids, with arms and processes extending into the air chambers of the mesophyll. Small angular crystals are embedded in the walls of the sclereids.

Vascular tissue. In the region of the veins, the spongy parenchyma cells form a compact tissue and sheath around the vascular tissues. Comment on the relative size of the xylem vessels in this hydrophyte compared with the mesophytes that you have been given to investigate.

Note the large proportion of phloem to xylem in the veins of this hydromorphic leaf.

***Zea mays* leaf**



Look at the prepared slide of *Zea mays* leaf. *Zea* is another example of a **C₄ plant**. Several anatomical features are commonly used to distinguish C₄ from C₃ plants.

Look at the leaf and identify the three major vein sizes (viz. large, intermediate and small). Monocot leaves contain two distinct sieve tube types and no secondary xylem.

The most notable of these is the presence of a **bundle sheath** surrounding the veins in the leaf, which usually **contain large, conspicuous chloroplasts**. Note that the mesophyll **radiates** from the bundle sheath - it looks wreath -, hence its German-origin name, the Kranz mesophyll. Another diagnostic feature which can be used to separate monocot from dicot foliage leaf, is the fact that when viewed in transverse section (i.e. such as the section that you should be examining now) all the veins in monocot leaves are cut in transverse plane, whereas typical transverse sections of dicots will have veins which are mostly cut obliquely. **Can you work out the reason why this should be so?**

Most monocot leaves have groups of **large, swollen epidermal cells**, interspersed with smaller epidermal cells. These large cells are called **bulliform cells**. These bulliform cells have a significant role in the life of the plant, in that in times of water stress, the leaf is able to roll up, due to the rapid loss of water from the cytoplasm of the bulliform cells. This results in a smaller portion of the leaf being exposed to the atmosphere and as a result, a lower rate of water loss due to transpiration or evaporation.

Core assignment

Draw low power plan diagrams (see page 28) which illustrate the basic structure of the leaves that you have examined, and the distribution of vascular bundles [**30**].

Write a short account (**no more than two** A4 pages maximum, 1.5 line spaced) in which you attempt to interpret the vascular trends which you could see in the specimens that you have been given to examine. Try to cross-reference the write up to the drawings that you made. [**30**].

Appendix A: Useful Laboratory techniques

TECHNIQUE 1: THE USE OF THE MICROSCOPE

Read these notes carefully and, whatever procedure you may have followed in the past, it is strongly recommended that you follow the methods as set out here.

Arrange the microscope so that you can use it in comfort when the left eye (for right-handed students) is at the eyepiece.

You should not have to lean across the bench or sit in a strained position nor should you have to move the microscope or your seat during observations. Your laboratory book should lie close to the microscope on the right hand side.

Make sure the L.P. (x10) objective is in position, turn on the lamp, open the diaphragm to its widest aperture and, looking through the eyepiece, adjust the light which is transmitted so that it is evenly distributed within the field of view.

By means of the sub stage adjustment, bring the condenser to its **highest position**, then lower it **very slightly** so that the whole field of view is brightly and evenly illuminated.

Partially close the diaphragm and adjust the lamp brightness to cut out glare.

Use of the higher power objectives.

The microscope which you have, will normally be equipped with not more than three objectives; a 3 to 4X, a 10X, and a 40X. The 40X objective is thus referred to as the high power objective. Some practicals may require the use of oil immersion objectives. Special precautions necessary for their use will be given to you, when the time arises. When the nosepiece is rotated to bring the H.P. objective into position, the specimen will still be visible, but may not sharply in focus (most student microscopes are not completely par focal). Re-focus by means of the fine adjustment, until the image is sharply focused.

Remember that adjustment of the condenser is just as essential for critical resolution, as is focusing.

When you have finished with the H.P., rotate the nosepiece, to bring the low power objective into position and re-focus with the fine adjustment. This practice will prevent the fine adjustment from being run to the end of its traverse.

Never insert a slide directly under the H.P. objective. Always focus using the L.P. first. Apart from obviating risk of damage to the H.P. lens, time will be saved because no two specimens will be of exactly the same thickness.

Care of the microscope.

The microscope is an expensive precision instrument; it is your responsibility to use it properly. Please observe the following simple guidelines.

If the microscope develops any faults or becomes damaged, report it to the instructor immediately. Do not on any account unscrew any of the components, particularly of the lens system.

Carry the instrument in an upright position and set it down gently.

If water or reagents are spilt on the stage, wipe them off immediately. All optical parts must be kept clean and dry. They should not be touched with the fingers or wiped with dusters, etc. Any cleaning should only be done with lens tissue, which is available on the instructor's bench).

The specimen must always be enclosed by a cover glass when examined with the H.P. objective.

After use, make sure the L.P. objective is in position and the instrument quite clean.

Do not leave any slides on the microscope.

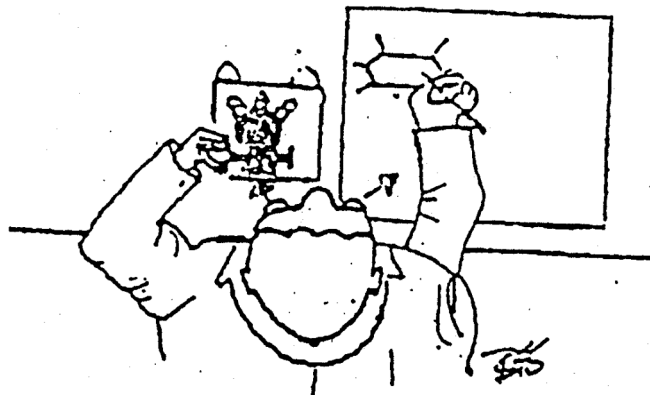


Figure 1 Diagram showing correct placement of microscope and drawing paper (for right-handed person).

TECHNIQUE 2: MOUNTING OF FREEHAND SECTIONS

Whenever you make mounts for microscope examination -

Make sure the slide and cover slip is quite clean.

Ensure that the specimen does not project beyond the edge of the cover slip.

Add just sufficient mounting medium to fill the area of the cover slip. If excess is present, mop up with absorbent paper. If insufficient is present, add a further drop at the side of the cover slip.

Lower the cover slip gently and avoid the inclusion of air bubbles.

Place the cover slip squarely in the centre of the slide.

Take some pride in making even the temporary mount neat and attractive.

If you think carefully you will appreciate that there is at least one scientific reason for each of these requirements.

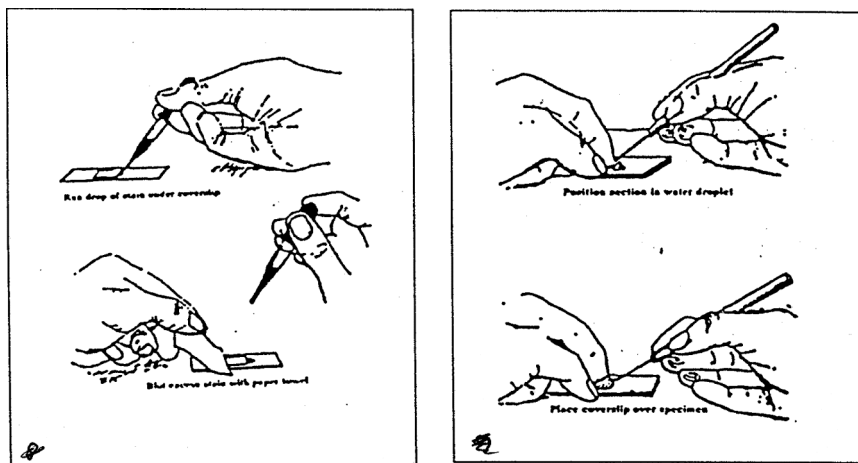


Figure 2 Shows placement of cover slip over specimen and irrigation: (a) a drop of stain placed next to cover slip (b) stain drawn by capillary action under cover slip. Irrigation:

TECHNIQUE 3: DRAWING CELL DETAIL

The drawing must be large, with clearly defined, non-fuzzy lines.

One particular cell must be represented, i.e. not a generalized impression.

The parts must be represented in their true proportions. Thus when making a large drawing of a single cell, the cell wall must be indicated by double lines, faithfully following any variation in wall thickness. (In later exercises, when cells are drawn to a smaller scale, single lines will normally be used).

Indicate the cytoplasm by stippling and try to show the approximate density of cytoplasm granules.

Pay particular attention to the "corners" of the cell and show how the walls are connected to adjacent cells.

Technique 4: the use of stains

The purpose of using a stain in microscopy, is firstly to render transparent objects more opaque and secondly, to find out something about their chemical composition. The affinity for a stain is largely dependant on the composition of the various parts of the cell and a single cell will differentially stain by virtue of its heterogeneous composition. For example, the protoplasts of the *Zebrina* guard cells were stained brown and the starch grains black by iodine solution. The cell wall became a yellowish colour, but was not very efficiently differentiated from the cytoplasm. It would however have been possible to stain the cell wall with another reagent, which had a specific affinity for cellulose. Thus whilst it is possible to differentiate some of the cell components by using a single stain or reagent, this can be done more effectively by using two (or more) stains, each more or less specific for a certain component. The two stains may be applied separately or may be combined in a single solution. Fabil stain consists of a mixture of dyes, incorporated in a mounting medium. The plant material, either fresh or preserved, is mounted directly in Fabil. It may be examined immediately, but the staining will be found to be better after about 5 to 10 minutes immersion, and continues to improve for some time. The main colour reactions are:

Cytoplasm and nuclei	deep blue
Cellulose and hemicellulose walls	pale or silvery blue
Lignin-impregnated walls	shiny yellow, orange or pink
Suberin-impregnated walls	crimson
Starch grains	black

Technique 5: section cutting

Study the diagrams and make sure you understand the different planes of sectioning, viz. transverse, longitudinal radial and longitudinal tangential.

Reference points, lines and planes

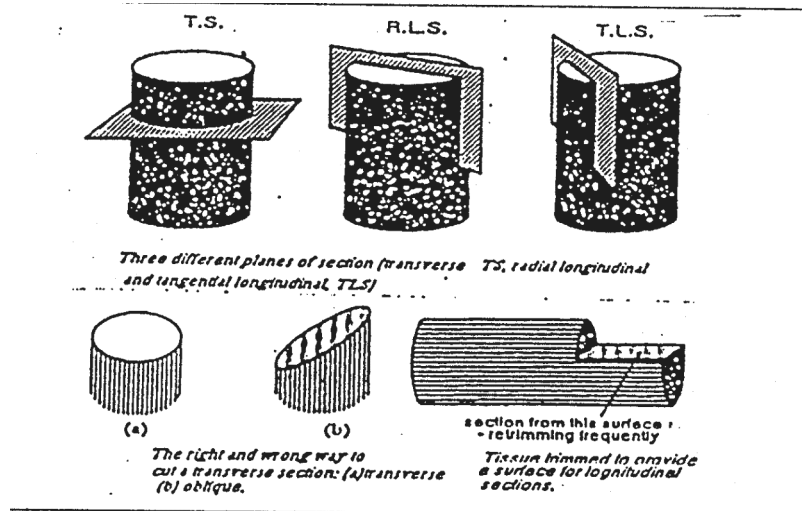


Figure 3 Illustrates planes of section. Please study carefully! Specimens, which are sectioned by supporting as illustrated on the previous page, are usually thin sections (about 15 - 25 microns).

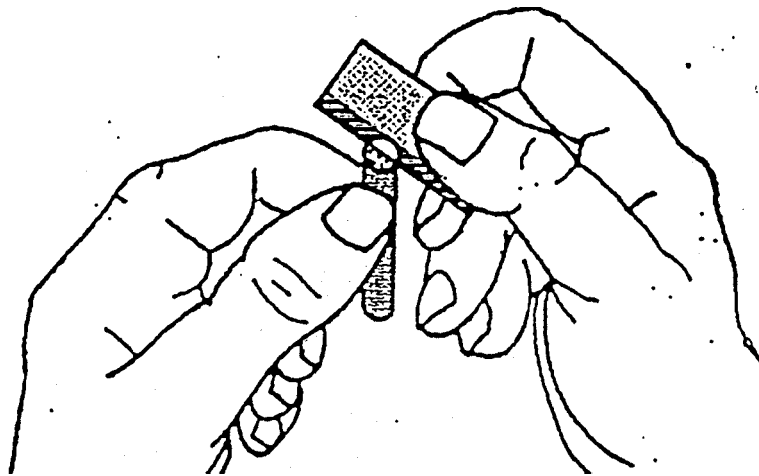


Figure 4 Sectioning with a single-edge razor blade (right-handed person). Blade is kept wet during cutting.

a) Whilst a single edge blade is preferable to a double edged one, not only for ease of holding but because they are usually made of thicker metal and therefore not so likely to bend during sectioning, one can cut very thin sections, with a double edged blade with practice.

b) At commencement of sectioning, trim the surface of the material level and truly orientated. Then cut several sections in rapid succession, transferring them to a watch glass as cut. It will be advantageous to rotate the material at intervals to counteract any tendency towards obliqueness in the sections.

c) Keep the blade and material wet whilst sectioning; use water for fresh material and 70% alcohol for preserved material. If the section dried at this or any subsequent stage, it would become full of air bubbles. *NOTE: Air bubbles often look like "cells" in that the edge of the bubbles approximate the average cell wall in thickness.*

d) It is often supposed that the thinner the section, the better. However, cells vary greatly in size and two equally thin sections might consist of only one, or of several layers of cells respectively. In the thinnest hand section of, for example, a stem, the very large cells of the pith may be cut in half, whilst in other parts of the section, the smallest cells may be two deep. The reason for attempting to cut the thinnest section possible, is to allow as much light to pass through the specimen, and to allow one layer of cells to be sharply focused. Within limits, it is as desirable for the section to be truly transverse as well as being thin.

e) The area of the section is determined by the variety of cell types and tissues present. The section must therefore include all cell and tissue types present within the specimen. The only exception to this, is when a complete section of an organ is required, in order that a plan may be drawn, to show the distribution of the cell and tissue types within the organ. In such cases, the section need not be particularly thin.

Technique 6: handling and staining of sections.

(a) As cut, transfer the sections to a watch glass by means of a fine paintbrush. For fresh material the watch glass should contain water, for preserved material, use 70% alcohol.

(b) Examine the sections: if thin enough, they will be almost transparent, but if too thick, they will be white and opaque.

(c) Using a fine paintbrush and not a needle, transfer two or three sections only to a clean slide.

(d) Add a drop of Fabil stain, just sufficient to fill the cover slip when it is lowered. (You will learn by experience, just how little is needed). Make sure that the stain covers the sections, before adding the cover slip, otherwise the cover slip may make a seal over the section and the stain will not penetrate.

(e) Do not stain sections in the watch glass: You won't be able to find them if they are immersed in a pool of Fabil.

(f) Use filter paper to mop up excess stain. Do not spill stain on clothes or books, as it cannot be removed.

Beware!!! Fabil is poisonous and caustic — wash off with water if it should get on your hands.

Technique 7: illustrating organ structure. The low power drawing

It is customary to illustrate the internal structure of organs by means of a diagrammatic plan, and a detailed drawing (hereafter referred to as the LP diagram and the HP drawing).

The LP Diagram shows the distribution of the zones of tissue within the structure.

It is advisable to have a “B” and an “HB” pencil for drawing.

(a) In the case of TS of stems or roots, draw half of the section. For TS of leaves, and all LS, draw the complete organ. A complete, but not necessarily thin section will be required for the latter.

(b) Commence by drawing an outline of the zones. It is very important to maintain the correct proportions - Note for example, the relative width of the cortex, and stele in stems and roots. Indicate accurately, the position, size and number of vascular bundles.

(c) The LP diagram should occupy at least half a page in your practical book.

(d) Now mark the position of the tissues. Cell structure is on no account to be shown in the LP diagram. Use the conventional shading, as illustrated below.

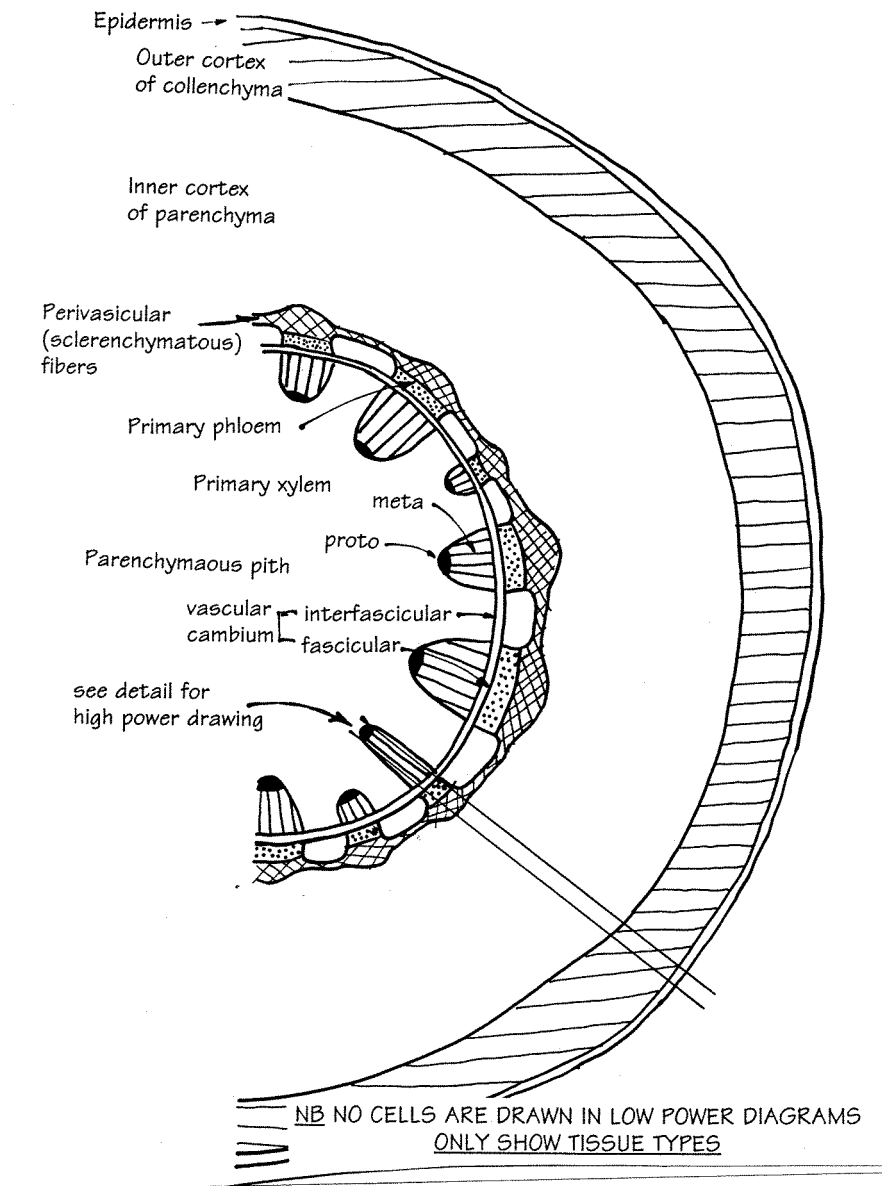
(e) Label your drawing as fully as possible (i.e., zones and tissues). Arrange the labels neatly and systematically, preferably going from the outer, to the innermost tissues as one reads down the page. You will find it convenient to arrange the labels on the right hand side of the page, with the plan of the organ on the left.

(f) Lines should be clear and not fuzzy.

(g) All labelling must be in pencil. Try to be neat and ensure that the lettering runs horizontally. Labels may be either printed, or written, according to what you can do most neatly. Rule indicator lines (without arrowheads) and terminate these unambiguously. Indicator lines should not cross one another and should go as directly as possible from the label, to the feature indicated.

(h) Although the plan is called an “LP” diagram, it is not intended that it should be based solely on the examination of the structure using the low power objective alone.

The conventional shading given below must be used in the LP diagram



Collenchyma		-hatched
Sclerenchyma		-cross hatched
Phloem		-stippled
Protoxylem		-solid
Secondary xylem		-radiating lines
All other tissues		-leave blank

Figure 5 The Low Power Drawing

NOTE: These symbols are merely to add emphasis to your drawing, they do not take the place of complete labelling.

Collenchyma hatched; Sclerenchyma cross hatched; Phloem stippled; Protoxylem black

Secondary xylem radiating lines; All other tissues leave blank

These shading used in the diagram on the following page adds emphasis in a pencil drawing: They are not intended to take the place of complete labelling.

Technique 8: illustrating organ structure, the high power diagram

HP Diagram: To show the structure of the tissues and their component cell types.

(a) Draw sufficient of the specimen to enable you to illustrate a representative sample of all the tissues and cell types present. A thin and truly orientated section is necessary, though it need not be complete.

(b) Commence by selecting the best part of your section and examine it under LP. Taking a full page, map out, with faint lines, the limits of the different tissues.

(c) The drawing should represent a continuous narrow sector, say 6 to 10 cells wide, and should not contain isolated or disconnected patches of tissues. There is one exception to this however, in the case of very wide zones of homogeneous tissues, for example, as found in the parenchymatous cortex of roots. It is permissible here to rule two parallel lines and writing between the lines "6 rows of cells omitted" as has been done in the illustration on the preceding page for example.

(d) The detailed study of cell and tissue structure must consist of an accurate drawing, i.e., it should not be diagrammatic. It must show the cells present in your preparation and must not in any way, be a generalised representation.

(e) To make a good HP drawing will at first be a tedious process. The epidermal and cortical cells are generally large and relatively easy to draw accurately. The smaller cells are more difficult e.g., phloem, tracheids fibres etc. Do not draw the epidermal / outer cortical cells too large—you may run out of paper.

(f) Work out a suitable scale before you start to draw. The smallest cells, e.g. in the phloem, cannot be represented with any degree of clarity at less than about 4mm diameter. This will mean that the largest cells (cortex / epidermis) may have to be about 2cm in diameter, to retain the proportions.

(g) Do not show any cell contents & do not use any shading except:

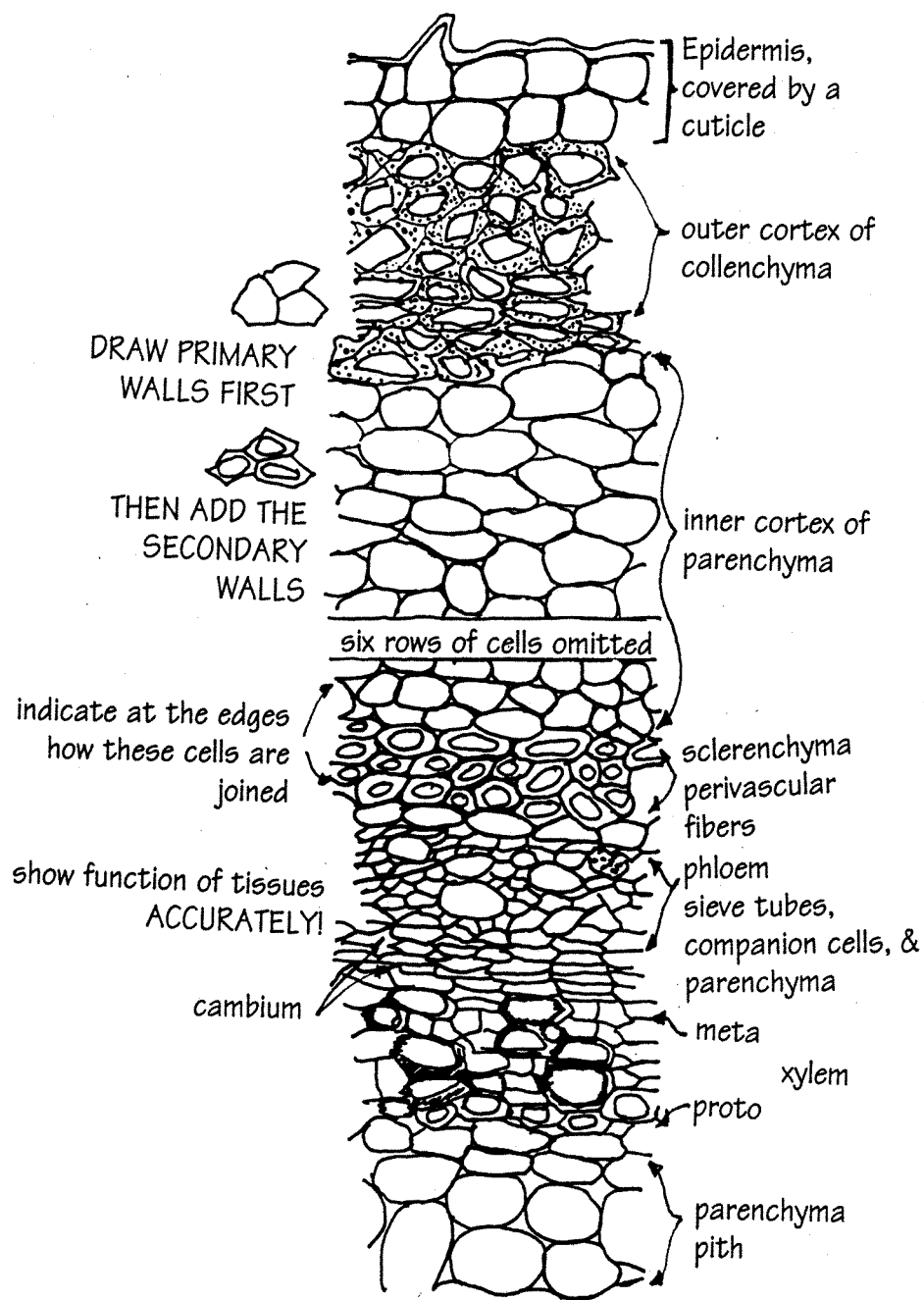
chloroplasts	-- (can be diagrammatic)
starch in starch sheath	-- (diagrammatic)
crystal inclusions	-- (can be diagrammatic)
sieve plates	-- (diagrammatic)
cytoplasm of companion cells	--(stipple)

(h) Use a single line for unthickened (e.g. primary) cell walls, and a double line for a thickened (e.g. secondary) wall. It is quite effective to use an "H" pencil for all primary walls, and an "HB" pencil for all secondary walls the secondary wall is usually drawn a little thicker than the primary wall—see the example on the preceding page.

(i) Draw the cell walls, and the cell cavities will draw themselves. This is particularly important in cell types such as collenchyma, sclerenchyma, and tracheary elements. Construct the walls by first drawing the primary wall. Then add the secondary wall. What you are doing is merely following the development process in your drawing.

(j) Pay particular attention to the shape of the cells and the way in which they fit together. Extend the walls at the edges of your drawing, to show how the cells join one another. Show the junctions between different cell and tissue types clearly and accurately.

(k) All drawings should be fully labelled. Observe the instructions (e) to (g) in the notes on the LP diagram.



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