41

Plant description, morphology and anatomy

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Plant form ranges from unicellular plants—for example, yeasts and some green algae—to the strongly differentiated higher plants. Examples of pharmaceutical interest may be found in most of the larger groups and a quick perusal of the families involved in Chapter 5 of this textbook illustrates this point.

Characteristically the higher plants consist in the vegetative phase of roots, stems and leaves with flowers, fruits and seeds forming stages in the reproductive cycle. Modifications of the above structures are frequently present—rhizomes (underground stems), stolons (runners with a stem structure), stipules, bracts (modified leaves), tendrils (modified stems), etc. Certain organs may appear to be missing or much reduced—for example, the reduction of leaves in some xerophytic plants.

It is most important that students acquire the ability to interpret morphological and anatomical descriptions of crude drugs as found in pharmacopoeias and allied works and also to record adequately the features of whole or powdered drugs and adulterants of commercial significance.

As indicated in Chapter 2, for convenience of study, drugs may be arranged not only according to families and chemical constituents, but also into such morphological groups as barks, roots, leaves, seeds, etc. Some drugs constitute more than one morphological part—for example, whole herbs and commercial 'roots', which may consist of both rhizomes and roots.

LEAVES AND TOPS ('HERBS')

These consist of stems (often limited in their girth by 'official' requirements) and leaves often associated with flowers and young fruits. All portions of such drugs need to be described.

Aerial stem. Note dimensions, shape, colour, whether herbaceous or woody, upright or creeping, smooth or ridged, hairs present or not and if so whether of the glandular or covering form. Note arrangement of tissues as seen in transverse section.

Position and arrangement of leaves. *Radical* (arising from the crown of the root) or *cauline* (arising from the aerial stem). In the Solanaceae note *adnation* (the fusion of part of the leaf with the stem). The arrangement may be *alternate* (e.g. lobelia), *opposite, decussate* (in pairs alternately at right angles; e.g. peppermint) or *whorled*.

Leaves, flowers and fruits. These can be described according to the schedules given below.

Structure of the aerial stem. The primary stem (Fig 41.1A) shows the following structure: epidermis, cortex, medullary rays, medulla and a vascular system taking the form of a dictyostele. The epidermis is composed of a single layer of compactly arranged cells and bears stomata. The cortex is usually parenchymatous, the outer layers of cells in aerial stems containing chloroplasts. The layers of cortex cells immediately underlying the epidermis may be collenchymatous, constituting a hypodermis. The endodermis is usually not well-differentiated in aerial stems, although a layer of cells containing starch (starch sheath) and corresponding in position to the endodermis may be defined. Underground stems often resemble roots in showing a more or less well-differentiated endodermis with characteristic Casparian strips (thickenings).

The pericycle may take the form of a complete or a discontinuous ring of fibres or may be parenchymatous and ill-defined. Pericycle fibres may form a cap outside each primary phloem group.

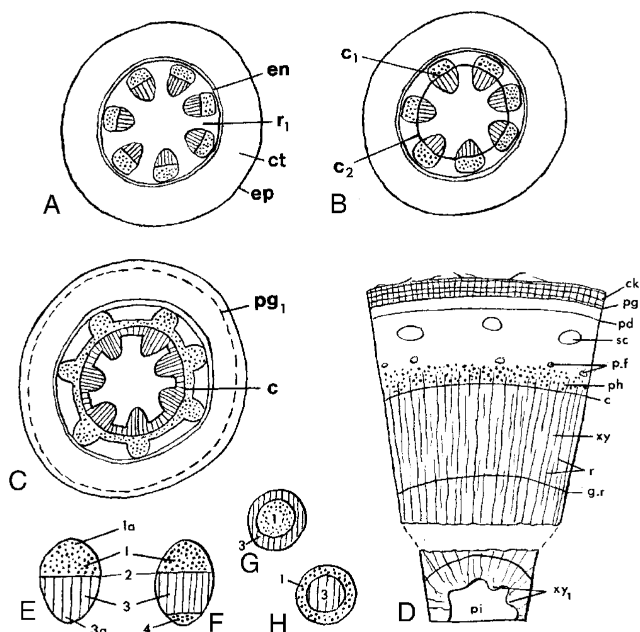

Fig. 41.1
Stem structure of dicotyledons (transverse section). A, primary structure showing seven vascular bundles; B, development of a complete cambial ring by formation of the interfascicular cambium; C, beginning of a secondary growth; D, stem after a number of seasons of growth, outer cork now present. E–H, types of vascular bundle: E, collateral; F, bicollateral; G, amphivasal; H, amphicribal. c, Cambium; c_1, fascicular cambium; c_2, interfascicular cambium; ck, cork; ct, cortex; en, endodermis; ep, epidermis; g.r, growth ring; pd, phelloderm; p.f, pericyclic fibres; pg, phellogen; pg_1, developing phellogen; pi, pith; r, rays; r_1, primary medullary ray; sc, sclerenchyma; xy, xylem; xy_1, primary xylem; 1, phloem; 1a, protophloem; 2, fascicular cambium; 3, xylem; 3a, protoxylem.

The vascular bundles of the dictyostele are usually collateral, but are in some cases bicollateral (Cucurbitaceae, Solanaceae, Convolvulaceae) (Fig. 41.1E–H). The xylem is differentiated centrifugally and the protoxylem is endarch; the phloem is differentiated centripetally and the protophloem is exarch (cf. the root). The differentiation, in dicotyledons, is usually incomplete, so that a zone of meristematic cells (the intrafascicular cambium) separates the primary vascular tissues. Such a bundle is described as open, in contrast to the closed bundle typical of monocotyledons. In the bicollateral bundle the intrafascicular cambium occurs between the xylem and the outer phloem group.

Secondary thickening is initiated by tangential divisions in the intrafascicular cambium. The daughter cells cut off on the inner side differentiate into xylem and those cut off to the outside into phloem. The amount of secondary xylem produced in both stems and roots, in general, exceeds the amount of secondary phloem. As the process of secondary thickening of the stem proceeds, its dictyostele is converted into a solid cylinder of secondary tissues. The intrafascicular cambia become linked to form a continuous cambial cylinder by the development of interfascicular cambia in the ray tissue (Fig. 41.1B, C). The cambial activity may spread out from the intrafascicular cambia across the rays, or in other cases cambial activity may originate at a median point in the ray and then by lateral extension from both intrafascicular and interfascicular cambia the cambial cylinder may be completed.

In woody perennials the cambial divisions are arrested during the winter but are renewed each spring. The xylem produced at different seasons varies in texture. The spring wood is characterized by abundance of relatively thin-walled large conducting elements; the autumn wood, by a high proportion of thick-walled mechanical elements such as wood fibres. A similar alteration between sieve tissue and phloem fibres may occur in the secondary phloem. With increase in girth the central core of xylem may become non-functional, dark in colour and packed with metabolic byproducts forming a heartwood or duramen. Sandalwood is the heartwood of *Santalum album* and is packed with volatile oil. The blocking of the vessels in the formation of heartwood occurs by the development of tyloses (see Fig. 42.6P).

The secondary increase in diameter of the vascular cylinder is accompanied by changes in the outer tissues. The epidermis and part or all of the primary cortex may be shed. A phellogen may arise in the epidermis, cortex or pericycle and give rise externally to cork and internally to a variable amount of phelloderm (Fig. 41.1D).

For the investigation of the anatomy of stems, transverse sections and radial and tangential longitudinal sections should be prepared from the drug previously moistened or soaked. For a study of the individual elements, disintegrated material should be used (see Chapter 43).

The following structures are constantly present in powdered stems: cork and vascular tissues in varying amount; abundant parenchyma often containing starch. Calcium oxalate and other cell inclusions may be present. Aleurone grains are absent.

The relative amounts, size, shape and form of the structural elements are of first importance in identification. The xylem elements, which are well-preserved in dry drugs, are of particular importance.

BARKS

As understood in commerce, barks consist of all tissues outside the cambium. In botany the term 'bark' is sometimes restricted to the 'outer bark'—that is, the periderm and all tissues lying outside it.

A young bark (Fig. 41.1) is composed of the following tissues.

1. Epidermis: a layer of closely fitting cuticularized cells with occasional stomata.
2. Primary cortex: a zone usually consisting of chlorophyll-containing collenchyma and parenchyma.
3. Endodermis: or inner layer of the cortex, which frequently contains starch.
4. Pericycle: which may be composed of parenchyma or of fibres. Groups of fibres often occur opposite each group of phloem.
5. Phloem: which consists of sieve tubes, companion cells and phloem parenchyma separated by radially arranged medullary rays.

In commercial barks the above structures have been modified by the activity of the cambium and the cork cambium or *phellogen*. Growth of the new tissues produced by the cambium causes the tissues of the primary bark to be tangentially stretched, compressed or torn. As these cells are stretched tangentially they may be divided by radial walls—for example, in the medullary rays. During this dilatation groups of parenchymatous cells in the cortex and phloem may be thickened into sclerenchymatous cells. The cambium produces secondary phloem, which often consists of alternating zones of sieve elements and phloem fibres. The pericycle is frequently ruptured, and parenchymatous cells which grow into the spaces may develop into sclerenchyma.

The cork cambium or *phellogen* may arise in the epidermis (e.g. willow), primary cortex or pericycle. The phellogen produces on its outer side *cork*, and on its inner side chlorophyll containing suberized cells which form the *secondary cortex* or *phelloderm*. These three layers are known as the *periderm*. If the cork cambium develops in or near the pericycle, a part of the whole of the primary cortex will lie outside the cork and will be gradually thrown off. *Lenticels* replace stomata for

purposes of gaseous exchange; and as the cork increases, the amount of chlorophyll-containing tissue decreases.

The natural curvature of the bark increases when the bark is removed from the tree and dried. Large pieces of trunk bark, especially if subjected to pressure, may be nearly flat. Terms used to describe the curvature are illustrated in Fig. 41.2. Some commercial barks (e.g. cinnamon and quillaia) consist of the inner bark only. In quillaia the dark patches often found on the outer surface are known as *rhytidome* (literally, 'a wrinkle'). This term is applied to plates of tissue formed in the inner bark by successive development of cork cambia.

Barks may be described under the following headings.

Origin and preparation. From trunk branches or roots. Whole or inner bark.

Size and shape

Outer surface. Lichens, mosses, lenticels, cracks or furrows, colour before and after scraping.

Inner surface. Colour, striations, furrows.

Fracture. Short, fibrous, splintery, granular, etc. The fracture depends largely on the number and distribution of sclereids and fibres. A bark frequently breaks with a short fracture in the outer part and a fibrous fracture in the phloem.

Transverse surface. A smoothed transverse surface, especially if stained with phloroglucinol and hydrochloric acid, will usually show the general arrangement of the lignified elements, medullary rays and cork. Sections, however, are more satisfactory and can be used for a microscopical examination of calcium oxalate.

Anatomy

The cork cells in transverse section are often tangentially elongated and arranged in regular radial rows. In surface view they are frequently polygonal. The cell walls give a suberin reaction; the cell contents frequently give a positive tannin reaction. The cortex is usually composed of a ground mass of parenchyma. An outer band of collenchyma often occurs. Secretion cells, sclereids and pericyclic fibres may occur scattered or in groups in the cortex. The cortical cells often contain starch or other typical cell inclusions such as calcium oxalate.

Sieve tubes, companion cells, phloem parenchyma and medullary ray cells are always present in the phloem, but these soft tissues may not be well-preserved in medicinal barks. The sieve tubes, unless well-developed, are observed only after special treatment. Secretion cells, phloem fibres and sclereids may or may not be present in the phloem.

Xylem tissue is usually absent but may be present in small amounts on the inner surface of the bark.

The following should all be carefully noted in the anatomical examination of barks: the presence or absence of outer bark (cork, phellogen, phelloderm); the structure, amount and site of origin of the cork; the extent, cell structure and cell contents of the cortex; the presence or absence and, if present, the distribution, size and form of sclereids, phloem fibres and secretion cells; and the width, height, distribution and cell structure and contents of the medullary rays. When calcium oxalate is present, its crystalline forms and their distribution should be studied.

Transverse and longitudinal sections should be prepared. The size and form of sclereids and phloem fibres are best studied in disintegrated material. Preparations treated with cellulose, lignin, starch, callus, oil, suberin and tannin stains should be examined.

The cell types mentioned above are discussed in Chapter 42.

Powdered barks. Powdered barks always possess sieve tubes and cellulose parenchyma. Cork, fibres, sclereids, starch, calcium oxalate and secretory tissues are frequently present. Xylem tissues are absent or only present in very small amount. Chlorophyll and aleurone grains are absent.

WOODS

Although few drugs consist solely of wood, no description of a stem or root is complete without an account of its wood. Wood consists of the secondary tissues produced by the cambium on its inner surface. The cells composing these tissues—the vessels, tracheids, wood fibres

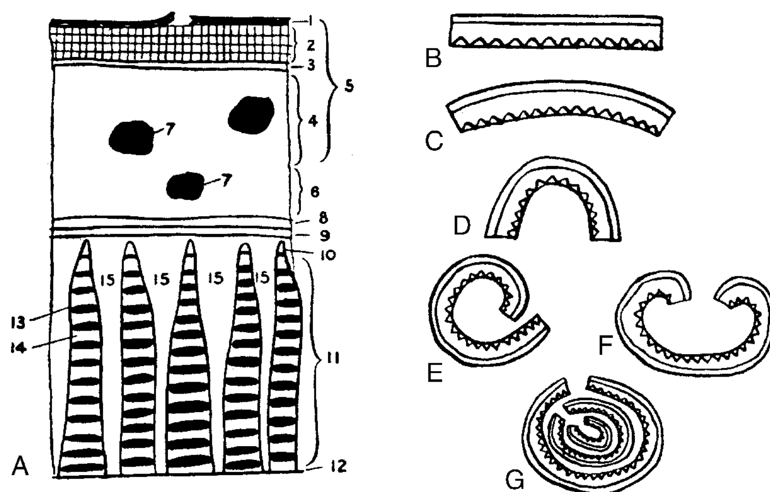

Fig. 41.2
Barks. A, diagram showing a typical arrangement of the tissues: 1, outer surface frequently showing lichens, lenticels and remains of primary tissues cut off by the cork; 2, cork; 3, cork cambium or phellogen; 4, phelloderm or secondary cortex; 5, periderm; 6, inner part of primary cortex; 7, groups of cortical sclerenchyma; 8, endodermis; 9, pericycle; 10, primary phloem; 11, secondary phloem; 12, cambium; 13, band of lignified fibres; 14, sieve elements; 15, medullary rays. B–G, shapes of barks: B, flat; C, curved; D, channelled; E, single quill; F, double quill; G, compound quill.

and parenchyma—are not necessarily all lignified. In some cases (e.g. the wood of belladonna root) non-lignified elements predominate. The distribution of the lignified elements may be ascertained by treating smoothed transverse, radial and tangential surfaces or sections with phloroglucinol and hydrochloric acid. In trees, the cells of the old wood frequently become coloured as they fill with waste products such as resins, tannins and colouring matters. This central region is called the *heartwood*, while the outer wood, which still retains its normal appearance and functions, is called the *sapwood*. Commercial guaiacum wood and logwood consist of heartwood.

In transverse section woods usually show annual rings each of which normally represents a season's growth. In some tropical species the annual rings are not well-marked, owing to the absence of a seasonal interruption in growth. The so-called *false annual rings* found in, for example, quassia are irregular rings formed by alternating zones of wood parenchyma and fibres. The width and height of *medullary rays* are of diagnostic importance in the case of Jamaica and Surinam quassias and rhubarbs. The *grain* of wood primarily results from the arrangement of the annual rings and medullary rays, but is modified by the wavy course of the wood elements which causes the wood to split irregularly. Irregular splitting is largely dependent on the number of lateral branches which cause knots in the wood.

Woods may be described under the following headings.

Size and colour. Note any differentiation into sapwood and heartwood. The latter may not be coloured uniformly (e.g. logwood).

Relative density. Woods vary considerably in this respect (e.g. guaiacum has a relative density of 1.33 and poplar one of 0.38).

Hardness and behaviour when split

Transverse surface. The lignified elements may show a markedly radiate arrangement or they may be irregularly scattered. Note distribution of wood fibres and wood parenchyma and of true and false annual rings. Measure the distances between medullary rays and between annual rings.

Longitudinal surfaces. Measure height of medullary rays.

LEAVES OR LEAFLETS

The following features can be used to describe leaves.

Duration. *Deciduous* or *evergreen*.

Leaf base. *Stipulate* or *exstipulate*; if stipulate, describe shape, etc; if sheath is present, describe it (e.g. *amplexicaul*—stem-clasping).

Petiole. *Petiolate* or *sessile*. If present, describe size, shape, colour, hairs, etc.

Lamina

1. Composition. If simple, whether *pinnate* or *palmate*. If compound, whether *paripinnate* (with an equal number of leaflets) or *imparipinnate* (Fig. 41.3).
2. Incision. The leaf may be more or less cleft, the amount being indicated by adding *-fid*, *-partite* or *-sect* to a prefix denoting whether the leaf is of a pinnate or a palmate type.
3. Shape. If the shape is obscured by drying, soak the leaf in warm water and spread it on a tile. The appropriate terms connected with leaf-shapes are given in Fig. 41.3.
4. Venation. *Parallel, pinnate* (feather-like), *palmate, reticulate* (net-veined).
5. Margin. See Fig. 41.3 for terminology.
6. Apex. See Fig. 41.3 for terminology.
7. Base. Symmetrical or asymmetrical; cordate, reniform, etc.

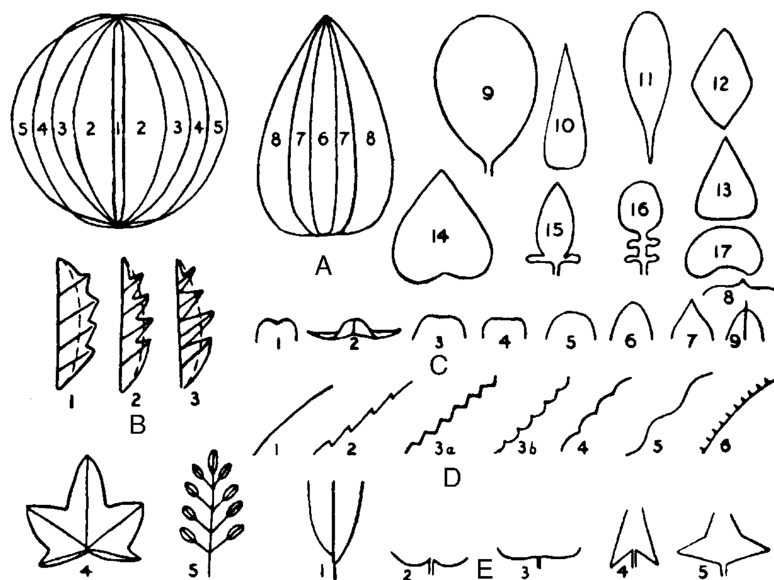

Fig. 41.3
Terms applied to leaves. A, Shape: 1, acicular; 2, elliptical; 3, oval; 4, oblong; 5, round; 6, linear; 7, lanceolate; 8, ovate; 9, obovate; 10, subulate; 11, spatulate; 12, diamond-shaped; 13, cuneate; 14, cordate; 15, auriculate; 16, lyrate; 17, reniform. B, Composition and incision: 1, pinnatifid; 2, pinnatipartite; 3, pinnatisect; 4, palmatifid; 5, imparipinnate. C, Apex: 1, emarginate; 2, recurved; 3, retuse; 4, truncate; 5, obtuse; 6, acute; 7, acuminate; 8, mucronate; 9, apiculate. D, Margin: 1, entire; 2, serrate; 3a and 3b, dentate; 4, crenate; 5, sinuate; 6, ciliate; E, Base: 1, asymmetric; 2, cordate; 3, reniform; 4, sagittate; 5, hastate.

8. Surface. Colour; glabrous (free from hairs) or pubescent (hairy); if the latter, whether hispid (with rough hairs), hirsute (with long distinct hairs) or with glandular hairs; punctate (dotted with oil glands). Note lines on surface of coca leaves, raised points on belladonna, press marks on Tinnevelly senna, etc. Note any differences between the upper and the lower surfaces.
9. Texture. Brittle, coriaceous, papery, fleshy, etc.

Anatomy

A study of the anatomy of the leaf reveals that there is a basic structural pattern yielding characters that enable the presence of a leaf to be detected in a powder. Other less general characters will make possible such distinctions as that between monocotyledonous and dicotyledonous leaves, and between xerophytic and mesophytic leaves. The more detailed anatomical characters will, when taken together, allow of the identification of the genus and ultimately of the species of leaf. A knowledge of the diagnostic characters of any leaf permits of the detection of contaminants and substitutes.

The leaf (Fig. 41.4) is built up of a protective epidermis, a parenchymatous mesophyll and a vascular system. The shape, size and wall structure of the epidermal cells; the form, distribution and relation to the epidermal cells of the stomata; the form, distribution and abundance of epidermal trichomes are all of diagnostic importance.

The mesophyll may or may not be differentiated into spongy mesophyll and palisade tissue. Palisade tissue may be present below both surfaces or occur only below the upper epidermis. In all green leaves the mesophyll cells are rich in chloroplasts. The mesophyll, although typically parenchymatous, may contain groups of collenchyma or sclerenchyma, secretion ducts or latex tissue, oil or mucilage cells, or hydathodes (water pores). Cells may contain inclusions such as crystals or calcium oxalate, the form, size and distribution of which may have importance.

The vascular systems of leaves fall into two main classes: the reticulate venation typical of dicotyledons and the parallel venation of monocotyledons. The structure of the individual veins is subject to considerable variation. The midrib bundle of the dicotyledonous leaf may be poorly or markedly differentiated. In leaves with a well-differentiated midrib the palisade tissue is usually interrupted in the midrib region and collenchyma frequently occurs above and below the midrib bundle. The main veins, in dicotyledonous leaves, are open and usually collateral (Fig. 41.4); less commonly they are bicollateral. The xylem faces towards the upper surface. Various degrees of secondary thickening of the midrib bundle are seen. The lateral veins are almost entirely collateral even in cases where the midrib bundle is bicollateral. The smallest veins often consist of xylem only. The veins of monocotyledonous leaves are closed bundles.

The midrib bundle is often, as in the Solanaceae, enclosed in an endodermis which may take the form of a starch sheath. The development of the pericycle is variable, in some cases being parenchymatous and containing secretion cells, in some cases consisting of a sheath of pericyclic fibres with their long axes parallel to the vein.

For the investigation of the structure of a leaf it is necessary to examine transverse sections of the lamina and midrib; portions of the whole leaf, including leaf margin, cleared in chloral hydrate; and surface preparations of both epidermi. Sections should be cleared, if necessary, and stained for cellulose and lignin. In individual cases it may be necessary to apply microchemical tests for mucilage, tannin, cutin, volatile oil, calcium oxalate or carbonate.

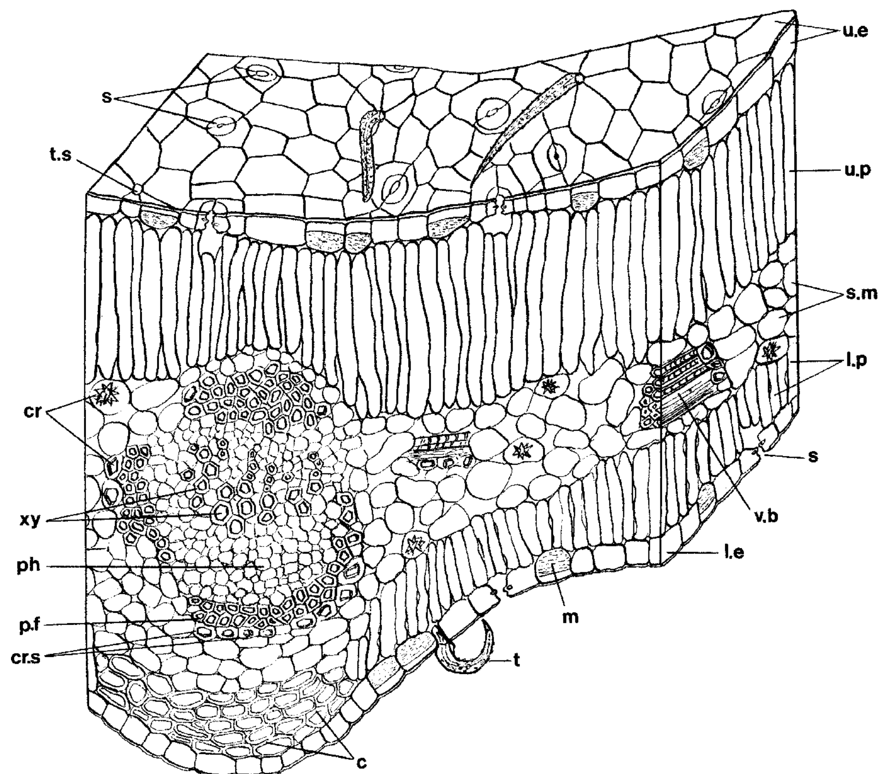

Fig. 41.4
Transverse section of senna leaflet: c, collenchyma; cr, calcium oxalate crystals; cr.s, crystal sheath; l.e, lower epidermis; l.p, lower palisade; m, mucilage cell; ph, phloem; p.f, pericyclic fibre; s, stomata; s.m, spongy mesophyll; t, trichome; t.s, trichome scar; u.e, upper epidermis; u.p, upper palisade; v.b, vascular bundle; xy, xylem vessels.

Powdered leaves. The following are consistently present: epidermis with stomata; cellulose parenchyma; not very abundant small-sized vascular elements and chlorophyll (except in bulb leaves). Structures frequently present are epidermal trichomes, glands, palisade cells, crystals of calcium oxalate, collenchyma and pericyclic fibres (see also Chapter 42).

For the differentiations of closely allied leaves it may be necessary to make determinations of such differential characters as vein-islet number, stomatal number, stomatal index and palisade ratio (q.v.).

INFLORESCENCES AND FLOWERS

The following features serve to describe the complex structure of flowers.

Type of inflorescence. *Racemose, cymose* or mixed (e.g. racemes of cymes in clove).

Axis or receptacle of inflorescence. The main axis of an inflorescence is called the *rachis*, while the branches bearing flower clusters and individual flowers are termed *peduncles* and *pedicels*, respectively. The term *receptacle of the inflorescence* must not be confused with the receptacle of the flower (see below). In the Roman chamomile the receptacle of the inflorescence is conical and solid, a membranous palea subtends each floret and the capitulum is surrounded by an involucre of bracts.

Type of flower. Monocotyledon or dicotyledon. Unisexual or hermaphrodite. Regular or zygomorphic. Hypogynous, perigynous or epigynous (Fig. 41.5).

Receptacle of the flower (thalamus or torus) is the extremity of the peduncle on which the calyx, corolla, etc. are inserted. When the receptacle is elongated below the calyx, it is called a *hypanthium*, or if below the ovary, a *gynophore* or stalk of the ovary (cf. clove).

Calyx. Note number of sepals if *polysepalous* or divisions if *gamosepalous*. *Caducous* (e.g. poppy) or *persistent* (e.g. belladonna). Describe colour, shape, hairs, etc., as for a leaf.

Corolla. Note number of petals if *polypetalous* or divisions if *gamopetalous*. Observe any special characteristics such as venation (henbane) and oil glands (clove petals).

Androecium. Note number of stamens; whether free or joined (*monadelphous, diadelphous*, etc.), *didynamous* or *tetradynamous, epipetalous*, etc. Dehiscence of anthers (valves, pores or slits).

Gynaecium. Note number of carpels; apocarpous or syncarpous; superior or inferior. Sizes and shapes of stigma, style and ovary. The enlarged base of the styles in the Umbelliferae is called a *stylopod*. Number of loculi, placentation (parietal, axile, free-central, etc.).

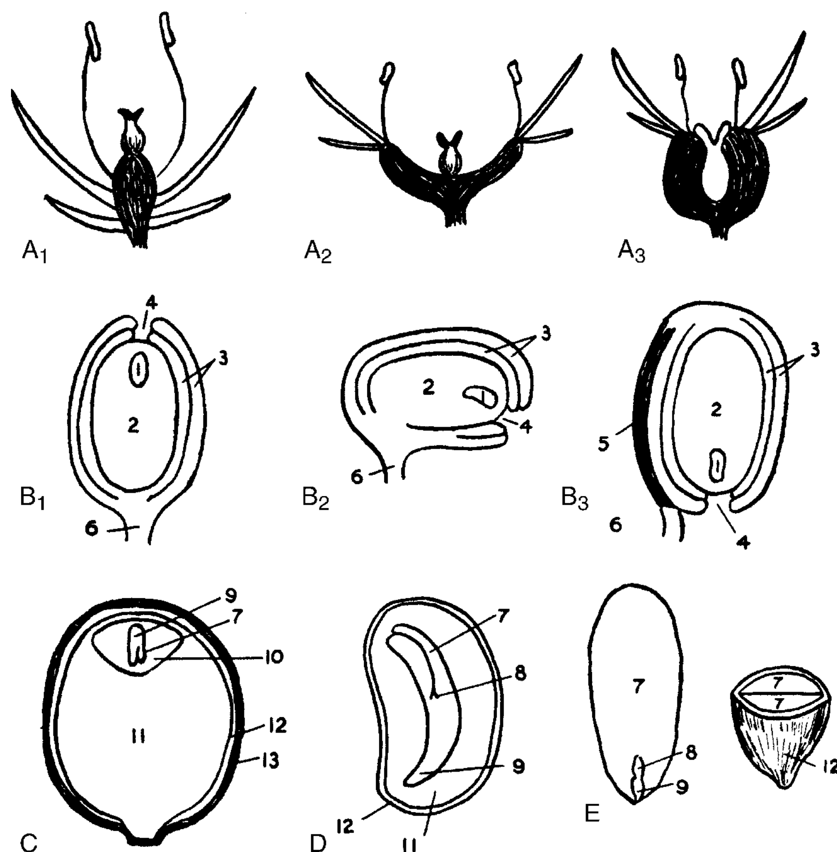

Fig 41.5
A_1, A_2 and A_3, hypogynous, perigynous and epigynous flowers; B_1, B_2 and B_3, orthotropous, campylotropous, and anatropous ovules; C, fruit of *Piper* with single albuminous seed; D, albuminous seed of *Papaver*, E, exalbuminous seed of almond. 1, embryo sac; 2, nucellus; 3, integuments; 4, micropyle; 5, raphe; 6, funicle; 7, cotyledon; 8, plumule; 9, radicle; 10, endosperm; 11, perisperm; 12, testa; 13, pericarp.

Ovules. Note number in each loculus; *orthotropous, campylotropous, anatropous* (Fig. 41.5).

Anatomy

The flower stalk or pedicel has a stem structure and in the powdered form exhibits the appropriate elements. The bracts, calyx and, to a lesser extent, corolla have a leaf structure and will yield such elements as epidermis with stomata, glandular and covering hairs, mesophyll cells, oil glands and crystals. The epidermal cells of the corolla often have a papillose or striated cuticle. Delicate coloured fragments of the corolla can often be distinguished in coarsely powdered drugs. A characteristic papillose epidermis may sometimes be present on the stigmas of the gynaecium. Characteristic fragments of the anther wall are diagnostic of the presence of flowers. Of first importance is the occurrence, size, shape and wall structure of pollen grains.

With powdered flowers the pollen grains, portions of the fibrous layer of the anther wall and the papillose epidermis of the stigmas are obvious features.

FRUITS

The following classification shows the principal types of fruit met with in pharmacognosy.

A. *Simple* (i.e. formed from a gynaecium with one pistil).
B. *Aggregate* (i.e. formed from more than one pistil, e.g. aconite).
C. *Collective* (i.e. formed not from one flower but from an inflorescence, e.g. fig).

1. Simple, dry, indehiscent fruits

(1) *Achene*. A small hard indehiscent fruit. The term is strictly only applied to those formed from one carpel, but is sometimes used for those formed from two carpels (e.g. the fruit of the Compositae). The latter is better termed a *cypsela*.
(2) *Nut*. This is similar to an achene, but is typically formed from two or three carpels (e.g. dock fruit).
(3) *Caryopis*. This is the type of fruit in which the testa and pericarp are fused (found in the cereals).

2. Simple, dry, dehiscent fruits

(1) *Legume*. A fruit formed from one carpel which splits along both dorsal and ventral sutures (e.g. senna).
(2) *Follicle*. A fruit from one carpel which dehisces by the inner suture only. Follicles are usually found in aggregates or etaerios (e.g. aconite and strophanthus).
(3) *Capsules*. Capsules are dry dehiscent fruits formed from two or more carpels. Some bear special names (e.g. the *siliqua* and *silicula* of the Cruciferae, and the *pyxis* or *pyxidium* found in henbane). The latter is a capsule which opens by means of a lid.

3. Schizocarpic or splitting fruits

A familiar example of this group is the *cremocarp*, the bicarpellary fruit of the Umbelliferae, which splits into two *mericarps*.

4. Succulent fruits

(1) *Drupe*. This is typically formed from one superior carpel (e.g. almond and prune). The inner part of the pericarp, which is called the endocarp, is hard and woody and encloses one seed.
(2) *Berry*. This fruit is formed from one or more carpels and the pericarp is entirely fleshy. It is usually many-seeded. Examples: nux vomica, colocynth, orange, lemon, capsicum. Special terms which are sometimes used are *pepo* for the berry of the Cucurbitaceae and *hesperidium* for that of the orange and similar rutaceous fruits.

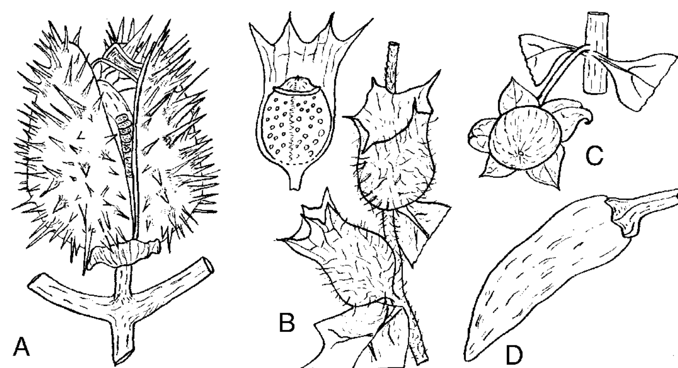

Fig. 41.6
Fruits of the Solanaceae. A and B, capsules: A, ripe fruit of *Datura stramonium*; B, pyxidia of *Hyoscyamus niger* with upper fruit showing calyx partly removed. C and D, berries of *Atropa belladonna* and *Capsicum* sp., respectively. All four fruits are basically bilocular but stramonium fruit becomes almost completely four-celled by the development of a false septum.

Examples of fruits of the Solanaceae are shown in Fig. 41.6.
The description of a fruit may be arranged as follows.

Class

See above.

Shape and dimensions

Adhesion. Superior or inferior. Fruits from inferior ovaries usually show floral remains at the apex (e.g. cardamom, fennel, unpeeled colocynth and lobelia).

Dehiscence. Dehiscent or indehiscent. Different types of dehiscence are shown by the legume, follicle, siliqua and the pyxidium and other capsules. Most capsules split longitudinally into valves which are usually equal in number to or double those of the loculi or placentae. Dehiscence is termed *septicidal* if the valves separate at the line of junction of the carpels or *loculicidal* if the valves separate between the placentae or dissepiment. In the latter case the placentae or dissepiment may remain attached either to the axis or to the valves.

Pericarp. Colour, texture, markings, number of sutures. Note whether uniform throughout or modified into epicarp, mesocarp and endocarp.

Placentation (e.g. marginal in senna, parietal in poppy, axile in cardamom, etc.).

Seeds. Number. Describe in detail (see under 'Seeds' below).

Other characters. Odour, taste, food reserves.

Anatomy

The pericarp is bounded by inner and outer epidermi which, in general, resemble those of leaves. The outer epidermis may bear stomata and hairs. In fleshy fruits the internal tissue is mainly parenchymatous, resembling the mesophyll of leaves. In dry fruits and fleshy dry fruits it usually contains fibres or sclereids. Secretory tissues such as vittae, oil ducts or cells, and latex tissue are commonly present in the pericarp of medicinal fruits. Husk of cardamoms can be detected by the presence

of pitted fibres, spiral vessels and abundant empty parenchymatous cells. The endocarp of almond, sometimes used as an adulterant, consists mainly of sclereids.

Portions of receptacle (e.g. the rind of colocynth), persistent sepals and flower stalk may be present.

SEEDS

Seeds may be produced from orthotropous, campylotropous or anatropous ovules (Fig. 41.5). Care must be taken to distinguish seeds from fruits or parts of fruits containing a single seed (e.g. cereals and the mericarps of the Umbelliferae). The seed consists of a kernel surrounded by one, two or three seed coats. Most seeds have two seed coats, an outer *testa* and an inner *tegmen*. The seed is attached to the placenta by a stalk or *funicle*. The *hilum* is the scar left on the seed where it separates from the funicle. The *raphe* is a ridge of fibrovascular tissue formed in more or less anatropous ovules by the adhesion of funicle and testa. The *micropyle* is the opening in the seed coats which usually marks the position of the radicle. An expansion of the funicle or placenta extending over the surface of the seed like a bag is known as an *aril* or *arillus*. A false aril or *arillode* resembles an aril, but is a seed coat. A *caruncle* or *strophiole* is a protuberance arising from the testa near the hilum.

The kernel may consist of the embryo plant only (*exalbuminous seeds*), or of the embryo surrounded by *endosperm* or *perisperm* or both (*albuminous seeds*) (Fig. 41.5). Endosperm and perisperm are tissues containing food reserves and are formed, respectively, inside and outside the embryo sac.

The description of a seed may be arranged as follows.

Size, shape and colour

Funicle, etc. Describe funicle and, if present, raphe and aril.

Hilum and micropyle. Size and positions.

Seed coats. Number. If present, describe arillode, caruncle or strophiole. Thickness and texture of testa; whether uniform in colour or not; smooth, pitted or reticulate. If hairs are present, describe their length, texture and arrangement. Mechanism for dispersal (e.g. awn of strophanthus).

Perisperm. Present or absent. Nature of food reserves.

Endosperm. Present or absent. Nature of food reserves.

Embryo. Size and position (e.g. straight in *Strophanthus*, curved in stramonium, folded in mustard). Size, shape, number and venation of cotyledons. Size and shape of radicle.

Anatomy

The testas of seeds often yield highly diagnostic characters. A highly diagnostic sclerenchymatous layer is often present (Fig. 41.7K, I). The number of cell layers, and their structure, arrangement, colour and cell contents are subject to characteristic variations. The epidermis of the testa is often composed of highly characteristic, thick-walled cells (Fig. 41.7A, B, E, J, L). It may bear characteristic hairs (Fig. 41.7M).

The storage tissues perisperm and endosperm, and in other cases cotyledons, are composed of uniform cells often containing characteristic cell contents (e.g. aleurone, starch, calcium oxalate, fixed oil, volatile oil). The cell walls are often considerably thickened (e.g. nux vomica).

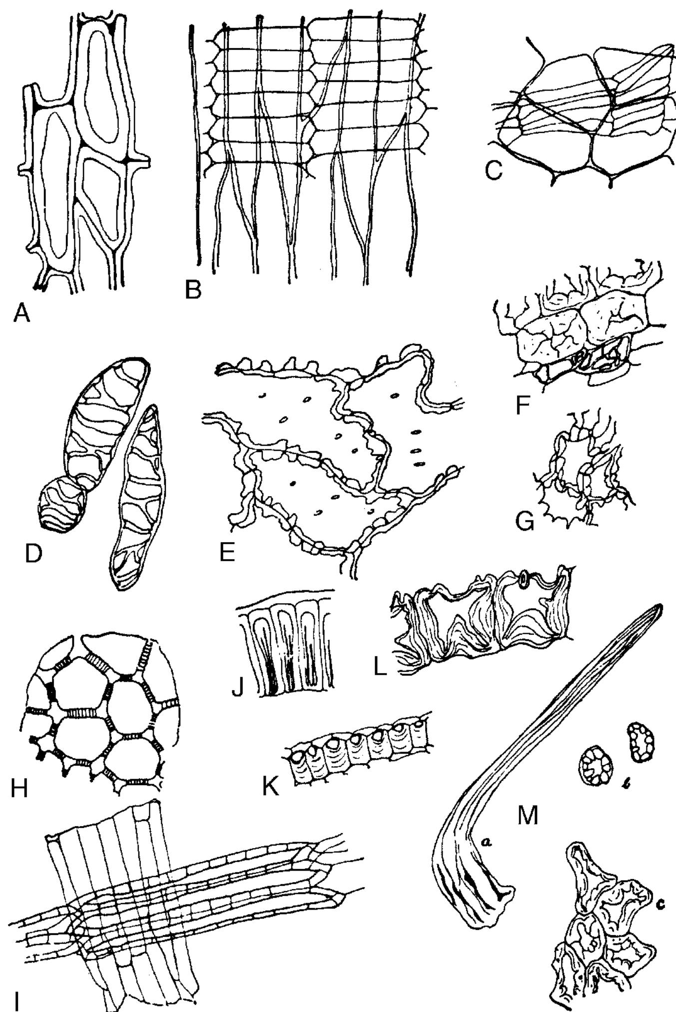

Fig. 41.7
Some diagnostic structures of fruits and seeds. A, lignified epidermal cells of testa of seed of *Lobelia inflata*; B, epidermis of testa of cardamom seed, with fragment of the underlying parenchymatous layer attached; K, sclerenchymatous layer of testa of cardamom seed in transverse section; C, 'parqueting cells' of inner epidermis of the pericarp of fennel, with parenchyma of mesocarp attached; D, lignified reticulate 'parenchyma' cells of the mesocarp of fennel. E, epidermis of capsicum seed in surface view; F, sclereids and reticulate cells of testa of colocynth seed; G, sclereids of same in surface view; J, epidermis of testa of colocynth seed in transverse section; H, pigment layer of testa of linseed in surface view; I, sclerenchymatous layer of testa of linseed seen in surface view and with hyaline layer adherent; L, epidermis of testa of stramonium seed in transverse section; M, lignified hair of nux vomica: a, whole hair; b, transverse sections of limb of hair; c, periclinal section through the bases of several hairs.

The radicle, plumule and leaf-like cotyledons yield little of diagnostic significance to the powdered drug.

Transverse and longitudinal sections of fruit and seeds should be prepared. Disintegration makes possible a study of the structure of the individual layers and elements and of structures such as vittae.

The variation in structure between different fruits and seeds is considerable. Aleurone grains, carbohydrate reserves and a little vascular tissue are constantly present in seeds. Fruits yield similar characters, except that the amount of vascular tissue is greater and lignified elements of the pericarp are often present.

SUBTERRANEAN ORGANS

Under this heading it is convenient to discuss: (1) stem structures such as corms, bulbs, stem-tubers and rhizomes and (2) root structures such as true and adventitious roots and root-tubers. Many drugs which are commonly spoken of as roots consist wholly or partly of rhizomes (e.g. rhubarb and gentian) and in many cases the gradual transition from stem to root makes an accurate differentiation of the two parts impossible.

Monocotyledonous rhizomes can be distinguished from dicotyledonous rhizomes by the scattered arrangement of their vascular bundles. Stem structures may usually be distinguished from roots by the fact that they bear buds and possess a well-marked pith. In underground organs chlorophyll is absent, and starch, when present, is usually abundant and in the form of large grains of reserve starch.

The following scheme may be used with suitable modifications for the description of most subterranean organs.

Morphological nature. Rhizome, root, etc.

Condition. Fresh or dry; whole or sliced; peeled or unpeeled.

Subaerial stems. Remains of subaerial stems occur in aconite, serpentary, etc. Note whether present in sufficient amount to constitute an adulteration.

Subterranean stems

1. **Size and shape**.
2. **Direction of growth and branching**.
3. **Surface characters**. Colour, stem scars, buds, cataphyllary leaves, roots or root scars, lenticels, cracks, wrinkles, surface crystals, evidence of insect attack, peeling, etc.
4. **Fracture and texture**. Flexible, brittle, hard, horny, mealy, splintery, etc.
5. **Transverse section**. Colour (cf. male fern); distribution of lignified and secretory elements (e.g. in ginger); relative sizes of bark, wood and pith. Note any abnormalities such as the star spots and absence of a lignin reaction in rhubarb.

Roots

1. **Kind**. True (i.e. developed from the radicle or its branches) or adventitious.
2. **Size and shape**. Tuberous, conical, cylindrical, etc.
3. **Surface characters**. Colour; cracks, wrinkles, annulations, lenticels, etc.
4. **Fracture and texture**.
5. **Transverse section**. Note absence of pith, whether the wood is markedly radiate or not, and any abnormalities such as are found in jalap and senega.

Food reserves and chemical tests

Odour and taste

Anatomy

Most of the important drugs derived from roots are those of dicotyledons and the following brief description of their fundamental structural pattern is restricted to that of the typical dicotyledonous type.

The primary root (Fig. 41.8A) shows the following structures: a piliferous layer composed of a single layer of thin-walled cells, devoid of cuticle and bearing root hairs formed as lateral outgrowths of the cells; a parenchymatous cortex, the innermost layer of which is differentiated into an endodermis; and a vascular cylinder or stele taking the form of a radial protostele or less frequently of a medullated protostele. The vascular tissues of the stele are enclosed in a single

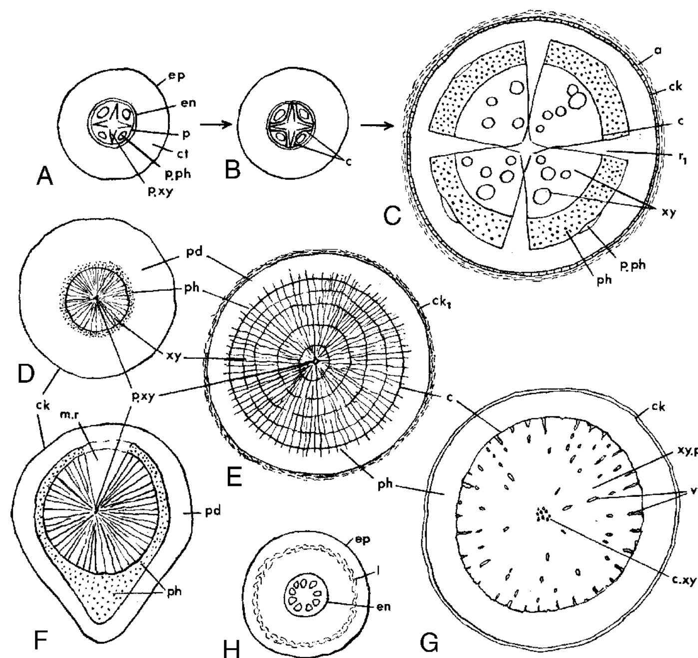

Fig. 41.8
Root strucutures in transverse section. A–C, initiation of secondary growth in liquorice root: A, primary structure; B, development of cambium; C, formation of secondary phloem and xylem and of the cork cambium. D–G, variations in root structure: D, ipecacuanha; E, *Rauwolfia serpentina;* F, senega; G, belladonna; H, veratrum, a monocotyledon wih no secondary thickening. a, Degenerative cortex; c, cambium; ck, cork; ck_1, stratified cork; ct, cortex; c.xy, central xylem; en, endodermis; ep, epidermis; l, lacuna; m.r, medullary ray; p, pericycle; pd, phelloderm; ph, phloem; p.ph, primary phloem, p.xy, primary xylem; r_1, primary medullary ray; v, xylem vessels, xy, xylem; xy.p. xylem parenchyma.

or many-layered pericycle. The protostele is composed of a central mass of xylem tissue with two or more radiating arms and of phloem groups located between the xylem arms. The xylem is differentiated in a centripetal direction, so that the protoxylem groups occupy the ends of the xylem arms and the metaxylem makes up the inner xylem mass. The number of protoxylem groups is usually fairly constant for a given species, but some variation is not uncommon (e.g. valerian). The xylem is described as diarch (*Solanum* spp.), triarch (alfalfa), tetrarch (liquorice, *Ipomoea* spp.) or polyarch, according to the number of protoxylem groups present. The central xylem cylinder is medullated in some cases (e.g. valerian). The phloem groups are usually separated from the xylem cylinder by a narrow zone of parenchyma ('fundamental parenchyma').

In many roots increase in diameter of the axis is accomplished by secondary thickening. Secondary thickening is initiated in the zone of 'fundamental parenchyma', the whole or part of which becomes meristematic. The derived cells mature as secondary phloem centrifugally and as secondary xylem centripetally. From the point of initial cambial activity there is a progressive tangential development, the cambia extending laterally until they reach the points where the protoxylem groups abut on to the pericycle. The pericycle opposite the protoxylem groups becomes meristematic and thus a continuous cambial cylinder is formed (Fig. 41.8B). The activity of the cambium opposite the protoxylem groups gives rise to the broad primary rays (Fig. 41.8C).

The cylinder of secondary tissues is composed of xylem and phloem elements which at first tend to be arranged in regular radial rows. This arrangement often becomes less regular, owing to irregular growth of the individual elements and further division and growth of the xylem and phloem parenchyma cells. The structure of the secondary xylem and phloem is described in Chapter 42.

Coincident with the development of the secondary vascular tissues other changes take place (Fig. 41.8C). The primary phloem groups are forced outwards and gradually obliterated. Divisions take place in the pericycle, so that it increases in diameter with the expansion of the vascular cylinder. Often the pericycle also increases in thickness, becoming many layers, and forms a 'secondary cortex'. The piliferous layer, cortex and endodermis become fractured and are cut off by the formation of a phellogen in the outermost layer of cells derived from the pericycle. At a still later stage a new phellogen may arise in the secondary phloem, with a consequent disintegration of the pericycle.

The structures of the primary and secondary roots of dicotyledons show many deviations from the general plan described above. Figure 41.8D–G indicates variations in root structure arising from the preferential development of certain tissues. Monocotyledons characteristically exhibit no secondary thickening (Fig. 41.8H). Jalap shows anomalous secondary thickening.

UNORGANIZED DRUGS

Many types of unorganized drugs are discussed in Part 5, namely: fixed oils, fats and waxes; volatile oils; resins, oleoresins, oleo-gum-resins, balsams and gums. To these must be added dried juices (e.g. aloes), latices (e.g. opium) and extracts (e.g. agar and catechu).

The following scheme may be used in their examination.

Physical state

Solid, semi-solid or liquid.

1. **If solid.** (a) Size and form: Tears, lumps, etc., and their approximate size and weight. (b) Packing: Paper, skins, leaves, plastic, etc. (c) External appearance: Colour, shiny or dusty; opaque or translucent; presence of vegetable fragments. (d) Hardness and fracture: Conchoidal, porous, etc. (e) Solubility in water and organic solvents. (f) Vegetable debris, if any, remaining insoluble (e.g. in myrrh and asafoetida). (g) Effect of heat: Does substance melt, char, sublime or burn without leaving appreciable ash? (h) Microscopical appearance of powder, sublimate (e.g. balsams) or insoluble matter (e.g. opium and catechu).
2. **If liquid.** (a) Colour and fluorescence. (b) Viscosity. (c) Density. (d) Solubility (e.g. of balsam of Peru in a solution of chloral hydrate).

Odour and taste

Chemical tests, chromatographic and spectroscopic characteristics

42

Cell differentiation and ergastic cell contents

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ERGASTIC CELL CONTENTS 560

Modifications to the basic structure of the living plant cell involving composition of the cell wall, cell shape and cell contents, are found in the various plant tissues and furnish those microscopical characters of drug plants which are of value in identification and in the detection of adulteration.

THE CELL WALL

The original cell wall may, during the differentiation of the cell, undergo various chemical modifications that profoundly change its physical properties. Principal among these are the deposition of further cellulose or hemicellulose and incrustation of the wall by lignin, cutin or suberin. Algal cell walls, which commonly contain pectin mixed with cellulose, xylose, mannose or silica, may contain also hemicellulose, alginic acid, fucoidin and fucin (Phaeophyta), geloses (Rhodophyta) and chitin.

Cellulose walls. Certain colour reactions can be applied for the recognition of cellulose cell walls. The colour reactions vary with differences in the relative proportions of cellulose, hemicellulose and pectin present.

1. Chlor-zinc-iodine gives a blue colour with true celluloses and a yellow with pectic substances. Walls containing these in different proportions stain blue, violet, brownish-violet or brown. Similar colours are obtained with iodine followed by concentrated acids.
2. Iodine, when used alone, gives no colour with true celluloses but may give a blue if hemicelluloses are present (e.g. in the cotyledons of tamarind seeds).
3. Ammoniacal solution of copper oxide dissolves true celluloses, and on pouring the alkaline liquid into dilute sulphuric acid the cellulose is precipitated. Walls containing hemicelluloses, etc., are incompletely soluble in this reagent.
4. Phloroglucinol and hydrochloric acid gives no pink or red colour with cellulose walls.

Lignified walls. Lignin is a strengthening material which impregnates the cell walls of tracheids, vessels, fibres and sclereids of vascular plants; it constitutes 22–34% of woods. Chemically, it is a complex phenylpropanoid (C_6–C_3) polymer which differs according to its source, lignin from dicotyledons being different from that of the conifers (Fig. 21.1). In the wall, it appears to occur chemically combined with hemicellulose and is built up in greatest concentration in the middle lamellae and in the primary walls. Lignified cell walls after treatment with Schultze's macerating fluid will show cellulose reactions.

For the identification of lignified walls the following tests are available:

1. On treatment with 'acid aniline sulphate' the walls become bright yellow.
2. Phloroglucinol and hydrochloric acid stains lignified walls pink or red. A similar colour is obtained when pentose sugars are warmed with this reagent.
3. Chlor-zinc-iodine stains lignified walls yellow.

Suberized and cutinized walls. Suberin and cutin consist of mixtures of substances, chiefly highly polymerized fatty acids such as suberic acid, $COOH[CH_2]_6COOH$, although the acids present in the two substances are not identical. These materials waterproof cells in which they occur. Suberin thickenings, such as are found in cork cells and endodermal cells, usually consist of carbohydrate-free suberin lamellae. Cutin forms a secondary deposit on or in a cellulose wall. Leaves are frequently covered with a deposit of cutin which may show characteristic

papillae, ridges or striations. Beneath the cuticle, the cellulose wall may also be impregnated with cutin (cutinized), so that these walls may show a gradation from pure cellulose on the inside, through layers of cellulose impregnated with pectin compounds and fatty substances, to the outer cuticle, which is free of cellulose. Waxes (largely esters of higher monohydric alcohols and fatty acids) occur with suberin and cutin. Unlike the latter, they readily melt on warming and are extractable with fat solvents. Such waxes in the form of minute rods or particles give a glaucous effect to the structures which they cover and are responsible for the 'bloom' of many fruits, stems, etc. Wax is found in larger amounts on the leaves of *Myrica*, and in the wax palms, *Copernicia*, it coats the leaves heavily (Carnauba wax).

The reactions of suberin and cutin are almost identical.

1. Chlor-zinc-iodine gives a yellow to brown colour.
2. Sudan-glycerin colours both suberin and cutin red, especially on warming. The reagent is made by dissolving 0.01 g of Sudan III in 5 ml of alcohol and adding 5 ml of glycerin.
3. Strong solution of potash stains suberin and cutin yellow. On warming suberin with a 20% solution of potash, yellowish droplets exude, but cutin is more resistant.
4. Diluted tincture of alkanna stains the walls red.
5. Concentrated sulphuric acid does not dissolve suberin or cutin.
6. Oxidizing agents. At ordinary temperatures concentrated chromic acid solution has little effect. When heated with potassium chlorate and nitric acid, the walls change into droplets, which are soluble in organic solvents or in dilute potash.

Mucilaginous cell walls. Certain cell walls may be converted into gums and mucilages. This gummosis (gummous degeneration) may be observed in the stems of species of *Prunus, Citrus* and *Astragalus*, in testas of many seeds (e.g. linseed and mustard) and in the outer layers of many aquatic plants. In the case of gum-yielding species of *Astragalus*, gummosis commences near the centre of the pith and spreads outwards through the primary medullary rays. The polysaccharide walls, excepting the primary membranes, swell and are converted into gum, the lumen, which frequently contains starch, becoming very small. When the stem is incised, whole tissues are pushed out by the pressure set up by the swelling of the gum. The commercial gum has a definite cell structure. The reaction of gums and mucilages is described below under 'Cell Contents'.

Chitinous walls. Chitin $(C_8H_{13}O_5N)_n$, a polyacetylamino-hexose, forms the major part of the cell walls of crustaceans, insects and many fungi (e.g. ergot). It gives no reactions for cellulose or lignin. When heated with 50% potash at 160–170°C for 1 h, it is converted into chitosan, $C_{14}H_{26}O_{16}N_2$, ammonia and acids such as acetic and oxalic. The mass may be dissolved in 3% acetic acid and the chitosan reprecipitated by the addition of a slight excess of alkali. Chitosan gives a violet colour when treated first with a 0.5% solution of iodine in potassium iodide, and then with 1% sulphuric acid. The test may be applied to shrimp scales, first freed from carbonate by means of 5% hydrochloric acid, to the elytra of beetles or to defatted ergot.

PARENCHYMATOUS TISSUE

Meristematic tissue is usually composed of cells characterized by isodiametric form (except in the case of the provascular tissues), by possessing a protoplast capable of division and a primary cell wall composed of cellulose. The fundamental parenchyma occurring in various parts of the plant is potentially meristematic, and such cells achieve maturity without further differentiation except for an increase in cell size and wall thickness and a restricted change of form. The pith, cortex and rays of the plant axis and the mesophyll of the leaves are composed, at least in part, of such parenchyma. The mesophyll cells often contain abundant chloroplasts, and may be differentiated into palisade and spongy mesophyll. An early stage of differentiation may be seen in the lignified pitted parenchyma constituting the pith of the stems of *Lobelia inflata* and *Cephaelis ipecacuanha*, and the pitted cellulose parenchyma of the pulp of *Citrullus colocynthis*.

THE EPIDERMIS

The epidermis consists of a single layer of cells covering the whole plant. The epidermis of the root constitutes the piliferous layer and that of the shoot is a highly differentiated and compact layer of cells. The epidermal cells, in contrast to the stomatal guard cells, are often devoid of chloroplasts. Epidermal cells show great variety in form, giving characteristic patterns when seen in surface view. In transection they are often flattened parallel to the surface, and square or rectangular in shape. The outer walls are often convex and the most markedly thickened.

The epidermis of the stems of trees and shrubs is usually obliterated early by the development of a cork cambium, but on the stems of herbaceous plants and in leaves, fruits and seeds the epidermis persists and often yields highly diagnostic characters.

For leaves in particular, the shape of the epidermal cells in surface view and in section (Fig. 42.1A–D), the nature and distribution of the wall thickening, the presence or absence of cuticle and its form, the distribution and structure of the stomata, the presence or absence of well-differentiated subsidiary cells to the stomata, the presence of characteristic cell inclusions such as cystoliths, the presence or absence and form, size and distribution of epidermal trichomes and the presence and distribution of water-pores should all be carefully noted in describing the characters of an epidermis.

The structures of the epidermis and stomata are of first importance in the microscopical identification of leaves (see Fig. 42.2). Straight-walled epidermal cells are seen in, for example, jaborandi, coca and senna leaves; wavy-walled epidermal cells in stramonium, hyoscyamus and belladonna; beaded walls in *Lobelia inflata* and *Digitalis lanata*; a papillose epidermis in coca leaf. A thick cuticle is developed in *Aloe* leaf and bearberry leaf; a striated cuticle in belladonna, jaborandi, *Digitalis lutea* and *D. thapsi*. Mucilage is present in the epidermis of senna and buchu leaves. Cystoliths of calcium carbonate occur in the epidermal cells of Urticaceae and Cannabinaceae; sphaero-crystals of diosmin occur in buchu epidermis (Fig. 42.1B).

The stomata may be surrounded by cells resembling the other epidermal cells (anomocytic, formerly ranunculaceous, type), but in other cases definite subsidiary cells may be distinguished. Three main types are distinguishable: the anisocytic (formerly cruciferous) type, with the stoma surrounded by three or four subsidiary cells, one of which is markedly smaller than the others; the paracytic (formerly rubiaceous) type, with two subsidiary cells with their long axes parallel to the pore; and the diacytic (formerly caryophyllaceous) type with two subsidiary cells, with their long axis at right angles to the pore of the stomata (Fig. 42.2). There are variations among these types (e.g. the actinocytic type, in which the subsidiary cells are arranged along the radii of a circle) and altogether some 31 types have been recognized. (For a survey of the classification of morphological types of stomata see M. Baranova, *Bot. Rev*., 1992, **58**, 49).

Often, when viewed under the light microscope as cleared preparations, the outlines of the epidermal cells and stomata do not appear as definite as the line drawings (Fig. 42.2) might suggest. This is due to

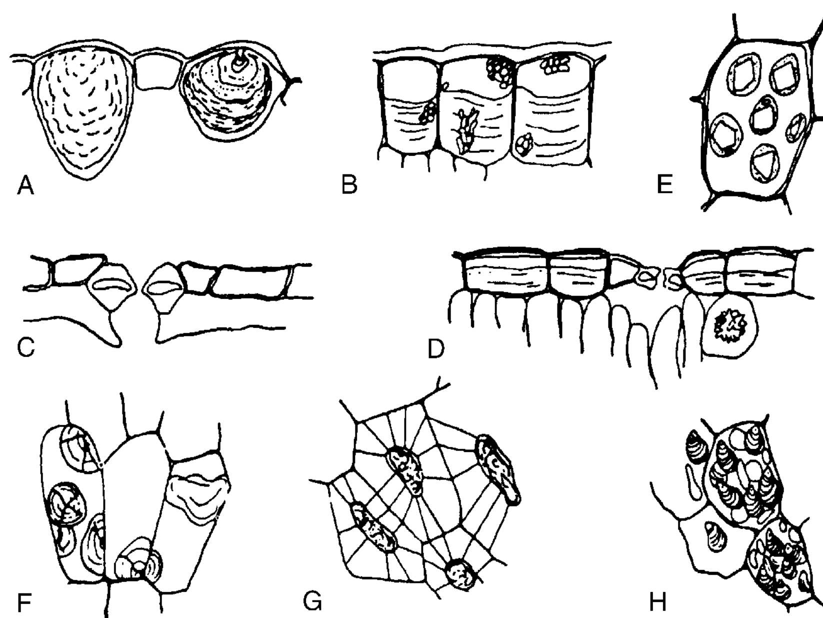

Fig. 42.1
A, Epidermal cells of *Urtica dioica* containing cystoliths of calcium carbonate. B, Epidermal cells of the leaf of *Barosma betulina* showing sphaerocrystalline masses of diosmin and a thick deposit of mucilage on the inner tangential walls. C, Cells of the lower epidermis of *Arctostaphylos uva-ursi* showing thick cuticle and sunken stomata. D, Upper epidermis of *Cassia angustifolia* showing mucilage cells and stomata; a cell of the underlying mesophyll contains a cluster crystal of calcium oxalate. E, Aleurone grains showing crystalloid and globoid, from the endosperm of the seed of *Ricinus communis*. F, Sphaerocrystalline masses of inulin in dahlia tuber. G, Cells of the endosperm of the seed of *Strychnos nux-vomica* showing walls of reserve cellulose, traversed by plasmodesmata. H, Parenchymatous cells from the rhizome of *Zingiber officinale* containing starch grains.

the convoluted arrangements of cells on the leaf surface and is illustrated by the scanning electron micrographs included in the digitalis and Solanaceae descriptions in Part 5.

The distribution of stomata between the upper and lower epidermis shows great variation. The stomata may be entirely confined to the lower epidermis, as in *Ficus* species, bearberry, boldo, buchu, coca, jaborandi and maté leaves. The leaves of savin show stomata confined to two localized areas of the lower surface. The floating leaves of aquatics have stomata confined to the upper epidermis. Sometimes they are evenly distributed on both surfaces; most commonly they are more numerous on the lower surface. For ‘stomatal number’ and ‘stomatal index’ see Chapter 43.

The epidermis of fruits and seeds may yield characters of diagnostic value (see Fig. 41.7). The outer and inner epidermi of the pericarp of the umbelliferous fruits are highly characteristic structures. Characteristic cells with thickened pitted walls form the outer epidermis of the pericarp in vanilla, juniper and capsicum. The outer epidermi of the pericarp of coriander and vanilla contain prisms of calcium oxalate. A striated cuticle is seen in aniseed, caraway and star anise fruits. Thickened palisade-like cells form the epidermis of the testa of colocynth and fenugreek seeds. Characteristic elongated tapering cells form the epidermis of cardamoms. Thickened lignified cells form the epidermis of lobelia seed, and mucilage cells that of linseed and of white and black mustard.

EPIDERMAL TRICHOMES

Most leaves and many herbaceous stems, flowers, fruits and seeds possess hairs or trichomes of one kind or another. Many show hairs of more than one type. Hairs may be grouped into non-glandular or clothing hairs, and glandular hairs. Clothing hairs may be unicellular or multicellular. Unicellular hairs vary from small papillose outgrowths to large robust structures (Figs 42.3, 42.4). Multicellular hairs may be uniseriate, biseriate or multiseriate or complicated branched structures (Fig. 42.4). The chemical nature of the cell wall, and the presence of pits or protuberances or of cell inclusions, such as cystoliths, should be noted.

Glandular hairs may have a unicellular or a multiseriate stalk; the glandular head may be unicellular or multicellular (Fig. 42.5). The cuticle of the gland may be raised by the secretion (Fig. 42.5E and F). In peppermint the oil secretion beneath the cuticle contains crystals of menthol. A particular type of hair is often characteristic of a plant family or genus—for example, biseriate hairs of the form shown in Fig. 42.4J are common in the Compositae, while glandular hairs such as Fig. 42.5A, B and C are found in the Solanaceae, and such as Fig. 42.5E in the Labiatae. For types of hairs found on seeds, see sections on cotton, strophanthus seeds and nux vomica seeds.

Trichomes serve a number of functions, which include physical and chemical protection for the leaf against microbial organisms, aphids and insects, and the maintenance of a layer of still air on the leaf surface, thus combating excess water loss by transpiration. The secretions of glandular trichomes of certain genera constitute important materials for the perfumery, food and pharmaceutical industries; some secretions contain narcotic resins and others give rise to skin allergies. The sesquiterpenes of the capitate and non-capitate glandular trichomes of *Helianthus annuus* are antimicrobial and the glandular trichomes of some *Solanum* species contain sucrose esters of carboxylic acids such as 2-methyl-propanoic and 2-methylbutyric acid, which are aphid deterrents. The isolated secretory cells of the pellate glandular trichomes of *Mentha piperita* can carry out the *de novo* synthesis of monoterpenes. These studies have been facilitated by improved methods of trichome microsampling.

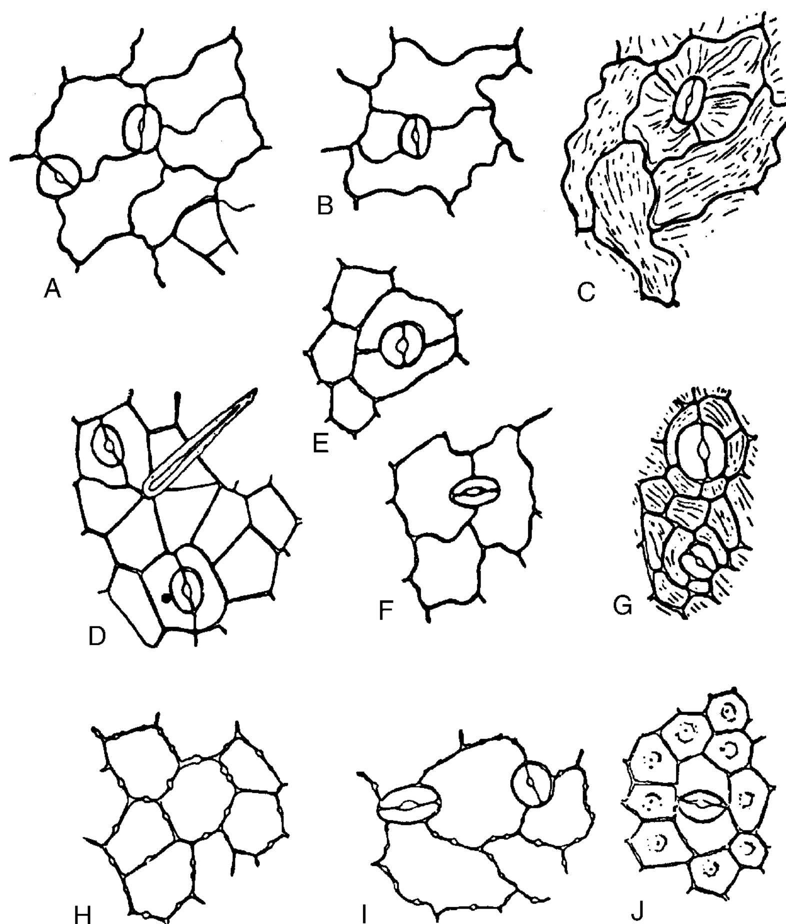

Fig. 42.2
Epidermis of leaves: A, lower epidermis of *Digitalis purpurea*; B, lower epidermis of *Hyoscyamus niger*, C, upper epidermis of *Atropa belladonna*; D, lower epidermis of *Cassia angustifolia*; E, lower epidermis of *Rosmarinus officinalis*; F, lower epidermis of *Mentha piperita*; G, lower epidermis of *Pilocarpus jaborandi*; H, upper epidermis of *Lobelia inflata*; I, lower epidermis of *Digitalis lanata*; J, lower epidermis of *Erythroxylum coca*. A, Anomocytic type of stomata; B and C, anisocytic type of stomata; D, paracytic type of stomata; E and F, diacytic type of stomata; G, actinocytic type of stomata.

THE ENDODERMIS

The endodermis is a specialized layer of cells marking the inner limit of the cortex. A typical endodermis is usually present in roots, in aquatic and subterranean stems and in the aerial stems of certain families (e.g. Labiatae and Cucurbitaceae). Leaves and aerial stems often show a starch sheath, probably representing a modified endodermis.

The cells of the endodermis appear in transverse section four-sided, oval or elliptical and often extended in the tangential direction. The cells are longitudinally elongated, with the end walls often transverse. A primary endodermis, such as can be studied in lobelia stem, is characterized by the deposition, in the radial walls, of special modified material (resembling cutin) in the form of a Casparian strip. Subsequently, a suberin lamella may be laid down within the primary wall, giving a secondary endodermis. This may be followed by the deposition of a secondary wall of lignocellulose, giving a tertiary endodermis, as in *Aletris* and *Smilax*. The structure of the endodermis is of value in differentiating between the commercial species of *Smilax*.

CORK TISSUE

As the plant axis increases in diameter, a cork cambium or phellogen usually arises which, by its activity, produces new protective tissues, known collectively as periderm, which replace the epidermis and part or all of the primary cortex. The cells of the cork cambium undergo tangential divisions giving rise externally to phellem or cork tissue and internally to phelloderm or secondary cortex. Usually, only a limited production of phelloderm occurs, so that the number of cork layers greatly exceeds the number of phelloderm layers. However, wide secondary cortex is seen in ipecacuanha root (Fig. 41.8) and taraxacum.

In roots the cork cambium arises in the pericyle; in stems it may arise in the epidermis or the subepidermal layer or be deep-seated. The first-formed cork cambium may be functional throughout the life of the plant and may itself keep pace with the increase in girth, giving rise to an even smooth bark. A persistent cork cambium, failing to increase in diameter, gives rise to the fissured bark of the cork oak and cork elm. Often, however, the first-formed cork cambium has only a limited period of activity and is replaced by secondary cambia of more deep-seated origin; this process may be repeated again and again.

Cork tissue is built up of a compact mass of cells, usually rectangular in transverse sections (Fig. 21.13C) five- or six-sided in surface view (see Fig. 21.13E) and often arranged in regular radial rows. The cell wall is composed of inner and outer cellulose layers and a median suberin lamella, or of a suberin lamella laid down upon the primary cellulose wall. The cellulose layers may be lignified, as in cassia bark. The mature cork cell is dead, impermeable to water and often filled with dark reddish-brown contents rich in tannins and related substances.

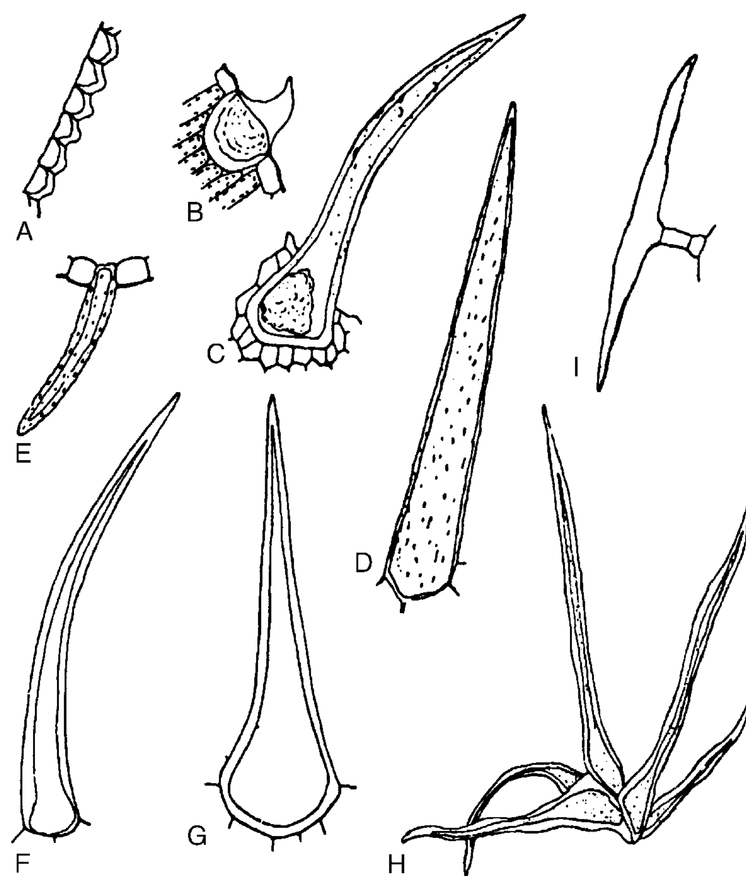

Fig. 42.3
Epidermal trichomes. A, Papillae of lower epidermis of *Coca* leaf. B–G, Unicellular hairs; B, papillose epidermal cell with cystolith from leaf of *Cannabis*; C, cystolith clothing hair from floral bract of *Cannabis*; D, *Lobelia inflata* leaf; E, senna leaf; F, lignified hair of *Ailanthus*; G, comfrey. H, Group of unicellular hairs from *Hamamelis* leaf. I, T-shaped hair of *Artemisia absinthium*.

The presence of cork cells in powdered drugs may show adulteration or use of low-quality or improperly peeled drug (e.g. cinnamon, ginger and liquorice).

The formation of cork puts out of action the stomatal apparatus, and involves the formation of special breathing pores or lenticels. The lenticels are larger in size and smaller in number than the stomata they replace. The simplest form of lenticel consists of a mass of unsuberized thin-walled cells which become rounded off and are known as complementary tissue. Often, however, in the lenticel area, the cork cambium gives rise not only to complementary tissue, but also, alternating with it, to diaphragms of suberized cells, with well-marked intercellular air spaces.

COLLENCHYMA

Collenchyma is a living tissue, directly derived from parenchyma, but having greater mechanical strength. The walls are thickened, the thickening being composed of cellulose and being laid down in longitudinal strips commonly located at the angles of the cells. The cells are usually four- to six-sided in transverse section, axially elongated when seen in longitudinal section. Their walls, being composed of cellulose, have considerable plasticity, and, hence, collenchyma constitutes the typical mechanical tissue of herbaceous stems and of the petioles and midribs of leaves. Collenchyma is present above and below the midrib bundle in many leaves (e.g. senna, stramonium, hyoscyamus, belladonna, digitalis and lobelia); in the wings of lobelia stem; in the cortex of cascara bark; and in the pericarp of colocynth and capsicum.

SCLEREIDS

Sclereids or stone cells are sclerenchymatous cells approximately isodiametrical in shape. The walls of the typical sclereid are thick, lignified, often showing well-marked stratification and traversed by pit-canals which are often funnel-shaped or branched. The cell lumen is usually small, sometimes almost completely obliterated. Cell contents of diagnostic significance may be present (e.g. prisms of calcium oxalate in calumba, starch grains in cinnamon).

Sclereids commonly occur in the hard outer coats of seeds and fruits and in the bark and pericyclic regions of woody stems. They occur isolated or in small groups in quillaia and calumba, in larger groups in cascara (Fig. 21.13) and wild cherry bark (Fig. 25.3) or in definite sclereid layers, as in cinnamon (Fig. 22.10) and cassia bark. The absence of sclereids from frangula and cinchona barks aids in their microscopical identification. The presence of elongated sclereids in powdered ipecacuanha is diagnostic of the presence of stem; lignified sclereids, which are present in clove stalk (Fig. 22.12), should be almost absent from powdered cloves. Characteristic sclereids are present in the rind and seed coat of colocynth (Fig. 41.7F and G).

Attention can appropriately be called, in this section, to the sclerenchymatous layer of the testas of linseed (Fig. 41.7I) and cardamoms (Fig. 41.7K); to the pitted fusiform sclerenchymatous cells of

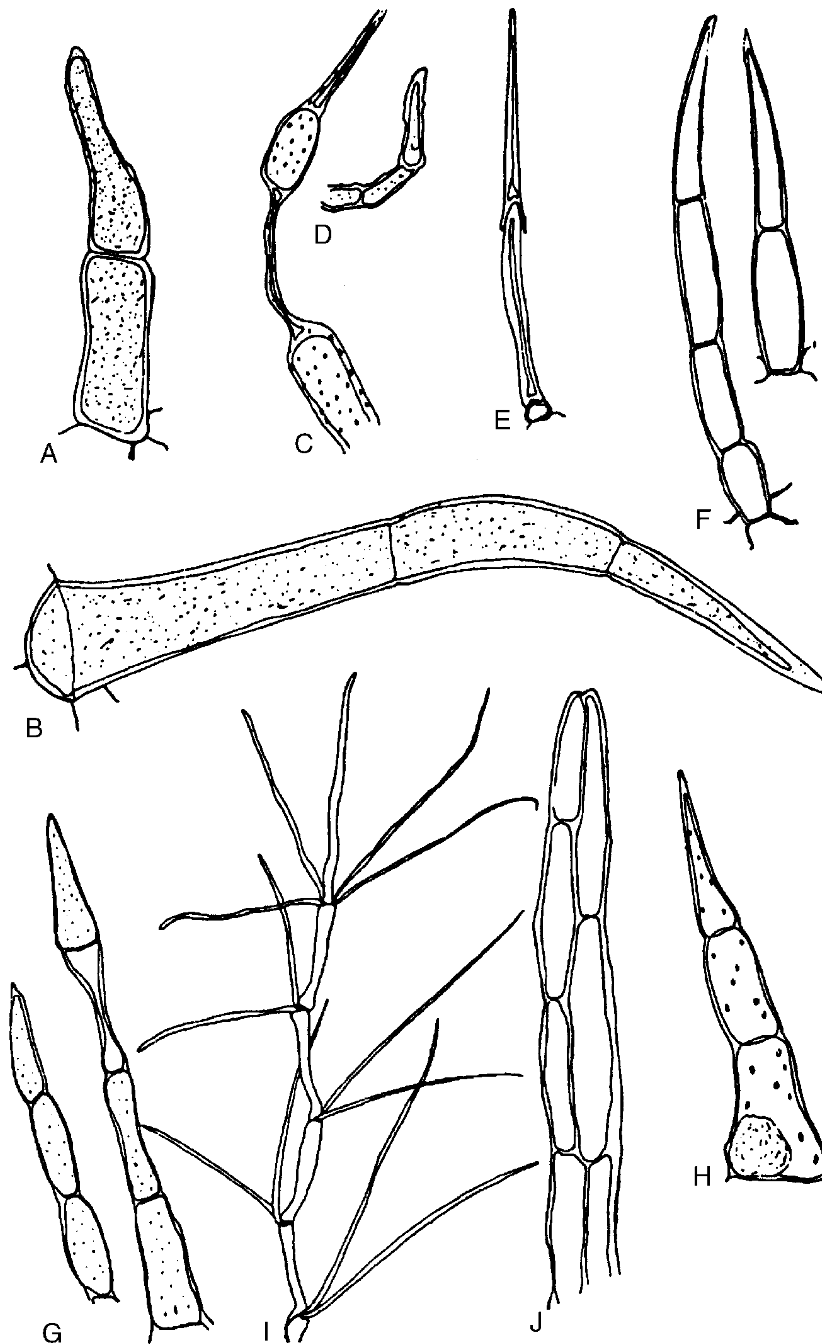

Fig. 42.4
Epidermal trichomes. A–H, Uniseriate clothing hairs; I, multicellular branched hair; J, biseriate hair. A, *Datura metel*; B, *Datura stramonium*; C, *Mentha piperita*; D, *Thymus vulgaris*; E, *Plantago lanceolata*; F, *Hyoscyamus niger*; G, *Digitalis purpurea*; H, *Xanthium strumarium*; I, *Verbascum thapsus*; J, *Calendula officinalis.*

the mesocarp of coriander (Fig. 22.6); to the lignified reticulate cells occurring in the mesocarp of fennel (Fig. 41.7D) and dill, and in the inner part of the testa of colocynth (Fig. 41.7F); and to the lignified idioblasts seen in the lamina of hamamelis leaf.

FIBRES

Tissue composed of spindle-shaped or elongated cells with pointed ends is known as prosenchyma. When cells of this kind are thick-walled, they are known as fibres. The cell wall may be composed of almost pure cellulose or may show various degrees of lignification in the form of sclerotic or sclerenchymatous fibres.

Fibres are developed from a single cell, the fibre initial, which during its development grows rapidly in the axial direction. During this period of growth the tips of the elongating cells may push past one another, a process known as 'gliding growth' and made possible by a modification in the state of the middle lamella. Most mature fibres are unicellular, but occasionally transverse septa develop (e.g. ginger). Fibres are best differentiated on the basis of the tissue in which they occur (i.e. as cortical fibres, pericyclic fibres, xylem fibres or phloem fibres).

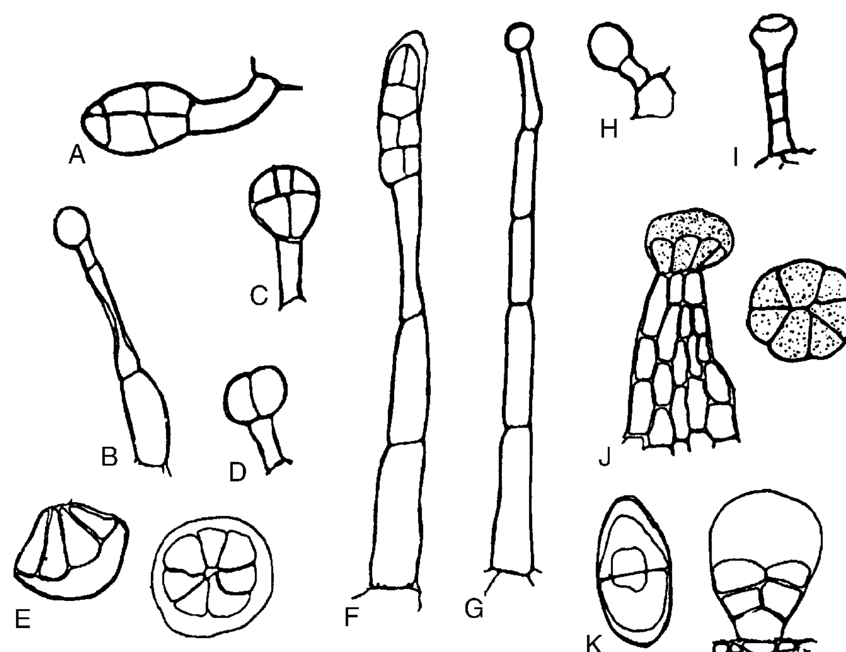

Fig. 42.5
Glandular hairs: A and B, *Atropa belladonna*; C, *Datura stramonium*; D, *Digitalis purpurea*; E, multicellular labiate glandular hair; F, *Hyoscyamus niger*; G and H, *Primula vulgaris*; I, *Digitalis lutea*; J, *Cannabis sativa*; K, *Artemisia maritima*.

Frequently, fibres are differentiated in the pericycle; thus flax consists of the *pericyclic fibres* of *Linum usitatissimum* and hemp of the pericyclic fibres of *Cannabis sativa*. The cell wall of the flax fibre is composed of almost pure cellulose; in hemp some lignification has taken place.

Isolated groups of pericyclic fibres occur in lobelia stem and in cinnamon bark. The meristeles of clove hypanthium are enclosed in an incomplete sheath of pericyclic fibres. The lignified, moderately thick-walled pericyclic fibres, accompanied by a parenchymatous sheath of cells containing prisms of calcium oxalate, constitute an important diagnostic character of senna leaf (Fig. 21.10). The presence of pericyclic fibres in the midrib of the leaves of *Digitalis lutea* and *D. thapsi* contrasts with their absence in *D. purpurea* and *D. lanata*.

Xylem fibres may be regarded as being directly derived from tracheids, and intermediate forms, having a limited conducting function and known as fibre-tracheids occur (Fig. 42.6A–C). The fibre-tracheid has smaller pits, thicker walls and usually more tapering ends than the typical tracheid. Wood fibres have thicker walls and pits reduced to minute canals. Occasionally, wood fibres are septate (Fig. 42.6D). Cells having a fibre-like form with living contents and simple pits but which are really fusiform xylem parenchyma cells are termed 'substitute fibres'. The mature wood fibre is a dead lignified element. The autumn wood is usually characterized by containing a higher proportion of wood fibres than the spring wood. The ground mass of secondary xylem of *Picroena excelsa* is built up of compactly arranged thick-walled wood fibres and the secondary xylem of liquorice (Fig. 23.11) contains wood fibres arranged in bundles, which alternate with the small groups of vessels and are enclosed in a sheath of xylem parenchyma containing prisms of calcium oxalate. The secondary xylem in gentian, rhubarb and jalap is free from fibres.

Phloem fibres may occur in both primary and secondary phloem; they may or may not be lignified. Their thickened walls are traversed by simple pits, in contrast to the fine-bordered pits of the wood fibres. Phloem fibres constitute the 'hard bast' of earlier writers. Jute consists of the phloem fibres from the stems of various species of *Corchorus*. The phloem fibres of liquorice resemble those of the xylem in being enclosed in a crystal sheath. The distribution, abundance, size and shape of the phloem fibres constitute important characters for the differentiation of medicinal barks. Phloem fibres occur isolated or in irregular rows in the barks of cinnamon (Fig. 22.10), cassia and cinchona (Fig. 26.33). The phloem fibres of cinnamon can be differentiated from those of cassia by their smaller diameter. In barks, in which fibres occur isolated or in rows, the area of fibres per gram of powdered bark can be made a criterion for determining the amount present in mixtures. The phloem fibres of cinchona constitute a prominent feature of the powder; they are large (80–90 μm in diameter), are fusiform in shape and have very thick walls, conspicuously striated and traversed by funnel-shaped pits (Fig. 26.33). The secondary phloem of cascara, frangula and quillaia is composed of alternating zones of hard and soft phloem. The phloem fibres of cascara are accompanied by a crystal sheath (Fig. 21.13); those of quillaia are characterized by their tortuous, irregular outline and often exhibit enlarged and forked apices. Fibres are absent from the phloem of gentian and ipecacuanha.

XYLEM

The primary xylem is composed of protoxylem and metaxylem. Secondary growth in thickness of the stem and root of gymnosperms and dicotyledons is accompanied by the formation of secondary xylem. The structural elements of xylem are tracheids, vessels or tracheae, xylem fibres, xylem parenchyma and rays.

The *tracheid* is derived from a single cell and can be regarded as the basic cell type of xylem tissue. It takes the form of an elongated water-conducting cell, with a lignified and variously thickened and pitted cell wall (Fig. 42.6A). At maturity it is a dead element. The pits are bordered (Fig. 42.6L–N), although in some cases the borders are so narrow that the pits appear simple. In gymnosperms the pits are confined to the radial walls.

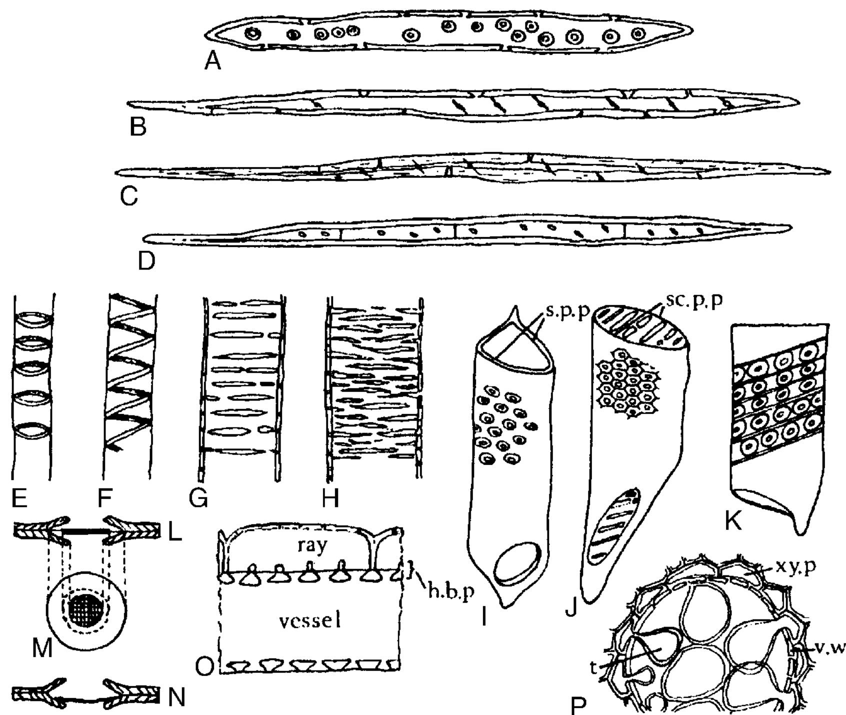

Fig. 42.6
Xylem components. A, Tracheid; B, fibre-tracheid; C, xylem fibre; D, septate fibre; E, annular vessel; F, spiral vessel; G, scalariform vessel; H, reticulate vessel; I, vessel segment with round bordered pits and simple perforation plate (s.p.p.) at either end; J, vessel segment with hexagonal pits caused by crowding, and scalariform perforation plate (sc.p.p) at either end; K, vessel segment with bordered pits and band of tertiary thickening. L, M, N, structure of the bordered pit; L and N, sections show overarched secondary wall, pit membrane and central torus, the latter, in N, closing the pit mouth; M, surface view of same; O, half-bordered pit pairs (h.b.p.) connecting a vessel and ray cells; P, vessel in transverse section showing development of tyloses. t, Tylosis; v.w, vessel wall; xy.p, xylem parenchyma.

The character of the secondary wall thickening enables us to distinguish annular, spiral, scalariform and reticulate tracheids. Transition forms between these types are not uncommon. Annular and spiral tracheids occur most frequently in protoxylem; scalariform and reticulate tracheids most frequently in metaxylem and secondary xylem. True vessels are absent in gymnosperms, in which the secondary xylem consists of a homogeneous tracheidal system only broken by narrow medullary rays and in some cases by a slight development of xylem parenchyma. Cellulose wadding is made from high grade sulphite pulp usually prepared from coniferous wood, and when examined microscopically shows typical coniferous tracheids, with bordered pits and a small amount of wood parenchyma. The tissues are completely delignified in the preparation of the pulp. Tracheids occur in the secondary xylem of some angiosperms (e.g. ipecacuanha).

Vessels or *tracheae* constitute the fundamental conducting elements of the xylem of the angiosperms. The vessel is derived from a vertical series of cells, in which increase in diameter and dissolution of the end-walls occurs so that a continuous tube is formed. The most primitive type of vessel consists of a vertical series of tracheid-like segments in which some of the scalariform pits of the adjacent end-walls have broken down to give slit-like openings; the most advanced type of vessel shows complete dissolution of the end-walls of the constituent segments (see Fig. 42.6I–K). The vessels of the protoxylem show annular or spiral thickening, those of the later-formed xylem scalariform and reticulate thickening (Fig. 42.6E–H). The secondary wall thickening is composed of lignocellulose. Larger vessels may have a complete secondary wall perforated only by pits. These pits are subject to considerable variation in size, form and crowding and sometimes bands of tertiary thickening are laid down within the secondary wall (Fig. 42.6K).

Spiral and annular vessels are typical of protoxylem, and usually occur in the protoxylem of stems and roots, in small vascular bundles and in the veins of leaves. Thus, small amounts of such vessels are seen in gentian, clove, squill and most leaves (e.g. senna, belladonna, hyoscyamus and stramonium). Spiral and scalariform vessels occur in lobelia stem. Reticulate vessels occur in gentian, ginger and rhubarb, those of the last two drugs being almost non-lignified. Vessels showing numerous bordered pits occur in quassia, jalap, sandalwood, hydrastis and the stems of belladonna and aconite.

The living meshwork of the secondary xylem is made up of *rays* and *xylem parenchyma* which permeate the dead mass of mature vessels, tracheids and wood fibres. The xylem parenchyma cells are often axially elongated, sometimes thin-walled but often with walls showing thickening and lignification. The walls are traversed by simple pits or, where the cells abut on vessels or tracheids, by half-bordered pits. Xylem parenchyma may function as a storage tissue, the cells becoming blocked with starch (as in ipecacuanha). The xylem parenchyma cells may grow into the vessel cavities and form tyloses which block up the vessel and render it non-functional, a process which occurs in the development of heartwood (Fig. 42.6P). The distribution of xylem parenchyma may be diffuse, vasicentric when it forms sheaths around the larger vessels, or terminal when a zone of xylem parenchyma is formed towards the end of each year's growth. The formation of concentric zones of xylem parenchyma may give rise to 'false annual rings', as in quassia. In transverse sections the medullary rays appear radially arranged and where the ray cells about on to vessels they may possess half-bordered pits (Fig. 42.6O).

PHLOEM

The structural elements of phloem include sieve tubes, companion cells, phloem parenchyma and secretory cells. The sieve tube is the conducting element of the phloem. It is formed from a vertical series of elongated cells, interconnected by perforations in their walls in areas known as sieve plates. The perforations may be restricted to smaller areas, sieve fields, several of which are contained in each sieve plate. The sieve plates may occur in the end-walls or lateral walls of the sieve tube (Fig. 42.7). The mature sieve plate is coated with a film of callus, which may increase in amount and form a callus pad completely blocking the sieve plate (Fig. 42.7D). The development of the callus pad may render the sieve tube permanently functionless; in other cases the callus pad formed in the autumn is redissolved in the spring. The mature sieve tube lacks a nucleus, but while functional contains cytoplasm. Sieve tubes may often be detected by recognition of the callus pads, which show typical staining reactions.

1. Alkaline solution of corallin: stains callose red.
2. Aniline Blue: stains callose blue.
3. Chlor-zinc-iodine: stains callose a reddish-brown.
4. Solution of Ammoniacal Copper Nitrate *BP*: does not dissolve callose.
5. Solution of potash: as even a cold 1% solution of potash dissolves callose, this should not be used as a clearing agent if it is afterwards desired to test the section for callose.

In view of their delicate structure and lack of lignification, sieve tubes are difficult to observe in commercial drugs. The sieve tubes of cascara bark can often be detected, even in the powdered drug, when stained with corallin soda. They are sometimes also to be observed in powdered gentian.

The companion cells are intimately associated with the sieve tubes both structurally and functionally. The sieve tube and the companion cells are derived from a common mother cell of the procambial strand in primary phloem or from a phloem mother cell derived from the cambium in secondary phloem. The phloem mother cell undergoes longitudinal division into two daughter cells of unequal size, the smaller of which becomes the companion cell. The companion cell is characterized by its dense protoplast and well-developed nucleus, and by possessing a thin cellulose wall.

The cells of the phloem parenchyma are usually axially elongated, although they may remain isodiametric and be arranged in linear series. They remain typically thin-walled.

The phloem often contains secretory cells (e.g. ginger, cinnamon, cassia and jalap). Laticiferous tissue may also occur in the phloem (e.g. lobelia and taraxacum) (Fig. 42.7E).

SECRETORY TISSUES

Secretory tissues include secretory cells, secretory cavities or sacs, secretory ducts or canals and latex tissue.

Oil cells occur in ginger (Fig. 42.8D), pepper, mace, cardamoms, cinnamon (Fig. 42.8G) and cassia. Large oil cells form an important diagnostic character of powdered sassafras root bark. Cells containing resins (Fig. 42.8H), oleoresins and mucilage are common. Enzyme storage cells occur in many endospermic seeds (e.g. the myrosin cells of the Cruciferae). Storage cells, crystal cells and tannin cells may also be considered under this heading.

Secretory cavities or *sacs* may arise by separation of the cells and subsequent formation of a secretory epithelium (schizogenously) or by breakdown of the cells forming a cavity not bounded by a definite epithelium (lysigenously). Schizogenous oil cavities occur in eucalyptus, lysigenous oil cavities in *Gossypium* species. Secretory products may appear in cells before the latter break down to give a lysigenous cavity. Schizolysigenous oil cavities occur in the Rutaceae and the Burseraceae. The oil cavity develops from a mother cell, which undergoes division to give daughter cells which separate, leaving a schizogenous central cavity. The walls of the cells surrounding this central cavity then break down, forming an oily secretion, and the cavity continues to increase in size lysigenously (Fig. 42.8E, F).

The vittae of the Umbelliferae are schizogenous oleoresin canals (Fig. 42.8A–C) and they occur in the stem, roots and leaves.

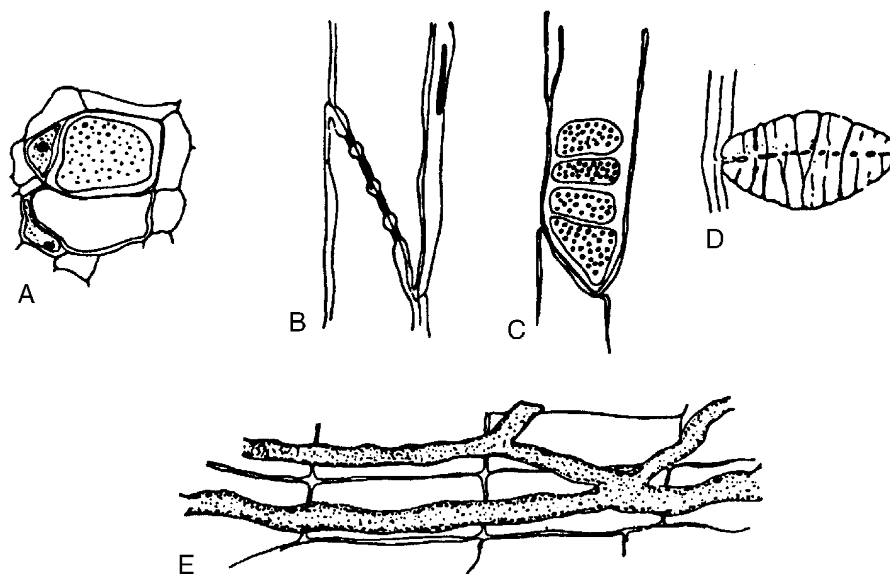

Fig. 42.7
Phloem elements. A, Sieve tubes and companion cells in transverse section, one of the sieve tubes showing a transverse sieve plate in surface view; B and C, respectively, tangential and radial longitudinal views of a sieve tube, showing an oblique sieve plate with four sieve fields; D, sieve plate in winter condition, showing deposit of callus; E, radial longitudinal view of laticifers in the root of *Taraxacum officinale*.

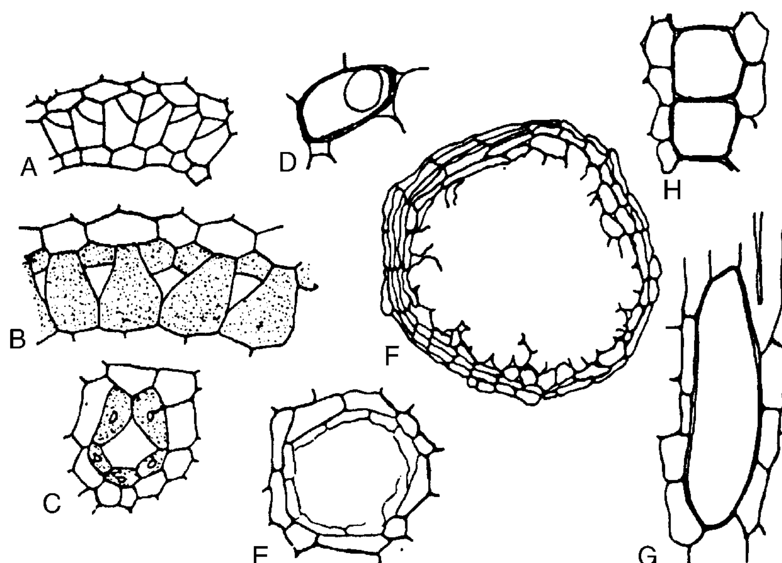

Fig. 42.8
Secretory cells and ducts. A, B, C, Stages in development of a typical schizogenous oil duct in the Umbelliferae (after Hayward); D, oil cell of ginger; E and F, schizolysigenous oil glands in *Barosma* and *Citrus*; G, oil cell of cinnamon seen in longitudinal section; H, resin cells of jalap.

The oleoresin ducts of *Pinus* species are also of schizogenous origin. Schizogenous oleoresin ducts which enlarge lysigenously occur in some members of the Leguminosae (e.g. *Copaifera*).

Latex (Laticiferous) tissue consists of either cells or tubes which contain a fluid with a milky appearance arising from the suspension of small particles in a liquid dispersion medium with a very different refractive index. The suspended particles vary in nature, and may be hydrocarbons composed of essential oils, resins and rubber. Alkaloids are present in the latex of Papaveraceae, the proteolytic enzyme papain in the latex of *Carica* (pawpaw) and vitamin B_1 in that of *Euphorbia*. Latex cells are typical of the Euphorbiaceae, Moraceae, Cannabinaceae, Apocynaceae and Asclepiadaceae. In the Euphorbiaceae the cells destined to form the latex systems are differentiated in the embryo. From these embryonic initials the branched tubular latex cells of the mature plant are developed. The latex cells have thickened walls and numerous nuclei, and contain latex in which characteristic dumb-bell-shaped starch grains may be present. The long, sinuous latex cells of *Cannabis sativa* are unbranched.

Laticifers are also formed by the partial or complete fusion of a longitudinal series of cells. They occur in Convolvulaceae, Campanulaceae and the suborder Liguliflorae of the Compositae. The Papaveraceae possess latex elements intermediate in structure between latex cells and vessels. The laticifers of *Ipomoea* consist of longitudinal rows of cells which retain their transverse walls. A similar condition is seen in *Sanguinaria*. In *Chelidonium* the marginal parts of the transverse walls persist; in *Papaver* and *Argemone* there is only slight evidence of the original transverse walls. The laticifers in the Liguliflorae take the form of a continuous non-septate series of passages usually occurring in the primary and secondary phloem. *Taraxacum officinale* (Fig. 42.7E) shows concentric zones of anastomosing latex vessels in the phloem of both rhizome and root.

It is often difficult or impossible to determine the mode of origin of the laticifers except by following their development from the embryonic or seedling stages. A further difficulty in delimiting elements of a laticiferous nature arises from their association in some plants with idioblasts containing tannins, mucilage, etc., and from the fact that latex material may also occur in schizogenous canals.

ERGASTIC CELL CONTENTS

The cell contents with which we are concerned in pharmacognosy are those which can be identified in vegetable drugs by microscopical examination or by chemical and physical tests. These cell contents represent either food-storage products or by-products of metabolism, and include carbohydrates, proteins, fixed oils and fats, alkaloids and purines, glycosides, volatile oils, gums and mucilages, resins, tannins, calcium oxalate, calcium carbonate and silica; being non-living, they are referred to as *ergastic*.

Starch

Starch occurs in granules of varying sizes in almost all organs of plants; it is found most abundantly in roots, rhizomes, fruits and seeds, where it usually occurs in larger grains than are to be found in the chlorophyll-containing tissues of the same plant. The small granules formed in chloroplasts by the condensation of sugars are afterwards hydrolysed into sugars so that they may pass in solution to storage organs where, under the influence of leucoplasts, large grains of reserve starch are formed. Starch is of considerable pharmaceutical importance and is fully discussed in Chapter 20.

Proteins

Storage protein occurs in the form of aleurone grains which are particularly well seen in oily seeds (e.g. castor seed (see Fig. 42.1E) and linseed). The simplest aleurone grain consists of a mass of protein surrounded by a thin membrane. Often, however, the ground mass of protein encloses one or more rounded bodies or globoids and an angular body known as the crystalloid. Aleurone grains are best observed after defatting and removal of starch, if these are present in large amount. Sections being examined for aleurone should be treated with the following reagents:

1. Millon's reagent stains the protein red on warming.
2. Iodine solution stains the ground substance and crystalloid yellowish-brown but leaves the globoids unstained.
3. Picric acid stains the ground substance and crystalloid yellow.

The endosperm cells of nutmeg each contain one large and several smaller aleurone grains. The large aleurone grains are 12–20 μm in diameter, and contain a large well-defined crystalloid. Aleurone grains, containing globoids, are present in the endosperm and cotyledons of linseed. Some of the aleurone grains of the endosperm of fennel contain a minute cluster crystal of calcium oxalate; others contain one or more globoids.

Fixed oils and fats

Fixed oils and fats are widely distributed and occur in both vegetative and reproductive structures. They often occur in seeds, where they may replace the carbohydrates as a reserve food material, and are not uncommonly associated with protein reserves. As lipids, fats form an essential component of biological membranes.

Reserve fats occur in solid, frequently coloured or crystalline masses which melt on warming. Feathery crystalline masses of fat occur in the endosperm of nutmeg. Fixed oils occur as small highly refractive drops. Oil globules, associated with aleurone grains, can be well seen in the cotyledons of linseed and colocynth and in the endosperm of nux vomica and umbelliferous fruits. Oils and fats are soluble in ether-alcohol, but, with a few exceptions, such as castor oil, are sparingly soluble in alcohol. They are coloured brown or black with a 1% solution of osmic acid, and red with a diluted tincture of alkanna. The latter stains rather slowly and should be allowed to act for at least 30 min. A cold mixture of equal parts of a saturated solution of potash and strong solution of ammonia slowly saponifies fixed oils and fats. After some hours, characteristic soap crystals may be observed. For a full discussion of fixed oils and fats, see Chapter 19.

Gums and mucilages

Gums, mucilages and pectins are polysaccharide complexes formed from sugar and uronic acid units. They are insoluble in alcohol but dissolve or swell in water. They are usually formed from the cell wall (e.g. tragacanth) or deposited on it in successive layers. When such cells are mounted in alcohol and irrigated with water, the stratification may often be seen (e.g. mustard and linseed).

Specific tests for these substances are at present lacking, but the following are useful. The official Solution of Ruthenium Red stains the mucilage of senna and buchu leaves, althaea, linseed and mustard. It also stains sterculia gum but has less action on tragacanth. A lead acetate medium can be used to prevent undue swelling or solution of the substance being tested. Some forms of mucilage are stained by the *BP* Alkaline Solution of Corallin, e.g. that found in squill. Others are stained by chlor-zinc-iodine or methylene blue dissolved in alcohol and glycerin.

The pharmaceutical gums are described in Chapter 20.

Volatile oils and resins

Volatile oils occur as droplets in the cell. They are sparingly soluble in water but dissolve in alcohol (cf. fixed oils). They resemble fixed oils (q.v.) in their behaviour towards osmic acid and tincture of alkanna, but they are not saponified when treated with ammoniacal potash.

Resins may be associated with volatile oil or gum, or may be found in irregular masses which are insoluble in water but soluble in alcohol. Resins, oleoresins and gum resins are usually secreted into secretory cavities or ducts. They stain slowly with diluted tincture of alkanna. For details of volatile oil and resin-containing drugs see Chapter 22.

Tannins

Tannins are widely distributed in plants and occur in solution in the cell sap, often in distinct vacuoles. If it is desired to study the distribution of the tannins in the plant, the sections must be cut dry, since tannins are soluble in water and alcohol. If sections of galls are so cut and mounted in clove oil, plates of tannin may be observed. Sections containing tannins acquire a bluish-black or greenish colour when mounted in a dilute solution of ferric chloride. For tannin-containing drugs see Chapter 21.

Alkaloids and glycosides

These important secondary metabolites are rarely visible in plant cells without the application of specific chemical tests.

Crystals

Various crystalline deposits may occur in plant cells.

Calcium oxalate. Oxalic acid rarely occurs in the free state in plants but is extremely common as its calcium salt in the form of crystals. It is dimorphous and is found either as the trihydrate, belonging to the tetragonal system of crystals, or as the monohydrate, belonging to the monoclinic system.

Crystals of the tetragonal system form as a result of supersaturation of the cell sap with calcium oxalate. They have all three axes at right angles to one another; two of the axes are equal in length and the third, or principal axis, may be either shorter or longer. They are illustrated in Fig. 42.9A–D; and in addition to these forms, the tiny sandy crystals or microcrystals found in the Solanaceae (Fig. 26.8) and other families probably belong to this system. In the monoclinic system the crystals have such forms as shown in Fig. 42.9E–I, and result from an excess of oxalic acid in the cell sap. They have three unequal axes with the two lateral axes at right angles to one another, but one only of these is at right angles to the third axis. These crystals shine more brightly when viewed in polarized light than do the trihydrate crystals.

Usually it is sufficient to describe the general form and size of the crystals, without reference to a crystallographical class. The most common forms encountered are prisms (senna, hyoscyamus, quassia, liquorice, cascara, quillaia, rauwolfia, calumba); rosettes (rhubarb, stramonium, cascara, senna, clove, jalap); single acicular crystals (ipecacuanha, gentian, cinnamon); bundles of acicular crystals (squill); microsphenoidal or sandy crystals (belladonna).

When calcium oxalate is present, it is important that the types of crystal, their size and distribution be recorded. Cascara shows cluster crystals generally distributed in the ground mass of parenchyma and prisms confined to the rows of parenchymatous cells forming a sheath round the fibres (Fig. 21.13). The prisms of calcium oxalate in calumba are contained in the sclereids.

The cells containing calcium oxalate may differ from those surrounding them in size, form or contents, and are often referred to as idioblasts.

Calcium oxalate is usually present to the extent of about 1% in plants but in some structures such as the rhizome of rhubarb it may exceed 20% of the dry weight. It often forms a character of considerable diagnostic importance. The solanaceous leaves may be distinguished from one another, belladonna by its sandy crystals, stramonium by its cluster crystals, and henbane by its single and twin prisms. Similarly, phytolacca leaves and roots, which both possess acicular crystals, are distinguished from belladonna leaves and roots, which have sandy crystals. Other instances of the diagnostic importance of calcium oxalate are given under the individual drugs and no attempt is made to give here more than a few selected examples, which will be extended by the student in further reading.

Sections to be examined for calcium oxalate may be cleared with chloral hydrate or caustic alkali, as these reagents only very slowly dissolve the crystals. The polarizing microscope will often assist in the detection of small crystals. Crystals may be identified as

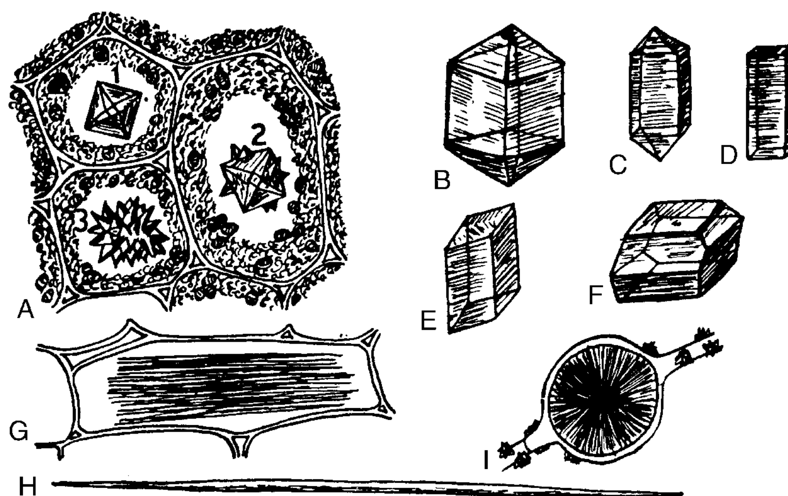

Fig. 42.9
Calcium oxalate. A–D, Crystals of the tetragonal system; E–I, crystals of the monoclinic system. A3, A rosette crystal formed of tetragonal crystals as seen in A1 and A2; D, a tetragonal prism; E, a monoclinic prism; G, raphides; H, a single needle crystal; I, a sphaerocrystal. (After Thoms' *Handbuch der Pharmazie*.)

calcium oxalate if they are insoluble in acetic acid and caustic alkali, soluble in hydrochloric and sulphuric acids without effervescence and show, after solution in 50% sulphuric acid, a gradual separation of needle-like crystals of calcium sulphate at the site of the original crystals.

Calcium carbonate. This may be found embedded in or incrusted in the cell walls. Concretions of calcium carbonate formed on outgrowths of the cell wall are termed cystoliths. They occur in the orders Urticaceae, Moraceae, Cannabinaceae and Acanthaceae, and in some of the Combretaceae and Boraginaceae. Well-formed cystoliths are seen in the enlarged upper epidermal cells and in the clothing hairs of the lower epidermis of the leaf of *Cannabis sativa* (Fig. 42.3). When the mineral substance of the cystolith is dissolved out in dilute acid, there remains a small, often stratified, basis composed of cellulose. Calcium carbonate can be identified by the fact that it dissolves with effervescence in acetic, hydrochloric or sulphuric acid. If 50% sulphuric acid is used, needle-shaped crystals of calcium sulphate gradually separate.

Hesperidin and diosmin. These occur as feathery-like aggregates or sphaerocrystalline masses in the cells of many of the Rutaceae and in isolated plants of other families. Crystalline masses of diosmin are present in the upper epidermal cells of buchu leaves (Fig. 42.1B). These crystals are insoluble in organic solvents but soluble in potassium hydroxide.

Silica. This substance forms the skeletons of diatoms (see 'agar' and 'kieselguhr'), and occurs as an incrustation on cell walls or as masses in the interior of cells (e.g. in the cells of the sclerenchymatous layer of cardamom seeds). Silica is insoluble in all acids except hydrofluoric. It may be examined by igniting the material and treating the ash with hydrochloric acid, the silica remaining unaltered.

43

Techniques in microscopy

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The microscopical characters of many drugs have already been described, but it will be realized that microscopical techniques require considerable skill; years of experience are necessary to acquire a really good knowledge of the microscopy of drugs, foodstuffs and other plant materials. It is first necessary to learn how to use a microscope properly and to understand the purpose of the different reagents used in the examination of crude drugs. The preparation of systematic and illustrated reports is also important.

Mountants for specimens

Definition, particularly of colourless structures, is increased by choice of a mountant of refractive index different from that of the object. A mountant of lower refractive index is to be preferred so that the outline shadow is on the side away from the object. The ratio refractive index of object to refractive index of mountant should be of the order of 1.06. The value of this relative refractive index for cellulose (cotton) to water is 1.17, to chloral hydrate solution (5:2) 1.08 and to glycerin 1.06. This serves to emphasize the value of chloral hydrate and glycerin as mountants for plant structures.

THE MICROSCOPE

Magnification and field of view

For work in pharmacognosy, microscopes are usually fitted with two objectives, 16 mm and 4 mm, two or three eyepieces and a condenser. The procedure for making microscopical measurements is described below. Different combinations of eyepiece and objective give different magnifications and fields of view, as indicated in the table below.

When using the microscope, it is useful to know the size of the fields of view. For instance, if we know that using a 4 mm objective and a ×6 eyepiece our field of view is approximately 0.5 mm, or 500 µm, the size of objects such as the *Arachnoidiscus* diatom in agar (100–300 µm) or the large rosette crystals of calcium oxalate in rhubarb (up to 200 µm) may be roughly estimated. For accurate measurement, however, an eyepiece micrometer or camera lucida is used.

Apparatus for making microscopical measurements and drawings to scale

Microscopical measurements can be made using a stage micrometer in conjunction with an eyepiece micrometer, camera lucida or microprojector.

Micrometers

Two scales are required, known, respectively, as a *stage micrometer* and an *eyepiece micrometer*. The stage micrometer is a glass slide 7.6 × 2.5 cm (3 × 1 inch) with a scale engraved on it. The scale is usually 1 or 1.1 mm long and is divided into 0.1 and 0.01 parts of a millimetre. The eyepiece micrometer may be a linear scale (Fig. 43.1A and the scale 0–10 in Fig. 43.1B) or it may be ruled in squares. The value of one eyepiece division is determined for every optical combination to be used, a note being made in each case of the objective eyepiece and length of draw-tube.

To do this, unscrew the upper lens of the eyepiece, place the eyepiece micrometer on the ridge inside, and replace the lens. Put the stage micrometer on the stage and focus it in the ordinary way. The two micrometer scales now appear as in Fig. 43.1B, when the 4 mm objective is in use. In the example figured, it will be seen that when the 7 line of the stage micrometer coincides with the 0 of the eyepiece, the 10 of the stage coincides with 7.7 of the eyepiece. As the distance between 7 and 10 on the stage scale is 0.3 mm, 77 of the small eyepiece divisions equal 0.3 mm or 300 µm; therefore, 1 eyepiece division equals 300/77 or 3.9 µm.

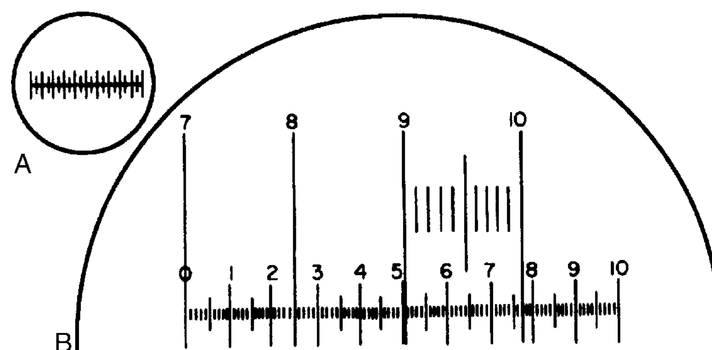

Fig. 43.1
A, Eyepiece micrometer; B, eyepiece micrometer superimposed on portion of stage micrometer scale.

Focal length of objective	Initial magnifying power	Approximate magnification (field of view in brackets) with eyepiece		
		×6	×10	×15
16 mm (2/3 inch)	10	62 (2.0 mm)	110 (1.1 mm)	155 (0.89 mm)
4 mm (1/6 inch)	45	285 (0.5 mm)	490 (0.25 mm)	690 (0.21 mm)

Camera lucida

Various forms of apparatus have been designed so that a magnified image of the object under the microscope may be traced on paper. The Swift-Ives camera lucida and the Abbé drawing apparatus are examples. The former fits over the eyepiece, and when in use light from the object passes direct to the observer's eye through an opening in the silvered surface of a prism. At the same time, light from the drawing paper and pencil is reflected by a second prism and by the silvered surface, so that the pencil appears superimposed on the object, which may thus be traced.

When the instrument is in use, the illumination of both object and paper must be suitably adjusted and the paper must be tilted at the correct angle to avoid distortion. The Abbé drawing apparatus utilizes, instead of the adjustable prism, a plane mirror carried on a side-arm; with the mirror at 45° to the bench surface, no inclined board is necessary.

To make measurements or scale drawings, the divisions of a stage micrometer are first traced on paper and then, using the same objective, eyepiece and length of draw-tube, the object to be recorded is traced.

The above camera lucidas which have served pharmacognosists well in the past do not now appear to be available commercially. This seems apparent from some of the line illustrations now submitted for publication!

Microprojection

With a suitable apparatus objects beneath a microscope can be transmitted to a screen or on to paper. Suitable particles can then be traced at the desired magnification.

Photomicrography

Modern research microscopes have built-in facilities for photomicrography; with less expensive or with older microscopes it is necessary to fit a suitable camera to the equipment. The photographic record may seem, at first sight, to dispense with the older tracing techniques discussed above. However, although the photographic record is suitable for thin sections of plant material, rarely is it completely so for powdered drugs. A photograph contains everything visible in a particular field of view and much of this is usually uninformative; rarely does a single field of view show all the diagnostic features of the powdered drug to best advantage, and also, owing to limitations on depth of focus, structures such as large fibres, vessels, trichomes, etc. are seldom seen with all details completely in focus. Fragments of disintegrated material adhering to, or partly covering, the element photographed are also confusing to the inexperienced observer.

Polarization

The apparatus consists of a polarizer, or Nicol prism, fitting below the microscope stage, and a similar prism forming the analyser fitting above the objective. As in the case of the polarimeter, with which students will be familiar, one Nicol is kept stationary while the other is rotated. With geological microscopes, polarizers are usually permanently fitted, but for botanical work the analyser is usually fitted when required, either between the objective and nosepiece or over the eyepiece. Less cumbersome are discs cut from polaroid sheets; one of these can be of a size to fit into the filter holder of the microscope and the other to rest on the eyepiece.

When both the polarizer and analyser have their diagonal surfaces parallel, the ray of plane polarized light is transmitted by the analyser. If now the polarizer is revolved, the light diminishes in intensity until at a position 90° from the first it is entirely extinguished, the polarized light being now totally reflected by the analyser. This position, when the diagonal surfaces of the two Nicols are at right angles, is termed 'crossed Nicols'.

Isotropic substances are characterized by having the same physical properties in all directions (e.g. gases, liquids and isometric crystals). Such substances are monorefringent (i.e. they have only one refractive index). Isotropic substances are not visible, however they be oriented, when examined between crossed Nicols. They in no way affect the polarized light passing through them from the polarizer.

Anisotropic substances exhibit different physical properties according to the direction along which they are examined. Such substances show more than one refractive index. The great majority of crystalline materials show birefringence. When a uniaxial crystal is placed with its optical axis horizontal to the stage and examined between crossed Nicols, then as the stage is rotated it will alternately shine bright (or coloured) and disappear. Through the 360° it becomes invisible (i.e. shows extinction) four times. The examination of crystals between crossed Nicols enables us to determine their crystal system (see 'Calcium oxalate' Chapter 42). The crystal is placed with its axis parallel to the longer diagonal of the polarizing Nicol. If the crystal belongs to the tetragonal system, the polarized light passes unchanged and on reaching the analyser is completely absorbed,

the field appearing dark (i.e. extinction takes place). Conversely, monoclinic crystals show extinction only when the vertical axis makes an angle with the diagonal of the Nicol known as the extinction angle.

Many crystalline substances show brilliant colours when examined in polarized light (e.g. asbestos, sucrose, cinnamic acid). Starch grains often show a black cross, a phenomenon due to the crystalline refraction of the material. Polarized light is useful for the detection of calcium oxalate, especially when only small quantities are present in the tissues under examination. It appears bright on a black background.

Phase-contrast microscopy

This has proved particularly useful for the examination of living cells, the constituents of which normally show little differentiation. Monochromatic light is employed; light directly transmitted through the sample is reduced in intensity and the deflected light is brought half a wavelength out of phase with the transmitted light. Strong contrasts in the material under examination are thereby obtained without reduction in the resolving power of the microscope.

Ultraviolet microscopy

The limit of resolution of any microscope is governed by the wavelength of the beam employed; the shorter the wavelength, the smaller the object which can be resolved. The ultraviolet microscope with lenses of fused quartz will transmit radiation down to the wavelength of 240 nm. The images produced are recorded photographically. The instrument has been valuable in the study of cell division and differentiation.

Electron microscopy

Just as a beam of light can be focused by an optical lens, so a stream of electrons can be focused by an electromagnet acting as a lens. Objects placed in the path of the electrons produce an image which can be recorded either on a fluorescent screen or on a photographic plate. Both the focal length and the magnification can be varied by regulation of the field strength, which is controlled by the current passing through the lens. Good stabilization of the lens current is essential for the best lens performance. Because gas molecules will cause a scattering of electrons, electron images are formed only in a high vacuum (less than 10^{-4} mmHg). Although commercial electron microscopes were available in 1939, it was not until the 1950s that their potential could be fully exploited for biological work. The breakthrough in this field centred on the preparation of ultra-thin sections of biological tissue by the use of glass knives and on the development of suitable staining, fixation and embedding materials. To prevent complete scattering of the electrons by the tissue, sections of the order of 20–200 nm are used and a buffered solution of osmium tetroxide is commonly employed for fixation and staining. Unstained cell components of a tissue have a fairly uniform electron scattering power, similar to that of the embedding medium, so that little contrast of the image is obtainable. However, the incorporation of electron-dense atoms (osmium) into the cell organelles enables a good degree of contrast to be obtained on the electron micrographs of the sections. There is, at present, no objective way of determining how much the fine structure of cells is altered by the fixation methods employed, but indirect correlation of the results obtained with those from other techniques is reassuring.

The light microscope gives magnifications of the order of ×1000 with a resolution, set by the wavelength of the light employed, down to about 0.2 μm for visible light; no further magnification of the image can increase the detail. The theoretical limit of resolution of the electron microscope is similarly governed by the wavelength of the electrons (about 0.003 nm) and in practice electron microscopes give resolutions to about 0.4 nm. Magnifications of ×10 000 to ×24000 are commonly employed and to show all the available detail on high-quality electron micrographs, prints at magnifications of around ×500 000 may be required.

Much knowledge of the detailed structure of the living cell has only been made possible by the advent of the electron microscope. For the routine examination of vegetable drugs the light microscope with polarizing attachment is generally fully adequate, but scanning electron micrographs at a much lower magnification than the above can be extremely useful for depicting structural details not obvious with the light microscope, for example, maize starch and digitalis (Fig. 23.17).

Drawings for publication and thesis work

For the detailed steps involved in the preparation of these line drawings, see earlier editions of this book.

PREPARATION OF DRUGS FOR MICROSCOPICAL EXAMINATION AND GENERAL USE OF REAGENTS

The following aims should be kept in mind for the microscopical examination of crude drugs.

1. The determination of the size, shape and relative positions of the different cells and tissues.
2. The determination of the chemical nature of the cell walls.
3. The determination of the form and chemical nature of the cell contents.

Dried material often requires softening by exposing it to a moist atmosphere (leaves) or by boiling in water (roots and barks). Botanical sections of the plant material may need to be made (cut either by hand or with a freezing microtome). Sections of the dry material may be necessary for the examination of mucilage or water-soluble cell components. Disintegration serves for the isolation of specific tissues and bleaching and defatting techniques for observing deeply coloured materials and fatty seeds respectively. Almost certainly, clearing reagents will be required together with a range of suitable stains for cell walls and cell contents.

Any report should state what characters appear to be of the greatest diagnostic importance and these should be illustrated by suitable sketches.

Distribution of tissues

A general idea of the distribution of tissues can be obtained by the examination of transverse and radial and tangential longitudinal sections. Such sections should first be mounted in water or dilute glycerin. Subsequently sections should be cleared by means of chloral hydrate or other clearing agents (see below) and some stained as follows.

Phloroglucinol and hydrochloric acid. Mount the section in a 1% solution of phloroglucinol in ethanol (90%) and allow to stand for about 2 min; remove any alcohol which has not evaporated with a piece of filter paper; add concentrated hydrochloric acid, cover and examine. All lignified walls stain pink or red.

Hydrochloric acid is a powerful clearing agent and it must be remembered that it will dissolve many cell contents, including calcium oxalate. The vegetable debris of catechu contains phloroglucinol and in this case the wood stains on the simple application of hydrochloric acid.

To prevent damage to the microscope either by liquid contact or by vapours, preparations mounted in concentrated hydrochloric acid should be free of excess acid and must be removed from the microscope stage as soon as possible.

Chlor-zinc-iodine solution. The reagent, often somewhat slowly, stains cellulose walls blue or violet, lignified or suberized walls yellow or brown, and starch grains blue.

Clearing, defatting and bleaching

Structures are frequently obscured by the abundance of cell contents, the presence of colouring matters and the shrinkage or collapse of the cell walls. Therefore, reagents are used for the removal of cell contents, for bleaching and for restoring as far as possible the original shape of the cell wall. If the microscopical examination is to be made from the section mounted in the clearing agent, the refractive index of the latter is important. It may be advisable to wash the section and mount in a different medium. The commonly used mountants glycerin, alcohol, carbolic acid, lactophenol, clove oil and Canada balsam all have some clearing effect. The following clearing and bleaching agents are particularly useful.

Solution of chloral hydrate. This dissolves starch, proteins, chlorophyll, resins and volatile oils, and causes shrunken cells to expand. Chloral hydrate may be used, not only for sections but also for whole leaves, flowers, pollen grains, etc. It does not dissolve calcium oxalate and is therefore a good reagent for detection of these crystals.

Solution of potash. Solutions of potassium hydroxide, both aqueous and alcoholic, up to a strength of 50% are used for different purposes, but for use as a clearing agent a 5% aqueous solution is most generally useful. A 0.3% solution of potash may be used to dissolve aleurone grains. A 5% solution is much more powerful, and rapidly dissolves starch, protein, etc., causing the swelling of cell walls. Potash should be washed out as soon as clearing is completed, as more prolonged action is liable to cause disintegration (see below).

Ether–ethanol. A mixture of equal parts of ether and ethanol (96%) is useful for the removal of fixed oils, fats, resins, volatile oils, tannins or chlorophyll. Defatting is particularly necessary in the case of oily seeds such as linseed and strophanthus.

Solution of sodium hypochlorite. This solution is useful for bleaching dark-coloured sections such as those of many barks and for removing chlorophyll from leaves. When bleaching is complete, the sections should not be left in the reagent but should be removed and washed with water. Prolonged contact with solution of chlorinated soda causes the removal of starch and lignin, which may not be desirable.

Disintegration and isolation of tissues

The use of reagents for purposes of disintegration is based on their action on the cell wall, particularly the middle lamella. Woody tissues are usually disintegrated by means of oxidizing agents, as these oxidize away the middle lamella, which is composed mainly of lignin. Thus, dilute nitric acid has a marked disintegrating effect on wood, whereas dilute sulphuric acid has not. The middle lamella of cellulose cells is composed of pectic substances which are made soluble by dilute acids or dilute alkalis, which thus effect disintegration. Pure celluloses, however, are resistant to hydrolysing and oxidizing agents, and the stability of cellulose in boiling 5% potash is made use of for the separation of cotton from wool. Other materials such as mannans, galactans, pectin, hemicelluloses, gums and lichenin, which may occur in the cell wall, are much more readily attacked by hydrolysing agents. It will thus be seen that the composition of the 'crude fibres' (i.e. those tissues which remain after the material has been subjected to the action of hydrolysing agents under controlled conditions) is likely to vary in both amount and chemical nature in different drugs. However, quantitative comparisons of the crude fibres of different samples of the same drug are useful.

Potassium chlorate and nitric acid. The strength of the reagent and the time it is allowed to act must be varied according to the nature of the material. For woods (e.g. quassia) the material, in small pieces or thick sections, is immersed in 50% nitric acid. *Minute* quantities of potassium chlorate are added at intervals to maintain an evolution of gas. From time to time a fragment of the wood should be removed and teased with needles. When it breaks up readily, it should be washed free from acid and examined. The process should not be continued longer than is necessary, since prolonged bleaching causes more or less complete destruction of the lignin.

Chromic acid and nitric or sulphuric acid. The reagent usually consists of a mixture of equal parts of 10% chromic acid and 10% nitric or sulphuric acid. It is frequently used for the disintegration of sclerenchymatous tissues such as the testas of capsicum and colocynth seeds or for the separation of lignified hairs such as those of nux vomica and strophanthus.

Solution of potash or soda. As mentioned above, alkalis are used both for clearing and disintegrating. The material is usually digested with 5% potash on a water-bath until the more resistant cells can be teased out of the more or less completely disintegrated parenchyma. The method is useful for the separation of the heavy cuticularized epidermis of leaves and for the isolation of secretory tissue such as the vittae of umbelliferous fruits and the latex vessels of lobelia. Suberized and cutinized tissues are very resistant to the potash. Potash is also useful for the isolation of lignified elements such as are found in the veins of leaves, in senna stalks and in many barks.

Preparation of a crude fibre

For qualitative work the following procedure may be adopted. Mix about 2 g of the drug, in No. 60 powder, with 50 ml of 10% nitric acid in a casserole. Bring to the boil and maintain at the boiling point for 30 s. Dilute with water and strain through a fine filter cloth held over the mouth of a filter funnel. Transfer the washed residue to the casserole and boil for a further 30 s with 50 ml of a 2.5% solution of sodium hydroxide. Collect and wash the residue as before, mount and examine. It will be found that the tissues disintegrate readily and are in a condition well suited to microscopical examination.

Reagents

Directions for making the following reagents, if not given below, will be found in the appendices of the *BP*. Some of the uses of each are mentioned, but further details will be found elsewhere.

Ethanol. Different strengths are used for preserving material and for hardening. Alcohol acts as a clearing agent by dissolving oils, resins, chlorophyll, etc. It does not dissolve gums and mucilages (therefore a useful mountant for drugs containing them).

Alkanna tincture. A supply sufficient for a few months only should be made by macerating 1 part of alkanet root and 5 parts of alcohol 90% for 1 week, afterwards filtering. Stains oils and fats and suberized and cuticularized walls.

Chloral hydrate and glycerin combines the properties of chloral hydrate and glycerin and is therefore useful for slow clearing without heat. Preparations mounted in it may be left for some days without undue evaporation.

Chloral hydrate solution BP (chloral 80 g, water 20 ml). A valuable and widely used clearing agent. See above.

Chromic acid solution. 25% Chromic acid in water. See 'Disintegration and isolation of tissues'.

Chromic and nitric acids solution. 10% Chromic acid and 10% nitric acid. See 'Disintegration and isolation of tissues'.

Clove oil. A useful clearing agent for powders containing much oil.

Cresol. A suitable mountant for chalks, kieselguhr, etc.

Chlor-zinc-iodine solution (syn. Schulze's solution). Prepared by adding a solution of zinc chloride (zinc chloride 20 g; water 8.5 ml) dropwise to a solution of potassium iodide (1.0 g) and iodine (0.5 g) in water (20 ml) until a precipitate of iodine forms which does not disappear on cooling. This requires about 1.5 ml. Used as test for walls containing celluloses. Iodine solution followed by sulphuric acid gives similar results.

Copper oxide, ammoniacal solution of, BP. This solution must be freshly prepared. It causes swelling and solution of cellulose walls. The balloon-like swellings produced in raw cotton are best observed if the reagent be diluted with an equal volume of distilled water. This solution is commonly known as cuoxam.

Corallin, alkaline solution of, BP (syn. Corallin-soda). Stains the callose of sieve-plates and some gums and mucilages.

Ether–ethanol. A defatting agent.

Ferric chloride solution; iron (III) chloride solution BP. See 'Tannins'.

Glycerin, dilute. One volume of glycerin is mixed with two volumes of distilled water. A useful mountant for preparations which may be left for some time, as it does not dry up. It has some clearing action, but is much inferior in this respect to chloral hydrate. It can usefully be added to a mount cleared with chloral hydrate solution to prevent the formation of crystals. It is not a good mountant for starch, as the grains tend to become transparent and striations, etc. are difficult to see; water is preferable.

Hydrochloric acid. This (density *c*. 1.18; *c*. 11.5 M) is used in testing silk and preparations containing colchicine, and with phloroglucinol as a test for lignin.

Iodine water, BP (see BP Iodine Solutions R1 and R2). This gives a blue colour with starch and hemicelluloses. Iodine Tincture *BP*, followed by sulphuric acid, resembles chlor-zinc-iodine (q.v.).

Lactophenol BP. See 'Clearing'.

Mercury–nitric acid solution BP (syn. Millon's Reagent). Test for protein-containing materials e.g. aleurone grains, wool and silk.

Nitric acid 10%. See 'Crude fibre'.

Phloroglucinol solution. A 1% solution in 90% ethanol with hydrochloric acid as a test for lignin.

Picric acid solution. A saturated solution in water which is used to stain aleurone grains and animal fibres.

Potash solution. A 5% solution is commonly used for clearing and disintegrating (q.v.) and for the separation of cotton from wool. A 50% solution is used in testing for chitin in ergot and for eugenol in clove. A 2.5% solution is used for preparing crude fibre (q.v.).

Potassium cupri-tartrate, solution of (syn. Fehling's Solution). Used in testing for reducing sugars such as glucose.

Potassium iodobismuthate R1. Precipitates with alkaloids.

Potassium tetraiodomercurate solution BP (syn. Mayer's Reagent). A precipitant for most alkaloids.

Potassium chlorate and nitric acid (syn. Schultze's macerating fluid). Produces bleaching, disintegration and delignification.

Ruthenium red, solution of, BP. Stains many gums and mucilages. It must be freshly prepared.

Sodium carbonate solution BP. This is useful for the disintegration of fibres such as flax, where the use of an oxidizing agent is not required.

Sodium hypochlorite solution. The *BP* includes a strong and weak solution; for use see 'Clearing, defatting and bleaching'.

Sudan III (Sudan red) solution. A solution in equal parts of glycerin and alcohol, stains oils and suberized walls, and is useful in the examination of secretory cells and ducts.

Sulphuric acid 80%. Concentrated sulphuric acid causes rapid charring, but dilutions containing 80% or less form useful reagents. The behaviour of cotton, wool, chalks, calcium oxalate and sections of strophanthus seeds should be noted. The acid dissolves cellulose and lignified walls, but has little action on suberin.

Water, distilled. A useful mountant for starches. Sections which have been bleached with solution of chlorinated soda or similar reagent may be freed from the bubbles of gas which they frequently contain by placing them in freshly boiled distilled water.

POWDERED DRUGS

The systematic approach to the identification of powdered drugs can proceed in a number of ways; for organized drugs, however, all methods depend on the microscopical recognition of characteristic cell types and cell contents. Identification can then be made by reference to tables and appropriate illustrations, by the use of punched cards, and by employing a suitable computer program. For dealing with mixtures of drugs, greater skills and practice are required and only the first of the above approaches is applicable. When a tentative identification has been made, further confirmatory observations and chemical tests can be performed; most pharmacopoeial drugs now have TLC tests for identity.

Preliminary tests

1. Note the colour. *White*: acacia, tragacanth; *light yellow*: colocynth, peeled liquorice, ginger, quassia, squill; *light brown*: ipecacuanha, unpeeled liquorice, nux vomica, opium, fennel, gentian, cascara, coriander, cardamoms, jalap, linseed, aloes; *cinnamon brown*: cinnamon, catechu; *dark brown*: clove, Curaçao aloes; *dark reddish-brown*: nutmegs; *violet*: ergot; *red*: cinchona; *orange*: rhubarb; *pale green*: lobelia; *green*: henbane, belladonna, stramonium, senna, digitalis.
2. Note odour. The following are particularly characterized: ginger, fennel, gentian, opium, coriander, cardamoms, cinnamon, clove, nutmegs.
3. Taste (NB: Students should *not* taste powdered drugs without the consent of their supervisor. Adulterated or spoiled drugs may be harmful, others such as capsicum too pungent to taste, and alkaloid-containing drugs poisonous). *Aromatic*: coriander, cardamoms, cinnamon, clove, nutmegs; *aromatic and pungent*: ginger;

bitter: colocynth, quassia, nux vomica, gentian, aloes, squill, cinchona, rauwolfia; *sweet*: liquorice; *astringent*: catechu; *mucilaginous*: marshmallow root, slippery elm bark, ispaghula husk.

4. Mix a small quantity of the powder with a few drops of water and allow to stand. Aqueous extracts and inspissated juices such as catechu and aloes dissolve almost completely, while the gummy or mucilaginous nature of drugs such as acacia, tragacanth and linseed becomes apparent.

   Mix a small quantity of the powder with dilute sulphuric acid. Effervescence followed by solution occurs with chalk.
5. Press a small quantity of the powder between filter paper. An oily stain, spreading but persisting when the paper is heated in an oven, occurs with powders containing fixed oil. Volatile oil will give a stain, disappearing on heating in an oven.
6. Shake a little powder in half a test-tube-full of water, and if any marked frothing occurs, suspect saponin-containing drugs; boil gently and note the odour of any volatile oil evolved. Filter and divide into two portions which may be tested for tannins and for anthraquinone derivatives as follows.

   (1) Test for tannin (see Chapter 21, 'Tannins'). *Tannins absent* from quassia, squill, strophanthus, capsicum and ginger; *gallitannins present* in cloves and rhubarb; *phlobatannins present* in catechu, krameria, prunus serotina, cinnamon and cinchona.

   (2) Test for anthraquinones by shaking the aqueous extractive with ether, separating and adding to the ethereal solution about one-third of its volume of ammonia. After shaking, a pink colour is obtained in the aqueous layer with rhubarb, cascara and senna.

Microscopical examination

If the preliminary tests have shown that the drug dissolves or becomes mucilaginous in water, trials should be made with other liquids such as alcohol, olive oil or lactophenol until one is found in which the drug is insoluble. In most cases, however, the following procedure may be adopted.

Examination for starch. Mount in water, examine and sketch any granules observed and prove whether they are starch or not by irrigation with iodine water. Do not waste time trying to find other structures that are best seen in the following mountants.

Examination for epidermal trichomes and calcium oxalate solution. Mount in chloral hydrate solution, boil gently until clear, and examine. To ensure that calcium oxalate, if present in small quantity, is not overlooked, polarized light may be used.

Examination for lignin. Moisten the powder with an alcoholic solution of phloroglucinol and allow to stand until nearly dry; add concentrated hydrochloric acid, apply a cover-glass, and examine. Note the presence or absence of lignified vessels, fibres, parenchyma, sclereids or hairs (e.g. nux vomica). If vessels or fibres do not stain pink, suspect rhubarb or ginger.

A considerable amount of information should have been derived from the preliminary tests and the above three mounts, and the examination may be continued by the application of further microchemical tests (chlor-zinc-iodine, tincture of alkanet, ruthenium red, corallin soda, etc.). It is often advisable to defat oily powders, to bleach highly coloured ones and to prepare a crude fibre of those powders containing much starch.

Microscopical measurements of cells and cell contents should be made whenever possible and compared with those published in the literature.

The application of a knowledge of the anatomy of those plant organs occurring in drugs should make possible a recognition of the organs represented. This should be followed by reference to an atlas of vegetable powders or tables of the diagnostic characters of powdered drugs. The identity of a powder should not be regarded as established until it has been compared with one of known authenticity.

A series of tables to assist with the identification of powdered drugs, based on the presence or absence of starch, epidermal trichomes and calcium oxalate, will be found in earlier editions of this book (up to and including the 13th edition). Tables of histological characters of drugs are also given in Wallis's *Textbook of Pharmacognosy* (1967); in these a morphological arrangement is employed so that in order to commence the identification process the student must first decide to which group his unknown powder belongs. For the appropriate illustrations of the elements present in powdered drugs see Jackson and Snowdon (1990) in the literature cited in Chapter 2.

Computer-assisted identification of powdered drugs

In 1976 Jolliffe and Jolliffee published details of a computer program for the identification of 174 powdered drugs of organized structure (*Analyst*, **101**, 622). Further elaboration of the method followed as indicated in the literature quoted below. The procedure involves the examination of a single unknown powder in four different mountants for the presence or absence of eleven histological characters, namely, calcium oxalate (if present, type of crystal), aleurone, cork, lignified parenchyma, stomata (if present, which of six types), trichomes (if present, type and structure), vessels/tracheids (if present, lignified or non-lignified), sclereids, fibres, starch and pollen. The information is coded as a string of characters comprising six blocks of five digits, each of which indicates the absence or presence of the microscopical characters being examined. Processing involves a validity check of the input data and a comparison of the observed characters for the unknown with those of the 174 powdered drugs stored in the data bank. The output then gives those drugs having zero errors compared with the input followed by those having 1, 2, etc., errors. Allowance is made in the program for the fact that a particular feature of the drug might be present in such small amount that it could be missed by the inexperienced microscopist. For drugs where the computer cannot give a unique identification, simple distinguishing tests are suggested e.g. the measurement of starch grains and fibres for cassia and cinnamon. Contamination of the drug with moulds, mites, etc. does not affect the identification and the presence of additional material in the powder affording one characteristic only, e.g. starch, is accommodated.

In addition to organized single powders it should be noted that computer-aided identification programs have also been described for unorganized drugs, homoeopathic tinctures, textile and surgical dressing fibres, and food materials (see below).

Further reading

Bannerman HJ, Cox BJ, Musset JH 1982 Computer-assisted identification of unorganised drugs. Pharmaceutical Journal 228: 716–717 [CAIDUD]

Jolliffe GH, Jolliffe GO 1978 Microcomputer-aided identification of powdered vegetable drugs. Pharmaceutical Journal 221: 385–386 [POWDERS]

Jolliffe GH, Jolliffe GO 1979 'MICROAID'. Practical Computing 2: 120–132

Jolliffe GH, Jolliffe GO 1989 The microcomputer as an analytical aid in drug microscopy. In: Trease and Evans' Pharmacognosy (13th edition). Baillière Tindall, London, UK, pp 784–798

Stevens RG 1980 Computer-aided identification of textile and surgical dressing fibres. Pharmaceutical Journal 223: 293–294 [FIBRES/BAS]

QUANTITATIVE MICROSCOPY

In addition to the simple measurement of the sizes of tissues, cells and cell contents by means of the micrometer eyepiece or camera

lucida, it is possible to estimate the percentage of foreign organic matter in many powdered drugs by a lycopodium spore method which was developed by T. E. Wallis (Analytical Microscopy, 3rd ed., 1965) and subsequently adopted by the *Pharmacopoeia*. Although the method appears now to be little used, the principles involved, as cited below, are of interest. Other microscopical determinations which may usefully be made in certain cases are vein-islet numbers, palisade ratios, stomatal numbers and stomatal indices.

Lycopodium spore methods

Wallis showed that lycopodium spores are exceptionally uniform in size (about 25 μm) and that 1 mg of lycopodium contains an average of 94 000 spores. The number of spores mg^{-1} was determined by direct counting and by calculation based on specific gravity and dimensions of the spores. The methods gave values in good agreement. These facts make it possible to evaluate many powdered drugs, provided that they contain one of: (1) well-defined particles which may be *counted* (e.g. pollen grains or starch grains); or (2) single-layered tissues or cells the *area* of which may be traced at a definite magnification and the actual area calculated; or (3) characteristic particles of uniform thickness, the length of which can be measured at a definite magnification and the actual length calculated. Whichever method is adopted, mounts containing a definite proportion of the powder and lycopodium are used and the lycopodium spores are counted in each of the fields in which the number or area of the particles in the powder is determined. The method is somewhat laborious and has not been subjected to statistical assessment. For powdered pharmacopoeial drugs reliance is now placed on other methods. Details can, however, be found in the *BP* 1973 and earlier editions of this book. Classical examples of drugs to which it was applied are senna and linseed (area measurements), nux vomica (trichome-rib lengths) and pypethrum and ginger (counts).

Leaf measurements

A number of leaf measurements are used to distinguish between some closely related species not easily characterized by general microscopy.

Palisade ratio. The average number of palisade cells beneath each upper epidermal cell is termed the *palisade ratio*. Quite fine powders can be used for the determination.

Pieces of leaf about 2 mm square, or powder, are cleared by boiling with chloral hydrate solution, mounted and examined with a 4 mm objective. A camera lucida or other projection apparatus is arranged so that the epidermal cells and the palisade cells lying below them may be traced. First a number of groups each of four epidermal cells are traced and their outlines inked in to make them more conspicuous. The palisade cells lying beneath each group are then focused and traced. The palisade cells in each group are counted, those being included in the count which are more than half-covered by the epidermal cells; the figure obtained divided by 4 gives the palisade ratio of that group. The range of a number of groups from different particles should be recorded.

Drugs for which palisade ratios have been utilized include belladonna, stramonium, buchu, senna, digitalis and, for recognition as adulterants, xanthium and phytolacca.

Stomatal number. The average number of stomata per square millimetre of epidermis is termed the *stomatal number*. In recording results the range as well as the average value should be recorded for each surface of the leaf and the ratio of values for the two surfaces.

Fragments of leaf from the middle of the lamina are cleared with chloral hydrate solution or chlorinated soda. Timmerman counted the number of stomata in 12–30 fields and from a knowledge of the area of the field was able to calculate the stomatal number; the camera lucida method described for vein-islet numbers may also be used, the position of each stoma being indicated on the paper by a small cross.

Using fresh leaves, replicas of leaf surface may be made which are satisfactory for the determination of stomatal number and stomatal index. An approximate 50% gelatin and water gel is liquefied on a water-bath and smeared on a hot slide. The fresh leaf is added, the slide is inverted and cooled under a tap and after about 15–30 min the specimen is stripped off. The imprint on the gelatin gives a clear outline of epidermal cells, stomata and trichomes.

The early investigations by Timmerman (1927) indicated that stomatal numbers are usually useless for distinguishing between closely allied species, but that in certain cases the ratio between the number of stomata on the two surfaces may be of diagnostic importance. It is possible, for example, to distinguish *Datura innoxia* from other species of *Datura* by this means.

Stomatal index. The percentage proportion of the ultimate divisions of the epidermis of a leaf which have been converted into stomata is termed the *stomatal index*:

$$I = \frac{S \times 100}{E + S} \times 100$$

where S = number of stomata per unit area and E = number of ordinary epidermal cells in the same unit area. While stomatal number varies considerably with the age of the leaf, stomatal index is highly constant for a given species and may be determined on either entire or powdered samples. It is employed in the *BP* and *EP* to distinguish leaflets of Indian and Alexandrian sennas.

Pieces of leaf other than extreme margin or midrib are suitably cleared and mounted, and the lower surface examined by means of a microscope with a 4 mm objective and an eyepiece containing a 5 mm square micrometer disc. Counts are made of the numbers of epidermal cells and of stomata (the two guard cells and ostiole being considered as one unit) within the square grid, a cell being counted if at least half of its area lies within the grid. Successive adjacent fields are examined until about 400 cells have been counted and the stomatal index value calculated from these figures. The stomatal index may be determined for both leaf surfaces.

Rowson (1943 and 1946) found that stomatal index values may be used to distinguish between leaves of co-generic species (Table 43.1).

Vein-islet number. The term 'vein-islet' is used to denote the minute area of photosynthetic tissue encircled by the ultimate divisions of the conducting strands. The number of vein-islets mm^{-2} calculated from four contiguous square millimetres in the central part of the lamina, midway between the midrib and the margin, is termed the *vein-islet number*. When determined on whole leaves, the area examined should be from the central part of the lamina, midway between the margin and midrib.

Many leaves may be cleared by boiling in chloral hydrate solution in a test-tube placed in a boiling water-bath. Those which are difficult to clear in this way may, after soaking in water, be treated successively with sodium hypochlorite to bleach, 10% hydrochloric acid to remove calcium oxalate, and finally chloral hydrate.

A camera lucida or projection apparatus is set up and by means of a stage micrometer the paper is divided into squares of 1 mm^2 using a 16 mm objective. The stage micrometer is then replaced by the cleared preparation and the veins are traced in four contiguous squares, either in a square 2 mm × 2 mm or a rectangle 1 mm × 4 mm (Fig. 43.2). When counting, it is convenient to number each vein-islet on the tracing. Each numbered area must be completely enclosed by veins, and

Table 43.1 Stomatal index values.

Species	Stomatal index	
	Upper surface	Lower surface
Atropa acuminata	1.7 to **4.8** to 12.2	16.2 to **17.5** to 1.83
Atropa belladonna	2.3 to **3.9** to 10.5	20.2 to **21.7** to 23.0
Cassia senna	11.4 to **12.4** to 13.3	10.8 to **11.8** to 12.6
Cassia angustifolia	17.1 to **19.0** to 20.7	17.0 to **18.3** to 19.3
Datura inermis	18.1 to **18.3** to 18.7	24.5 to **24.9** to 25.3
Datura metel	12.7 to **17.4** to 19.4	21.2 to **22.3** to 23.9
Datura stramonium	16.4 to **18.1** to 20.4	24.1 to **24.9** to 26.3
Datura tatula	15.6 to **20.2** to 22.3	28.3 to **29.8** to 31.0
Digitalis lanata	13.9 to **14.4** to 14.7	14.9 to **16.1** to 17.6
Digitalis lutea	2.5 to **5.5** to 8.4	21.6 to **22.9** to 25.2
Digitalis purpurea	1.6 to **2.7** to 4.0	17.9 to **19.2** to 19.5
Digitalis thapsi	5.9 to **7.0** to 7.8	11.9 to **12.4** to 13.5
Erythroxylum coca	Nil	12.2 to **13.2** to 14.0
Erythroxylum truxillense	Nil	8.9 to **10.1** to 10.7
Phytolacca acinosa	Nil	**15.0**
Phytolacca americana	2.9 to **4.2** to 5.7	13.0 to **13.2** to 13.4

Fig. 43.2
The vein-islets of 4 mm² of the leaf of *Erythroxylum truxillense.* (After Levin.)

Table 43.2 Vein-islet numbers.

	Species	Range of vein-islet numbers	Average
Senna	*Cassia senna*	15–29.5	26
	Cassia angustifolia	19.5–22.5	21
Coca	*Erythroxylum coca*	8–12	11
	Erythroxylum truxillense	15–26	20
Digitalis	*Digitalis purpurea*	2–5.5	3.5
	Digitalis lanata	2–3.5	2.7
		3–8	4.4
	Digitalis lutea	1–1.5	1.2
	Digitalis thapsi	8.5–16	

Table 43.3 Veinlet termination numbers.

Atropa acuminata	1.4–3.5
Atropa belladonna	6.3–10.3
Cassia angustifolia	25.9–32.8
Cassia senna	32.7–40.2
Datura stramonium	12.6–20.1
Digitalis purpurea	2.5–4.2
Erythroxylum coca	16.8–21.0
Erythroxylum truxillense	23.1–32.3
Hyoscyamus niger	12.4–19.0

those which are incomplete are excluded from the count if cut by the top and left-hand sides of the square or rectangle but included if cut by the other two sides. For example, the vein-islets in Fig. 43.2 total 62 and the vein-islet number is therefore 15.5.

The vein-islet number frequently serves to distinguish closely related plants (Table 43.2).

Veinlet termination number. Hall and Melville (1951) determined veinlet termination number, which they define as 'the number of veinlet terminations per mm² of leaf surface. A vein termination is the ultimate free termination of a veinlet or branch of a veinlet'. By this character they distinguished between Peruvian and Bolivian coca leaves and between Alexandrian and Tinnevelly senna leaflets. The values are recorded in Table 43.3.

One practical difficulty in the measurement of vein-islet and veinlet-termination numbers is deciding exactly where, and if, a veinlet terminates. This may appear to vary according to the preliminary treatment a leaf has received.

At present, of the above leaf measurements, only stomatal index is employed officially. With the increasing number of whole herbs and leaves now being introduced into the *European* and *British Pharmacopoeias* and the need for standardization of the many herbal products of interest world-wide, a further investigation of the-possible usefulness of these leaf measurements might prove rewarding. For acceptance, the results from any future measurements would necessitate a more sophisticated statistical analysis than was probably afforded the examples quoted above.