RESEARCH PAPERS

VALERIANA OFFICINALIS LINN., ITS POLYPLOID FORMS AND THE STRUCTURE OF THEIR RHIZOMES AND ROOTS

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Skalinska[1] has noted that recent work has revealed the occurrence in Europe of three chromosomal forms of *Valeriana officinalis* Linn. The diploid form has been reported from the Continent only[2], whereas tetraploid and octoploid forms occur in Britain as well as upon the Continent. The present communication is a record of a histological study of tetraploid and octoploid forms, authentic specimens of which were provided by Skalinska. The work was further extended by examining a number of specimens grown for commercial use and others consisting of the dried rhizome and roots sold as "valerian root" of commerce for the preparation of medicines.

Materials

The materials used are as follows:—

1. Authentic samples of *Valeriana officinalis* Linn., tetraploid and octoploid forms, from Mme. Skalinska of the Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey.
2. Samples of fresh rhizome from Harold Deane, Esq., of Messrs. Stafford Allen and Co., Long Melford, Suffolk.
3. Fresh samples grown by T. E. Wallis at Mill Hill, London, N.W.7, for several successive seasons; the parent plant was obtained from H. Deane, Esq., of Long Melford.
4. Sample from Switzerland, Botanical Garden, Zurich.
5. Samples of leaves and flowers from herbarium sheets in the Herbarium at Kew Gardens, through Dr. C. R. Metcalfe.
6. Samples from the Museum of the Pharmaceutical Society of Great Britain.
7. Five commercial samples of "Valerian, B.P." from different wholesale houses in England.

Description of the Rhizome

The fresh rhizome grows vertically in the ground, it is bluntly cylindrical-obconical, about 3 cm. long and 2 cm. wide at the crown. Horizontal stolons arise in the axils of the scale leaves and bear other rhizomes at their extremities, some of the stolons are very short, so that three or four rhizomes often become grouped together to form a rather dense mass. The longer stolons may attain a length of about 10 cm. and a diameter

* The subject-matter of this communication formed part of a Thesis by one of us (P.K.S.) accepted for the degree of Ph.D. in the University of London.

of 5 mm.; they frequently ascend at the tips and grow out to form aerial stems; they have intermodes about 5 mm. long near the parent rhizome, but as much as 15 mm. in the more distal part; scale leaves and sometimes also adventitious roots occur at the nodes from some of which branch stolons may arise and these also may grow out to form aerial shoots. Very numerous pale-buff adventitious roots arise from all parts of the surface of the rhizome except the actual summit; they are about 15 to 20 cm. long and 2 to 3 mm. thick; the majority travel horizontally for a distance of about 10 cm. and then curve downwards; roots from the lowest part of the rhizome are not directly vertical but somewhat oblique. The apical 2 to 5 cm. of each root is more slender and has numerous fibrous branches.

The fresh rhizome has no valerianic odour but when broken or squeezed, a slight aromatic odour is perceptible; it has a slightly pungent aromatic taste.

Commercial valerian, sold as a drug, is dull brownish grey and consists of the dried rhizomes with stolons and and attached roots. To facilitate drying the larger rhizomes are cut longitudinally, more rarely transversely, into 2 or 4 pieces and show the same general characters as the fresh rhizomes. They are, however, much shrunken and in the pieces cut longitudinally the exposed pith may show about 4 to 9 transverse diaphragms separated by spaces or lacunae (see Fig. 1, *G* and *H*). The roots are so numerous that there is hardly any free surface between their bases at the surface of the rhizome; they are about 2 mm. in diameter, brittle and much curved and twisted and are longitudinally wrinkled. The smaller rhizomes are entire and many of the roots are broken off and lie loose in the drug. The stolons are reddish brown, much shrunken and about 2 mm. in diameter. Numerous small circular scars are present on the rhizomes and also upon the nodes of the stolons where roots have been broken off. The drug is hard and breaks with a short horny fracture; it has a strong characteristic odour and a sweetish, camphoraceous and ultimately slightly bitter taste.

Origin of the Lacunae

The microscopy of the rhizome has been well described by several pharmacognosists, the most detailed account being that of Moll and Janssonius[3]. An item of interest which does not appear to have been described is the mode of origin of the large horizontal disc-shaped lacunae in the pith. The large parenchymatous pith contains an abundance of starch; the cells measure, R and T* 48 to **75** to 115 μ and L 61 to **80** to 86 μ. In the middle part of the pith of older rhizomes there is a vertical series of lacunae. Each lacuna originates by the development of isolated cells without visible contents, followed by their multiplication and finally by the rupture of their cells walls (see Fig. 2). The large space or lacuna thus formed extends almost across the pith in the position

* When recording measurements, the letters L, R and T are used to indicate measurements in a longitudinal, radial and tangential direction, respectively, the directions having reference to the axis of the relevant plant member.

of one of the short internodes of the rhizome; between the lacunae diaphragms of parenchyma remain in the position of the nodes; young rhizomes are devoid of lacunae and diaphragms. The formation and position of these structures is shown in Fig. 1, *H*.

Sclereids of the Pith

The rhizomes of the tetraploid and octoploid forms are closely similar in their structure. Though the cells of the pith and cortical parenchyma are rather larger in octoploid than in tetraploid forms there is not sufficient

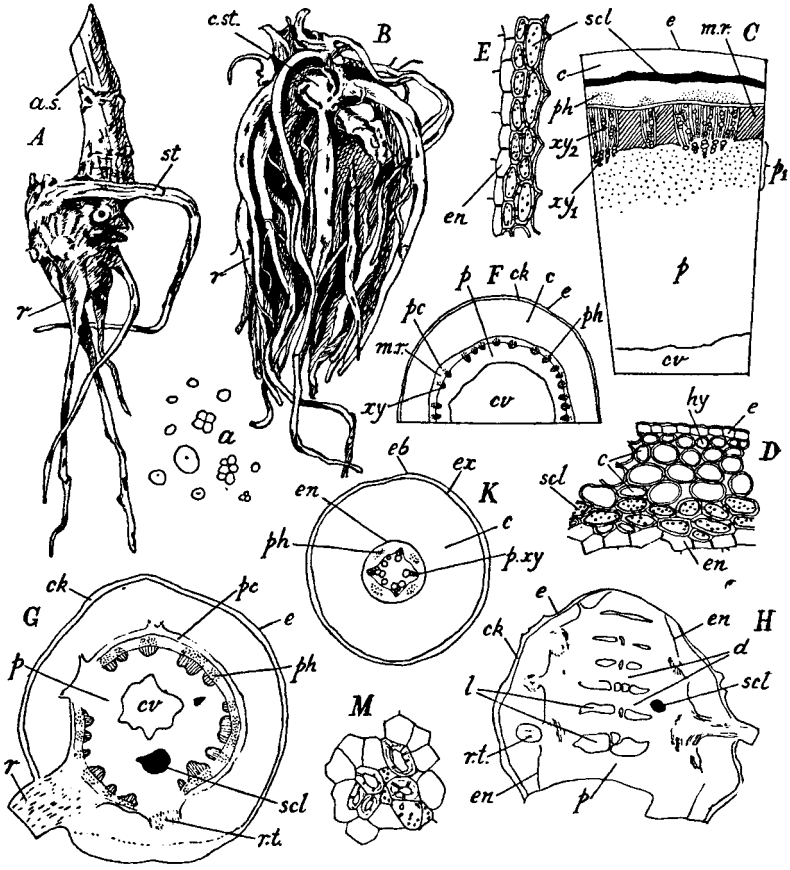

FIG. 1. *Valeriana officinalis* Linn. *A* and *B* dried commercial rhizomes, × 1; *C*, diagram of a transverse section of an aerial stem, × 6; *D*, outer tissues of stem in T.S., × 50; *E*, sclerenchyma and endodermis of stem in L.S., × 50; *F*, diagram of T.S. of a stolon, × 5; *G*, diagram of T.S. of a rhizome, × 5; *H*, diagram of L.S. of a rhizome, × 5; *K*, diagram of T.S. of a root, × 25; *M*, small group of sclereids from pith of a rhizome, × 35; *a*, starch, × 200; *a.s.*, aerial stem; *c*, cortex; *ck*, cork; *c.st.*, scar left by stolon; *cv*, cavity; *d*, diaphragm; *e*, epidermis; *eb*, epiblema; *en*, endodermis; *ex*, exodermis; *hy*, hypodermis; *l*, lacuna; *m.r.*, medullary ray; *p*, pith; p_1, lignified part of pith; *pc*, pericycle; *ph*, phloem; *p.xy*, protoxylem; *r*, root; *r.t.*, root trace; *scl*, sclereids; *st.*, stolon; *xy*, xylem; xy_1, primary xylem; xy_2, secondary xylem.

difference to be of real diagnostic value. The starch grains also have a maximum size of 12 to 14 μ in octoploid and of 8 to 10 μ in tetraploid plants. The difference of sizes of these cells and starch granules is of the same order as the error of measurement, so that they are useless as criteria for differentiation of the varieties. There is, however, one feature of the pith which possesses a well-defined diagnostic significance; this is the presence of groups of sclereids in the diaphragms of the pith of tetraploid plants (see Fig. 1, *G*, *H* and *M*) and their absence from octoploids. These sclereids are strongly lignified, pitted and heavily thickened, individual sclereids are isodiametric and measure about 30 to **50** to 60 μ in diameter. See Fig. 1, *M*.

These sclereids are briefly referred to by Karsten and Beneke[4] and by Gilg, Brandt and Schürhoff[5], but these authors do not say where they occur in the rhizome. They have been described and figured by Tschirch and Oesterle[6], by Flück and Haller[7] and by Flück, Schlumpf and Siegfried[8], all of whom note their occurrence in the diaphragms of the pith. Tschirch and Oesterle also state that when found in the powder, they indicate the presence of rhizome. It is also evident that all these authors have examined tetraploid plants.

Histology of the Root

(a) *Roots from Plants of the Current Season* (see Fig. 3)

Externally is a piliferous layer consisting of small subrectangular cells measuring about R and T 6 to **30** to 40 μ and L 40 to **60** to 102 μ. The outer walls are dome-shaped, yellowish, cuticularised and slightly lignified, the thickening of the wall showing striations due to layering of the secondary deposit; they may be prolonged as papillae or as short or long unicellular root-hairs which are from 20 to **53** to 82 μ long and 12 to 20 μ wide.

The exodermis over the greater part of the root consists of a single layer of large polygonal tabular cells; sometimes becoming locally increased, in the one to 3 centimetres nearest to the rhizome, to 2 to 4 layers over about one-eighth to one-quarter of the circumference, Figure 3, *E*. The cells have thin, suberised and lignified walls and are fairly uniform in size measuring R 20 to **41** to 53 μ, T 32 to **60** to 70 μ and L 32 to **60** to 82 μ. The cells of the exodermis contain globules of volatile oil, but occasional cells, scattered at irregular intervals, are nearly cubical in shape, about 35 to 40 μ across, and contain granular contents, which stain with Sudan-red and also stain deep brown with iodine and sulphuric acid.

The cortex consists of about 15 to 28 layers of cells; the outer 2 or 3 layers, just within the exodermis, are collenchymatous and smaller in size than the cells in the middle part of the cortex. Collenchymatous cells are prominent in the sections cut from the portion nearer to the rhizome where the roots have become fully developed. All the cells of the cortex contain abundance of starch and the walls are cellulosic. The measurements of the outer collenchymatous cells are R 16 to 40 μ, T 28 to 50 μ and L 8 to **88** to 135 μ; the larger cells of the middle region measure R 33 to **53** to 70 μ, T 40 to **60** to 70 μ and L 48 to **112** to 155 μ,

while those immediately outside the endodermis are about the same size as the collenchymatous cells. There are a few isolated cells whose contents become brownish with iodine and sulphuric acid, but these cells were not found in all sections.

The endodermis consists for the greater part of one layer of prominent regularly arranged cells with bright casparian strips; at places, over a very limited area, the endodermis consists of two layers of cells. The

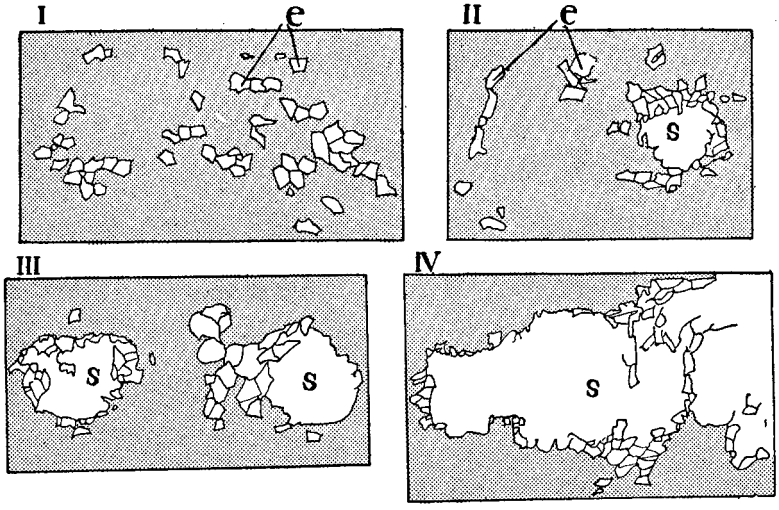

FIG. 2. *Valeriana officinalis* L., Rhizome. I, II, III and IV successive stages in the development of the lacunae between the diaphragms of the pith; all × 25. The shaded areas consist of parenchyma containing starch, e, cells without visible contents.

cell walls are suberised and take a reddish or brownish stain with Sudan-red. A very few small droplets of oil have been found in some sections, and there are some cells with granular contents which may take a black stain with iodine, but become brown on the further addition of sulphuric acid. The dimensions of the cells are R 12 to **20** to 28 μ, T 20 to **45** to 49 μ and L 32 to 50 μ. By treatment with iodine and sulphuric acid the endodermal layer becomes more prominent, the walls take a brown colour and passage cells can be seen opposite to the protoxylem groups and occasionally in other positions also.

The pericycle consists of 1 to 3 layers of large parenchymatous cells, which are sometimes slightly collenchymatous. The walls are of cellulose and the cells measure R 16 to **20** to 24 μ and T 24 to 32 μ.

The roots are at first mostly tetrarch, but sections taken from the portion nearest to rhizome show a polyarch structure with up to 19 bundles which may be discrete or more or less united into groups by slight secondary development of xylem vessels. Generally nearest to the rhizome, due to the secondary growth of xylem, the protoxylem comes to lie at the middle of each xylem group. If sections are taken at every 2·5 cm. of a long root, beginning from the rhizome, the number of bundles

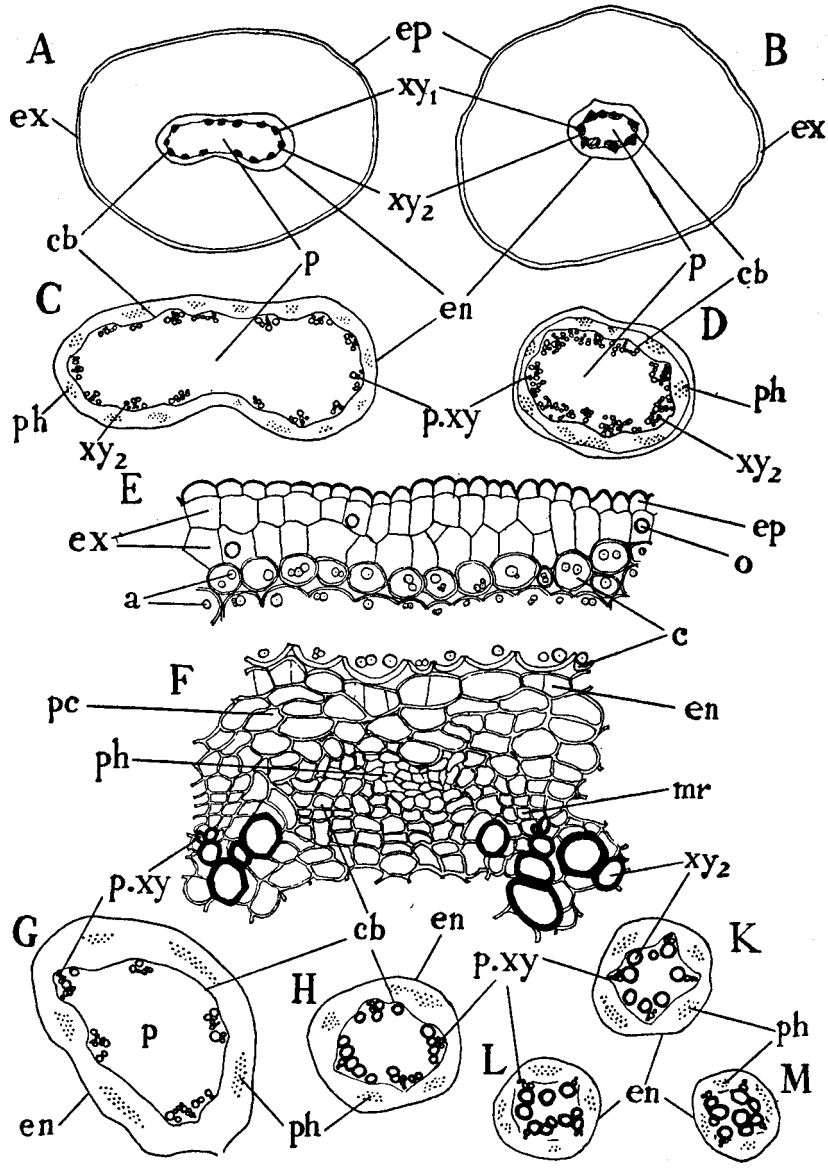

FIG. 3. *Valeriana officinalis* L., Root. *A*, diagrammatic transverse section of a long root, showing an oval stele × 10; *B*, diagrammatic transverse section of an externally similar root showing a nearly circular stele × 10. Both *A* and *B* were cut within 1·0 cm. of the rhizome. *C*, and *D*, steles from *A*, and *B*, respectively, both × 25. *E*, portion of the outer cortex from the same root as *G*, showing part of the exodermis where much of it has become 2 layered × 100. *F*, details of two xylem bundles and one phloem bundle from *A*, × 200. *G*, *H*, *K*, *L*, and *M*, diagrams of the steles of 5 successive transverse sections of the same root cut at distances of 1, 2, 3, 5 and 7 inches respectively measured from the rhizome, showing the gradual diminution in size of the pith until it practically disappears, all × 50. *a*, starch; *cb*, cambium; *c*, cortex; *en*, endodermis; *ep*, epiblema; *ex*, exodermis; *mr*, medullary ray; *o*, oil globule; *p*, pith; *pc*, pericycle; *ph*, phloem; *p.xy*, protoxylem; xy_1, primary xylem; xy_2, secondary xylem.

becomes gradually less till near the tip it is only four. Excluding the very small vessels of the protoxylem, the majority of the vessel-elements measure R and T 20 to **36** to 50 μ and L 164 to **205** to 287 μ. The phloem groups also increase by secondary development and nearest to the rhizome the phloem groups with the medullary rays between them form an almost continuous narrow ring outside the xylem. The medullary rays extending from the tips of protoxylem groups to the cortex are very clearly seen when stained with iodine and sulphuric acid since they take a contrasting blue colour. Starch grains in small numbers are found in the pericycle and phloem, but not in all the cells. In the older parts of the roots, the cambiform tissue consists of 3 to 4 layers of cells, the walls of which become light blue and the contents yellow with iodine and sulphuric acid.

Towards the tip of the root where it is usually tetrarch, there is no pith, though there may be one or two parenchymatous cells between the vessels of opposite bundles, Figure 3, *M*. In older parts of the root, as the number of xylem bundles increases, the parenchymatous cells also multiply so that near the rhizome a well marked pith is usually present; see Figure 3, *G*. The cells of the pith measure from R and T 24 to 32 μ and about L 40 μ, they have cellulose walls and contain starch in smaller amount than the cells of the cortex. Some of the root sections show steles of oval shape while others are nearly circular, Figure 3, *A* and *B*. Foder and Kichler[9] have stated that the Japanese variety of valerian can be distinguished from the European by the shape of the central cylinder; that of the Japanese valerian is oval while in European valerian it is round. However, since the roots of several of samples from England possess oval steles in the 4 cm. nearest the rhizome, this alleged distinction is not valid.

As there are two forms of British valerian, having octoploid and tetraploid nuclei respectively, the structure of roots from three tetraploid and four octoploid samples was examined to search for any anatomical features by which the two forms may be distinguished. No clearly marked differences in cell structure of the roots from the two forms could be discovered by which they can be certainly distinguished. However, one can obtain an indication of the degree of polyploidy by determining the maximum size of the starch grains present in the roots. For this purpose, softened roots (stored in glycerol 1 volume, water 3 volumes) were scraped with a needle and the starch so removed was mounted in lacto-phenol; the largest grains were selected by eye in a systematic search of the mount under a 16 mm. objective and as each was noted, its size was accurately determined under a 4 mm. objective. Proceeding in this way one finds that starch of the tetraploids has a maximum size of 18 μ, while that of the octoploids attains a maximum of 30 μ.

(b) *Roots from Rhizomes of Last Season, after the Aerial Plant has Fully Developed, Flowered and Fruited and Died Down*

Transverse sections of roots from old rhizomes showed a typical secondary development of the xylem and other tissues. At the centre

is a pith resembling that of the roots from daughter rhizomes; or sometimes a large central hollow has replaced much of the pith which is surrounded by a wide band of xylem having a width almost 1½ times the diameter of the pith. Medullary rays traverse the secondary xylem, one opposite to each protoxylem group, and the cells of the rays have become both thickened and lignified; the parenchyma of the xylem is also thickened and lignified so that a very hard core of xylem is formed. Outside the xylem is a cambium and a narrow band of phloem. The cortex is almost twice the width of the ring of xylem and is similar in structure and contents to that of the roots attached to rhizomes of the present season, see Figure 4. The great majority of the roots on the old parent rhizome show these features, but there are a very few roots, apparently formed during the current season, which resemble those of the daughter rhizome, as one would expect. Starch is equally abundant in the cortex of both old and young roots. The roots from tetraploid and octoploid plants are similar in structure and distribution.

The structure of the roots is described somewhat fully because Tschirch[10] has stated that two types of roots are present in valerian; Neuber[11] and Tschirch[12] have suggested that the roots should be named "hold-fast" and "storage or nourishing" roots respectively. Tschirch and Neuber further suggest that the presence of two distinct types of root is to be found in many plants. In valerian, however, it seems to be obvious that the difference is merely one of the age and that two physiologically differentiated types do not exist (see Fig. 4).

This conclusion is in agreement with the finding of Flaskamper[13], who states that his investigations show that the occurrence of "nourishing" and "hold-fast" roots in the sense of Tschirch's thesis cannot be maintained. Tschirch, in his "Handbuch", remarks in a note that "The objections of Flaskamper against the Heterorizie are overruled, the hold-fast roots are not an older stage of the nourishing root", but he offers no further evidence.

In commercial valerian there are few parent rhizomes bearing the remains of the large aerial stem. This is probably due to the fact that these old rhizomes become shrunken and tend to decay. We have, however, been able to find a very few parent rhizomes and the roots conform to the descriptions given above. Another point also deserves comment; Moll and Janssonius[3] make a distinction between roots growing vertically and those growing horizontally. We have been unable to confirm this view, but find no fundamental difference in the structure of the xylem of roots growing in these two directions.

Stolon (see Fig. 5)

The epidermal cells of the stolon measure about R 12 to 16 μ, T 16 to **28** to 36 μ and L 40 to 60 μ; the outer walls are slightly dome-shaped, thickened and have a striated cuticle. A phellogen arises in the outermost layer of the cortex and forms up to 3 or 4 layers of cork cells in the older stolons. The cells walls of the cork are thin, suberised and measure R 20 to **36** to 41 μ, T 41 to **45** to 49 μ and L 53 to **61** to 69 μ.

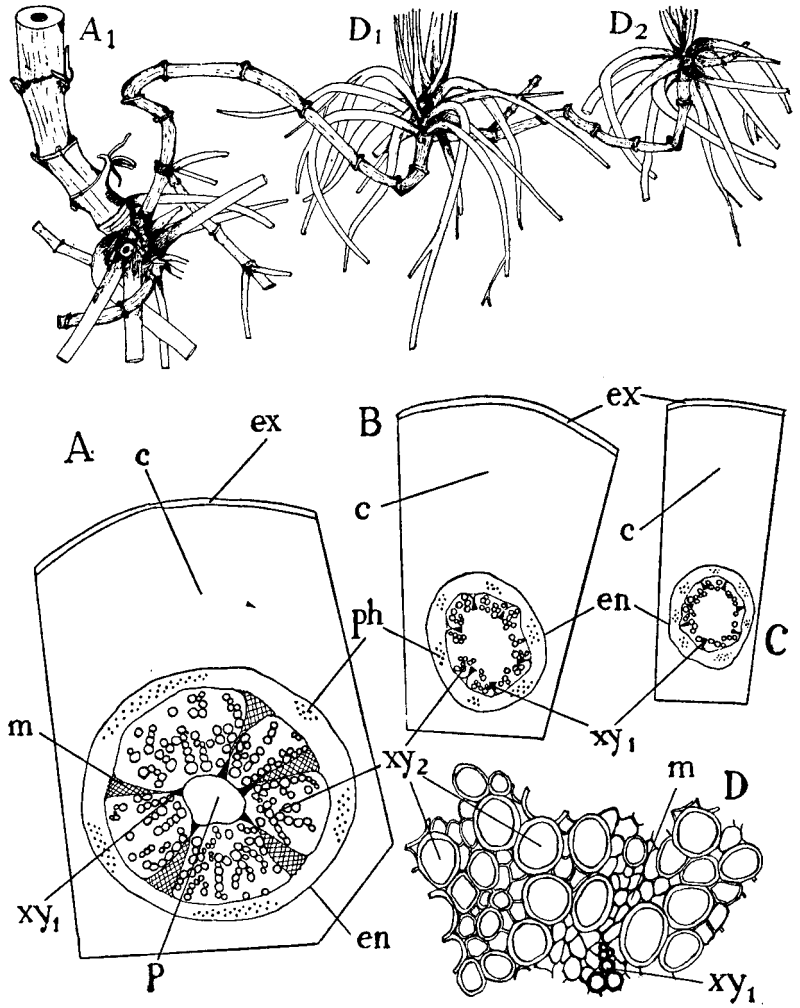

FIG. 4. Three plants of *Valeriana officinalis* Linn., octoploid, attached to one another by means of stolons. A_1 is a parent plant, with a thick woody aerial stem; D_1 is the first daughter plant, which arose from the parent plant during the succeeding season; D_2, another daughter plant grown the same year as D_1, but much younger. All $\frac{2}{3}$ natural size. The drawing shows how each stolon terminates in a new plant and turns at its extremity into an almost vertical direction when doing so. *A*, *B* and *C* are diagrammatic transverse sections from the roots of the plants A_1, D_1 and D_2, respectively, all × 25. *D*, portion of a xylem bundle from *A*, showing a primary xylem group together with some secondary xylem × 100. *c*, cortex; *en*, endodermis; *ex*, exodermis; *m*, medullary ray; *p*, pith; *ph*, phloem; xy_1, primary xylem; xy_2, secondary xylem.

The cortex consists of about 12 to 16 layers of parenchyma, the cells of the outer and innermost regions being somewhat smaller than those of the middle region; the walls are of cellulose and the whole tissue is filled with starch granules. Individual cells measure about R 20 to **40** to 82 μ, T 28 to **60** to 102 μ and L 70 to **94** to 106 μ.

The endodermis consists of one to two layers of cells which are 4–5-sided in transverse section, having suberised and slightly lignified walls and having the dimensions R 20 to **36** to 41 μ, T 28 to **41** to 48 μ and L 61 to **82** to 139 μ; some of the cells contain droplets of oil but there are no other visible contents.

The pericycle consists of 3 to 4 layers of small but thick-walled cellulosic cells which measure R and T 12 to 28 μ and L up to 48 μ. The stele contains a ring of 15 to 25 collateral bundles with wide medullary rays between them. There is a well-marked cambium which is continued across the medullary rays. The xylem vessels are strongly lignified and the vessel elements measure L 205 to 328 μ and R and T 16 to 32 μ, often being slightly wider tangentially. The pith occupies about one-half to three-fifths of the diameter of the stolon and consists of cellulosic parenchyma; usually there is a large hollow in the centre. The cells of the pith measure about R and T 28 to 65 μ and L 125 to 145 μ; they contain abundant starch grains which are mostly simple, but some are 2– to 3–compound, individual starch grain measure about 6 to 12 μ. In stolons showing marked secondary growth, the xylem bundles become closely approximated, eventually forming a closed ring, the medullary rays as well as the outer part of the pith becoming lignified.

No marked difference could be observed between the stolons of the octoploid and tetraploid forms, excepting that a hollow is more consistently present in the pith of the stolons of octoploid plants than in those of the tetraploid plants.

Aerial Stem (see Fig. 1, *C*, *D* and *E*)

The epidermis consists of slightly elongated polygonal tabular cells which measure about R 17 to 20 μ, T 20 to **33** to 43 μ and L 40 to **59** to 66 μ. The outer wall of the epidermal cells is more strongly thickened than the other walls; it is cuticularised but not lignified, being insoluble in cold sulphuric acid and giving no reaction with phloroglucin and hydrochloric acid. In the younger parts of the stem there are numerous trichomes mostly unicellular, but occasionally two celled. The unicellular trichomes are about 116 μ long and 26 μ wide at the base; the two-celled trichomes are longer and measure about 155 to 693 μ long and up to 36 μ wide; the surface of the trichomes exhibits numerous elongated cuticular warts and the base of some of them is slightly constricted. The older basal region of the stem bears a few trichomes only.

The cortex consists of 5 to 10 layers of rounded parenchymatous cells measuring about R 30 to **50** to 66 μ, T 53 to **73** to 76 μ and L 86 to **116** to 132 μ; the smallest cortical cells are those of the hypodermis; the dimensions of the cells of the remaining tissue gradually increase towards the interior as far as the layer of sclereids. The walls are of cellulose and starch is abundant in the young stem, but is absent from the cortex of the very mature woody stem close to the rhizome; the one or two layers of the cortex immediately adjacent to the endodermis gradually become thickened and lignified, with simple pits in the walls; so that in the oldest parts of the stem this layer forms a complete ring of

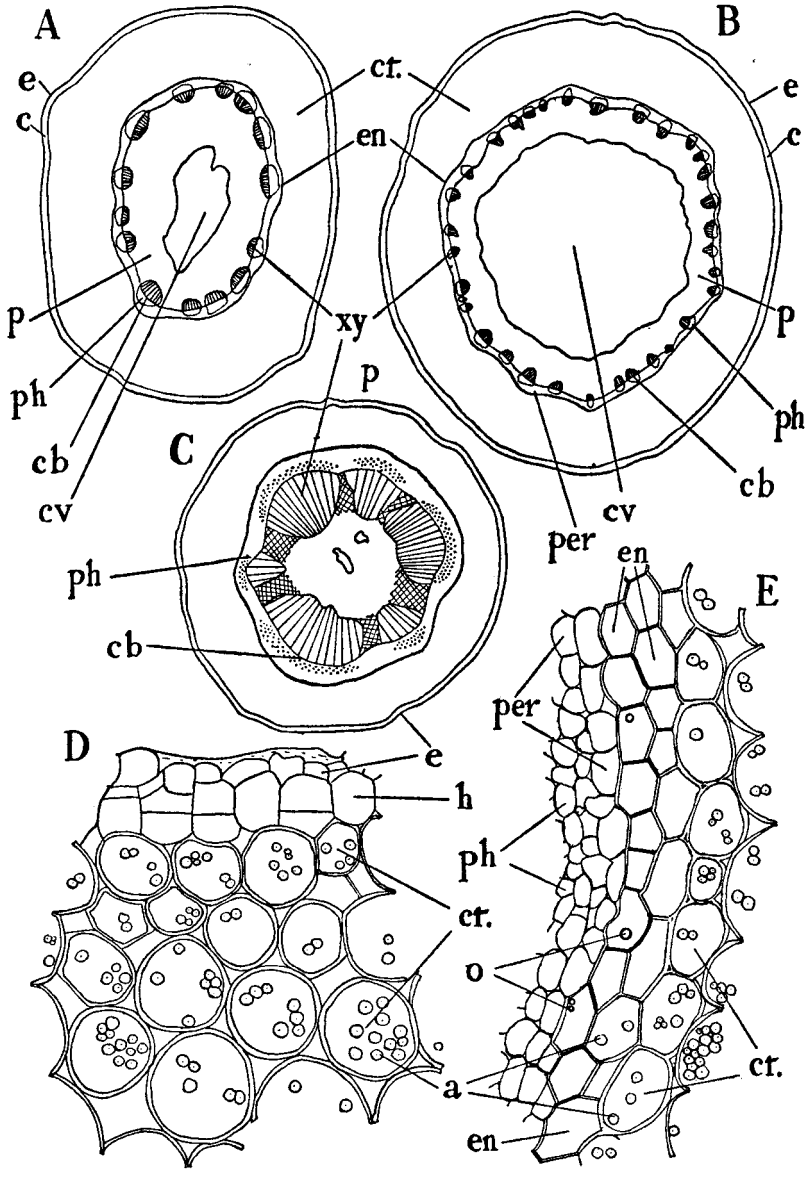

FIG. 5. *Valeriana officinalis* Linn., Stolon. *A*, diagrammatic transverse section of a younger stolon branching from the older stolon *C*. *B*, diagrammatic transverse section of a thick stolon from a young and vigorous rhizome. *C*, diagrammatic transverse section of an old stolon in its second year from a parent plant bearing a large aerial stem. *A*, *B*, and *C*, all × 12. *D*, epidermis and outer cortical layers from the stolon *B*. *E*, part of a transverse section of the stolon *B*, showing the endodermis and adjacent layers. *D* and *E*, both × 200. *a*, starch; *c*, cork; *cb*, cambium; *ct*, cortex; *cv*, cavity; *e*, epidermis; *en*, endodermis; *h*, hypodermis; *o*, oil; *p*, pith; *per*, pericycle; *ph*, phloem; *xy*, xylem.

sclerenchyma. These sclerenchymatous cells measure about R 36 to **46** to 50 μ, T 66 to **79** to 83 μ and L 53 to **70** to 149 μ; they contain no visible cell contents.

The endodermis consists of one or in some places of two layers of cells measuring about R and T 23 to **33** to 43 μ and L 66 to **83** to 99 μ; the cell walls are slightly lignified and cuticularised in the oldest part; they show a distinct casparian strip.

The pericycle consists of a layer of parenchymatous cells measuring about R and T 16 to 23 μ.

In the younger part of the stem there is a circle of numerous collateral bundles separated by wide medullary rays. In the hard and woody part of the stem nearest to rhizome secondary xylem has developed strongly, especially outside the primary xylem groups. The medullary ray tissue formed by the cambium becomes lignified and only a few narrow strips of secondary xylem and phloem are formed in the interfasicular region. The primary xylem groups stand out prominently when the section is treated with phloroglucin and hydrochloric acid, because they project into the pith and the parenchymatous cells between the vessels have not become lignified. All the parenchyma of the secondary xylem is lignified. The elements of the xylem vessels measure about R and T 17 to **40** to 56 μ and L 148 to **198** to 495 μ.

In the centre there is a large pith which is sometimes partially or almost entirely replaced by a central hollow. The cells measure R 116 to **149** to 165 μ, T 109 to **116** to 132 μ and L 132 to **149** to 205 μ; in the young stem they have cellulosic walls and contain starch. The starch grains of both cortex and pith are mostly 2– to 4–compound; single grains measure 3 to 8 μ. Starch is not found in the very thick woody part of the stem nearest to the rhizome and here also the cells of the outer part of the pith are lignified.

Sclereids in the Powder

The structure of the sclerenchymatous layer of the innermost part of the cortex of the aerial stem has been made a subject of special study by Flück and Haller[7] with the purpose of devising a criterion by which the amount of aerial stem present in powdered valerian can be determined. Since these cells and similar ones in the bases of the leaf-stalks are easily distinguished from the sclereids of the pith of tetraploid rhizomes, they can be accurately counted in powdered valerian. A certain amount of sclerenchyma occurs in some stolons, but it is different in form and, since the amount of stolon in commercial valerian is very small—about 0·5 to 1·0 per cent—any error it may introduce is negligible. Flück and Haller find 6·83 sq. cm. of sclerenchyma of stem base and leaf stalk per g. of aerial stem and they propose to exclude more than 5 per cent of stem bases from the drug by imposing a limit of not more than 0·35 sq. cm. of sclereids from the aerial stem per g.

Octoploid Valerian Plants

Evidence that the plant grown at Mill Hill from a rhizome supplied by Mr. H. Deane of Long Melford was an octoploid form was obtained

by measuring the diameters of its pollen grains, as suggested by Blakeslee[14] and also the lengths of its stomata, as used by Karpechenko[15] and quoted by Darlington[16]. These same data were successfully used by Rowson[17] when studying polyploid forms of belladonna and stramonium. Skalinska[18] examined cytologically valerian plants of this same strain and found them to be octoploid; she gave measurements for the pollen grains which she recorded as having a mean diameter of 56 μ with a maximum of 72 μ and 87 μ[1]. Skalinska did not state the mountant in which she examined the pollen grains; preparations mounted in lactophenol were therefore made from specimens in the Herbarium at the Royal Botanic Gardens, Kew, using plants which she had determined cytologically as tetraploid and octoploid. Leaves from the same specimens were cleared in solution of chloral hydrate (chloral hydrate 5, water 2) so as to determine the dimensions of the stomata, values which had not been recorded by Skalinska. The results obtained from these herbarium plants are given in the accompanying Table and the maximum diameters of the pollen grains agree with those recorded by Skalinska.

TABLE I

RECORDS OF LENGTHS OF THE LARGEST STOMATA OBSERVED AND OF THE GREATEST DIAMETERS OF POLLEN GRAINS OF SKALINSKA'S TETRAPLOID AND OCTOPLOID FORMS OF *Valerian officinalis* Linn. PRESERVED IN THE HERBARIUM AT THE ROYAL BOTANIC GARDENS, KEW, SURREY. ALL MEASUREMENTS ARE IN MICRONS

Tetraploid			Octoploid		
No. of plant	Stomata	Pollen grain	No. of plant	Stomata	Pollen grain
T_1	32 to 36	57 to 65	O_1	50 to 52	65 to 70
T_2	36 to 40	53 to 57	O_2	50 to 53	67 to 70
T_3	40 to 42	50	O_3	50	78 to 86

The measurements made of pollen grains from the Mill Hill plant mounted in lactophenol gave a maximum of 78 μ with one larger grain measuring 86 μ; the stomata from leaves of the same plant measured in preparations cleared with solution of chloral hydrate had a maximum length of 50 μ; these dimensions agree with the figures obtained from the octoploid plants of Skalinska's specimens.

These results confirm the validity of the conclusions drawn from the maximum dimensions of starch grains from the roots and from the presence or absence of sclereids in the pith of the rhizomes.

Maximum figures have been quoted in this work because their use has been strongly advocated by Macleod in his book, *The Quantitative Method in Biology*, 2nd edition, 1926, where he summarises observations made during a lifetime of research. His maximal measurements and the application he has made of them give very strong evidence of the utility of maximum values for characterising particular species, even when they are very closely related.

SUMMARY

1. A description is given of the fresh rhizome of *Valerian officinalis* Linn., with its stolons, offsets and adventitious root system.

2. The development of lacunae in the pith of the rhizome is shown to originate from cells having no visible contents by their multiplication and final breakdown to form spaces.

3. The occurrence of sclereids in the pith of the rhizome is shown to be characteristic of the tetraploid plant.

4. The anatomy of the roots is described and the differentiation into "holdfast" and "nourishing" roots is rejected, and it is suggested that these two postulated forms are really older and younger stages of the same roots.

5. The starch granules of tetraploid roots have a maximum size of 18 μ while those of octoploid roots have a maximum of 30 μ.

6. The anatomy of the stolon and of the aerial stem is described and figured and attention is directed to the proposed use of the area of lignified sclerenchyma in the powder as a means of excluding more than 5 per cent of aerial stem.

8. Data based on pollen grains and stomata are given for distinguishing tetraploid and octoploid plants.

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