HISTOLOGICAL ANALYSIS OF THE FULLY DEVELOPED ROOT IN SOME VALERIANA SPECIES

by

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In almost all pharmacopoeias, *Valeriana* root figures as an official and frequently used drug. Its anatomical structure was discussed already in the last century, among others by van Thiegem (9), Meyer (4), Zacharias (13) and, at the turn of the century, by Vidal (12) and Tschirch (10). As to the structure of the root, research workers have thrown light on many interesting details; however, our knowledge of the *Valeriana* root structure cannot be regarded as complete, particularly as far as certain matters of detail and differences among root types and species are concerned. In the meantime, some of the described partial results even have fallen into oblivion, as shown by descriptions in many pharmacognostic text-books of the last 20 years, and in anatomical characterizations of the *Valeriana* root published in one or the other pharmacopoeia. In these books the various root types of *Valeriana* are not specified and the root is characterized as triarch-pentarch. Such descriptions may lead to misinterpretations, and indeed did so on the occasion of some drug examinations. In order to eliminate such misinterpretations, we are going to review the histological conditions of the *Valeriana* root built up of consolidated tissues, as the first of our histological and histogenetic observations on three *Valeriana* species.

Material and Method

We have used for our examinations the root material of *V. officinalis* L. ssp. *collina* (Wallr.) Nyman, *V. officinalis* L. ssp. *exaltata* (Mikan) Soó and *V. sambucifolia* Mikan, grown in the Botanical Garden of Eötvös Loránd University. Immediately after collection, manual sections were made from part of the roots, stained with toluidene blue and fixed in glycerine-gelatine. Other roots were fixed in Bouin mixture (6); for microtomic serial sections vesuvine malachite green was used. The photographic records were made with the aid of planchromate eyepieces and objectives.

Results

Main root. — Although the main root of Valerianae is of no practical importance, — we still brief reference should make to its histological conditions, since in most cases the main root subsists even after the roots of shoot origin are fully developed (8) and can thus occur in examinations of the drug. In literature, the main root of *Valeriana* is mentioned by van Tieghem (9) only. According to our examinations, the main roots of all three species observed are of diarch structure, but the two xylem bundles merge into the vascular tissue described by Vidal (12) only in the zone above the root tip, while further upwards there is pith tissue between them. In the primary xylem bundles, under the protoxylem usually pressed together, some tracheae and tracheids come into being. The primary phloem bundle consists of a small number of sieve tubes and companion cells with thin walls and small lumina. After the consolidation of the primary bundles the formation of the lateral roots of a diarch structure sets in. In the main root, under the crumbling rhizodermis, similarly to the roots of shoot origin (see later), a characteristic hypodermis develops; however, in the case of the main root, no oil secretion was observed therein. In the cortical parenchyma, not more than 6—8 cell-rows broad, there is hardly any starch accumulated. The primary endodermisə is Casparian. On some main roots, immediately under the root-neck, triarch and even tetrarch structures were observed. In case of longeval main roots, a secondary thickening may also occur. (Photo 1.). In the structure of the main root of the three examined species no substantial anatomical differences wer found.

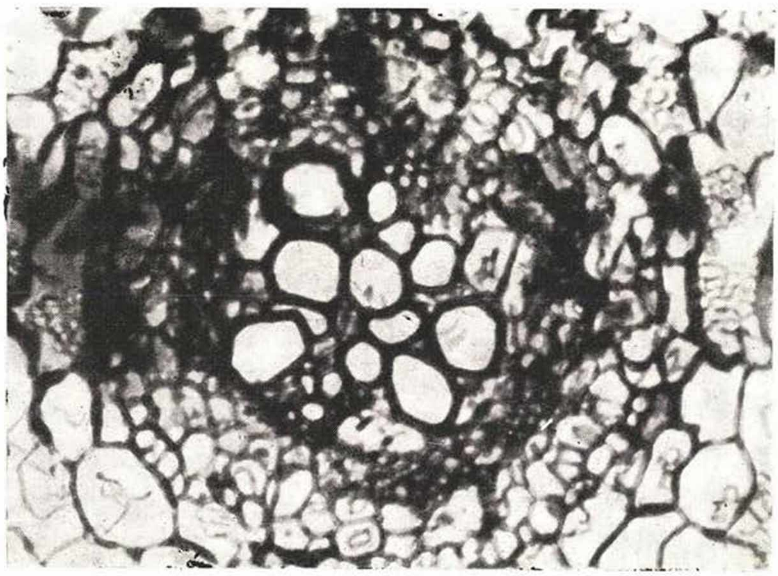

Photo 1. *V. officinalis* ssp. *exaltata*, secondarily thickened main root, transection. Oc. 6. 3 Obj. 16

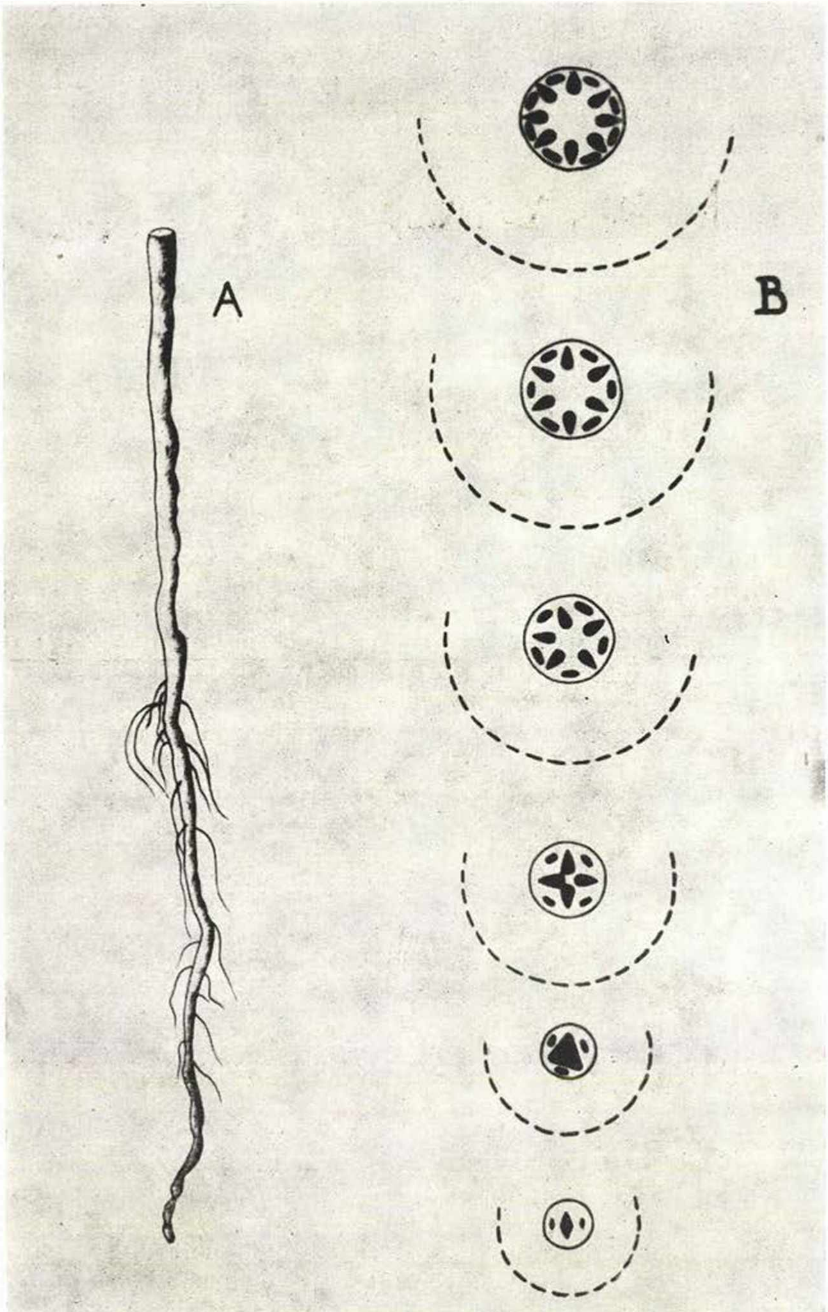

Fig. 1. *A)* Root of shoot origin of *V. officinalis* ssp. *collina.* 1 : 1
B) Schematic drawing of transections made of various levels of the root. 50 : 1

Primary formation of roots of shoot origin. — As already referred to, the roots of shoot-origin constributing the drug are described in most of the technical books as being of tri-pentarch structure, although v a n T h i e g h e m (9) already points to the number of bundles decreasing towards the root tip.

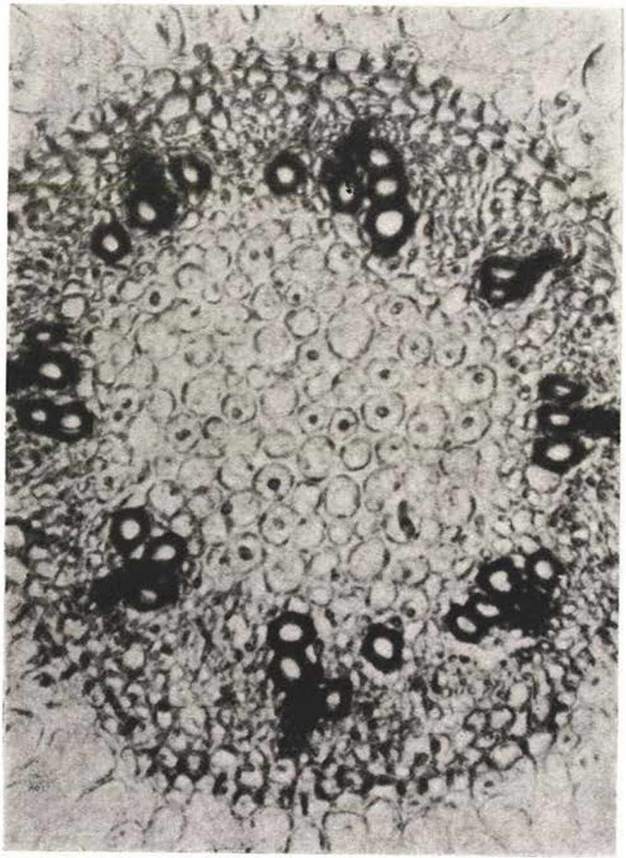

Photo 2. *V. officinalis* ssp. *exaltata*, transection of a young root of shoot origin from the zone under the stock. Oc. 6. 3, Obj. 16

According to our examinations, the roots of shoot-origin of the species of *Valeriana* are similarly to the main root diarch immediately above the root tip. In this region the xylem bundles are usually merged into one another, with no pith tissue between them. Further upwards the number of bundles gradually increases and so does the basic tissue stock of the stele too. The pith tissue, containing not much stored starch appears; the xylem bundles become gradually detached from each other, the ray widens out, while in the uppermost zone of the root, 6 to 12 primary xylem and/or phloem bundles are arranged around the broad pith tissue (Fig. 1.). What is described in literature as tri- or pentarch applies thus only to a certain zone of the root. The primary vascular tissue system of the xylem bundles consists of 3 – 5 tracheae of big lumina, pitted, with spiral and ring-type thickening and simple perforation, as well as of 6 – 8 tracheidae, all arranged under protoxylem elements of small lumina; no parenchyma formation was observed in the primary xylem. The phloem consists of some sieve tubes and companion cells of small lumina, with no supporting element appearing during the whole course of development. The formation of lateral roots begins after the consolidation of the primary bundles. The lateral roots are also of diarch structure and can become in dependence on their thickness, tetrarch at most. There was no secondary thickening to be observed in the lateral roots. Outwardly the stele is closed by an uniseriale pericambium. The primary roots have a Casparian endodermis (Photo 2.). Outside the endodermis follows a wide parenchymatic tissue region with many schizogenous intercellulars. Very much simple, concentric, secondary starch of 20 – 30 μ size is stored in the cells. Less frequently though we could also observe starch composed of a few grains of 8to 12 μ size. In all three species examined, we succeeded in observing in the cortical parenchyma the oil secreted in the cells with nonsuberized walls, as described by Fridvalszky (2).

Very early, already during the consolidation of the tissue region above the meristematic growing point, the external cell-row of the primary cortex built up of wide storing parenchyma begins its characteristic development, as a result of which a hypodermis comes into being, built up of tight-fitting cells with suberized walls, 4 or 5 times superior in size to those of the storing parenchyma of the primary cortex. The cell-wall structure of these cells was discussed by Zacharias (13) who has observed a lignine containing and a cellulose layer inside the suberine layer. The secretion of oil is preceded by the suberization of the cell-wall. The oil appears in the form of small droplets which usually merge into 1 – 2 large drops (Photo 3.). The primary cortex is covered with rhizodermis, the hairs of which are breaking off very quickly; in the basal parts of the cells some suber and subsequently lignine is deposited. This basal part of disintegrating rhizodermis cells can be observed during the whole life apan of the root in the form of irregular, decomposed cell-residues.

Comparative examination of the root of the three species revealet differences to exist in regard to the number of bundles, as well as to the width of the pith and of the cortex. These differences were in agreement with the morphological examination of the roots (5 – 8). In roots of shoot origin of *V. officinalis* ssp. *collina* immediately under the rise of the root max. 12 primary xylem, and/or phloem bundles were counted; the width of the pith amounted here to 18 – 20 and that of the primary cortex, to 30 – 32 cellrows. In *V. officinalis* ssp. *exaltata* we have found max. 9 primary xylem resp. phloem bundles, while the width of the pith and of the primary cortex amounted to 14 – 16 and 26 – 28 cell-rows respectively. The stele of the roots of *V. sambucifolia* was at most hexarch, the width of the pith and of the cortex amounted to 7 – 9 and 17 – 19 cellrows respectively.

Histological conditions of roots of secondary increase. – In the course of first-year vegetation, the roots of shoot origin of *Valeriana* do not begin to thicken until the second half of summer. The beginning of thickening is indicated by the gradual development of the sinuous cambium. Division begins under the primary phloem bundles, continues above the primary xylem bundles and ends with the division of the primary rays to result in a continuous cambium (Photo 4.). The starting function of the sinuous cambium follows the rhythm of cambium development, i. e. new elements are first produced under the primary phloem bundles: usually a trachea each of big lumen appears first on both ends of the primary phloem bundle. This process is immediately

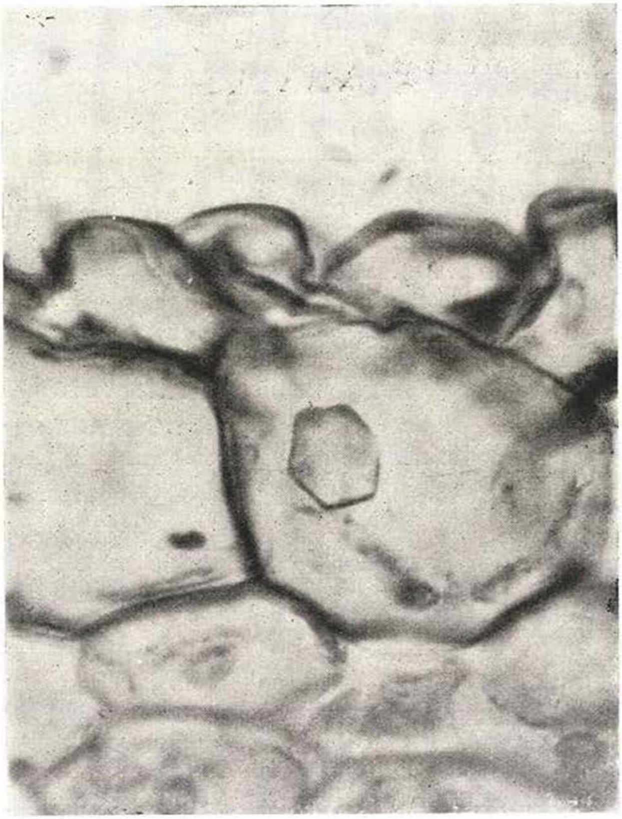

Photo 3. *V. officinalis* ssp. *exaltata*, transection of young root of shoot origin, in the hypodermis an oil drop deformed in the course of the microtechnical procedure Oc. 6. 3, Obj. 40

followed by the abstriction of secondary phloem elements and, towards the primary xylem bundles, by that of the fundament al tissue. The function of the root cambium is thus of a heterogenous nature white; the secondary vascular tissue system consists of collateral open bundles. The secondary transformation of the endodermis begins also at the time when the sinuous cambium takes shape. A radial division occurs in some of the cells (Photo 5.), while other cells of the endodermis are elongating first in a tangential and then, to a smaller extent, in a radial sense too; further on, their cell wall becomes suberized. As a final result of the secondary transformation of the endodermis, the strongly

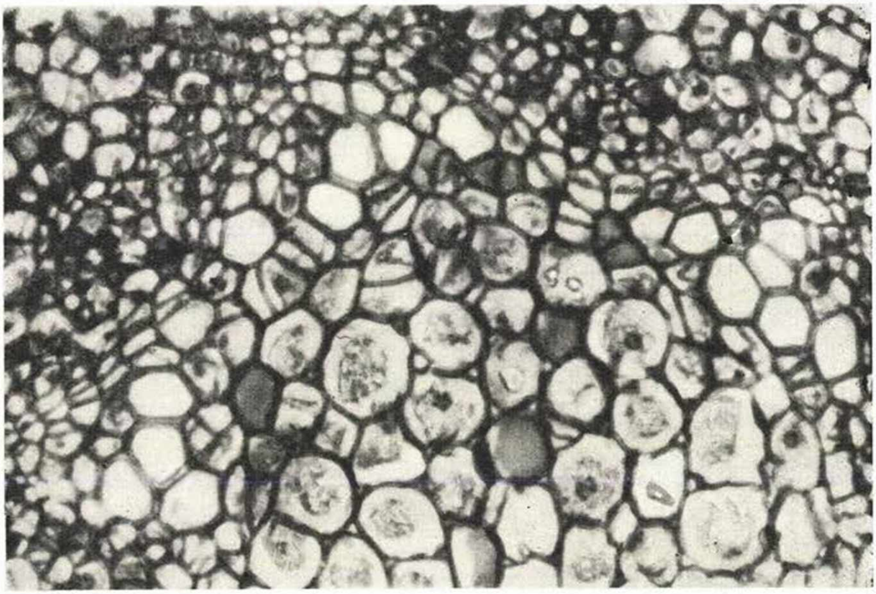

Photo 4. *V. officinalis* ssp. *exaltata*, transection of root of shoot origin: development of sinuous cambium. Oc. 6. 3, Ob. 16

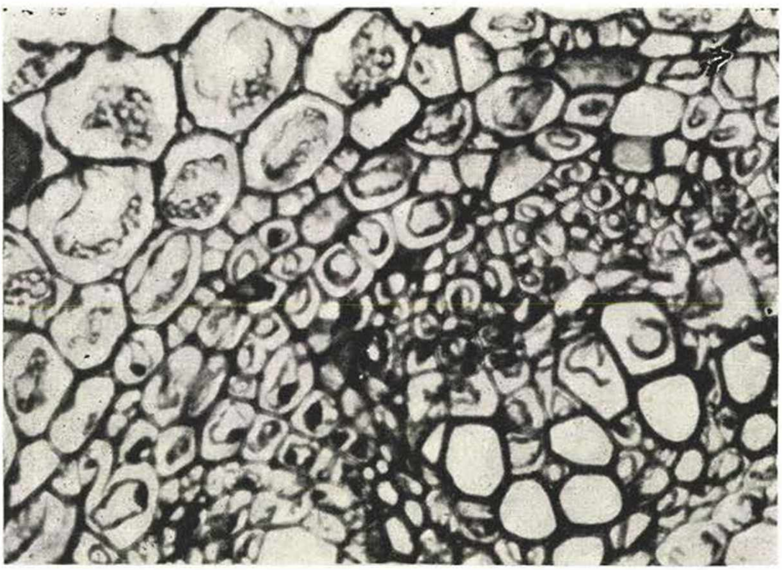

Photo 5. *V. officinalis* ssp. *exaltata*, transection of root of shoot origin: radial divisions in endodermis. Oc. 6. 3, Ob. 16

elongated big cells with suberized walls are linked up with thin walled cell chains (Photo 6.). We wish to specially emphasize that, from the very beginning of the secondary thickening of the roots, no Casparian endodermis can ever be observed; therefore, all representations or descriptions, mentioning a Casparian endodermis together with secondary vascular elements, must be considered as erroneous (e. g. 3.). The primary cortex remains throughout the entire secondary thickening. Prior to a further discussion of thickened

Photo 6. *V. sambucifolia*, root of shoot origin, transection: fully developed secondary endodermis. Oc. 6. 3, Obj. 16

roots, we have to divide the secondary roots into two groups, according to our examinations, i. e. the so-called storing roots and the tensile roots. Let us mention that as early as in 1905 Tschirch (10) refers to *Valeriana* as having two different sorts of roots of shoot origin; in the subsequent period however — maybe on account of the contestation by certain authors (1) —, the two different sorts of roots were hardly or not mentioned at all. In one of our previous publications (8) we have already discussed the ontogenetic and morphologic aspects of these two types of root, so we here only refer to the fact, that in the course of first-year vegetation the overwhelming majority of the roots is of the storing type, with only a few tensile roots being organized in the second half of summer. However, as the plants grow older, the number of tensile roots gradually increases at the expense of the storing roots, so that in several years old plants the tensile roots are predominant.

The *storing roots* are characterized by very wide primary cortex and excessively thin stele. The ratio of these two tissue regions is usually 4 : 1. A further characteristic feature is the very slow, protracted secondary thickening. Sometimes, the sinuous cambium develops already during first-year vegetation, but in most cases in the second year only, producing only a few vascular elements during its very slow retarded functioning (Photo 7.). Usually, some tracheae of big lumina and a few tracheidae are produced under the

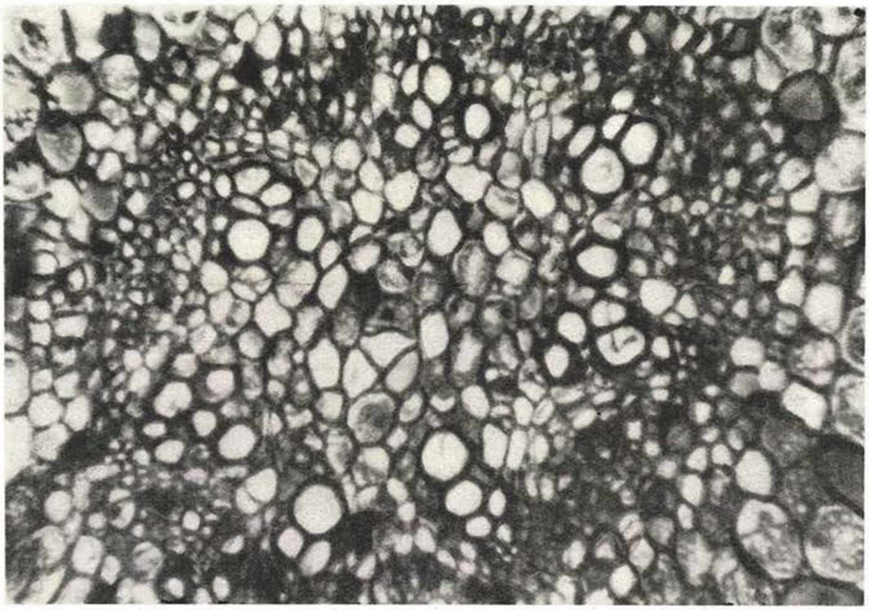

Photo 7. *V. sambucifolia*, transection of storing root: part of stele. Oc. 6. 3, Obj. 10

secondary phloem, consisting of a few sieve tubes and companion cells that are often pressed together.

There is no typical enporting element to be found in the storing roots. It should be stressed that the storing roots are never nearly attaining the thickening of even the youngest tensile roots. The pith tissue of the storing roots contains also secondary starch. An excessive amount of starch is stored not only in the secondary endodermis described above, but also in the very wide parenchymatic cortex. Covered mostly with starch, cells with ethereal oil contents can also be found in the primary cortex. In the hypodermis there is much oil, while the cells are usually intact, being covered by the basic part of the crumbled rhizodermis cells as by a protective tissue.

The *tensile roots* are characterized by a wider stele within a comparatively thin primary cortex. The ratio of the two tissue regions is usually 1 : 2. The

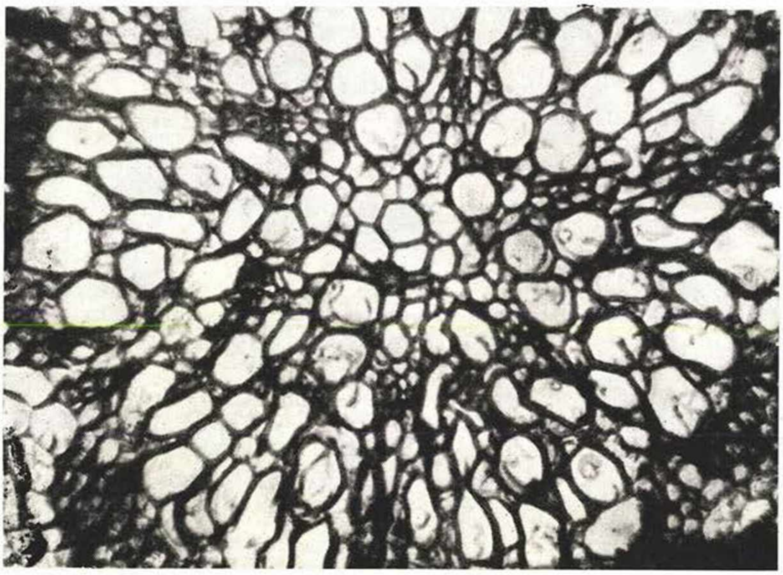

Photo 8. *V. sambucifolia*, transection of tensile root: part of stele.
Oc. 6. 3, Obj. 10

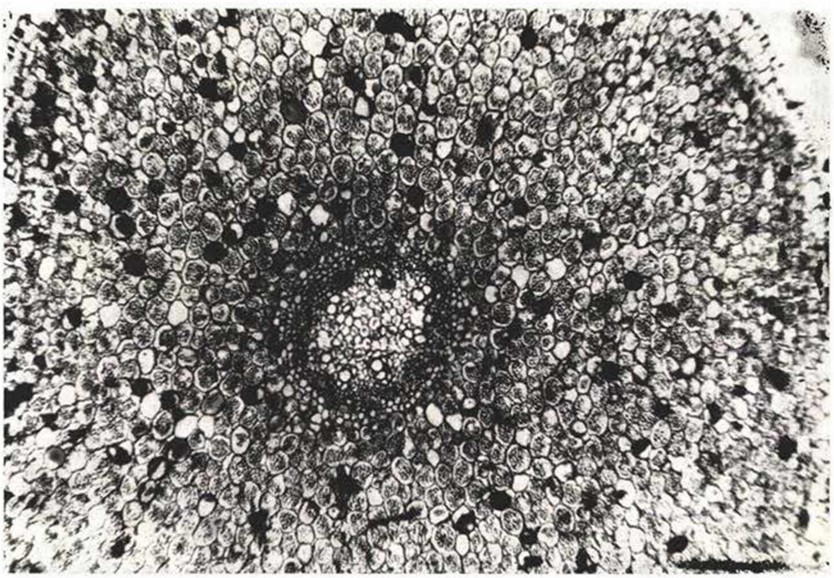

Photo 9. *V. officinalis* ssp. *collina*, transection of storing root.
Oc. 2. 5, Obj. 6. 3

thickening of tensile roots begins very soon and attracts attention with its extraordinary rapidity. After its development, the sinuous cambium produces secondary xylem at a high rate and, much slower, some secondary phloem elements. The phloem of tensile roots consists also of sieve tubes and companion cells, with no supporting elements either. Secondary xylem elements are abstricted in large number; they consist mostly of big-lumen tracheae arranged in pore rays, of a smaller number of tracheidae and transitory configurations, as well as of lignified xylemparenchyma cells. As a contrast to technical literature (**11**), we did not find any typical libriform fibre here either. In spite of this

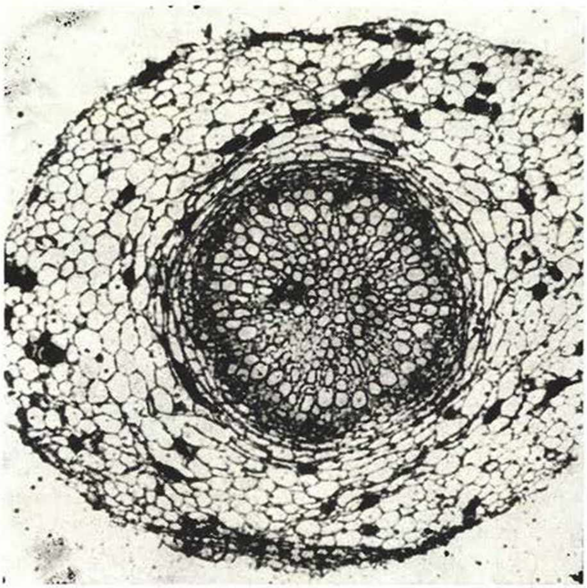

Photo 10. *V. officinalis* ssp. *collina*, transection of tensile root.
Oc. 2. 5, Obj. 6. 3

however, the xylem body of the tensile roots is of a considerable solidity, because the cells of the secondary rays produced above the primary xylem bundles at the border of the pith and often even the pith itself, consisting of a few cells only, become lignified in the course of thickening. No stored starch can be found in the pith tissue of the tensile roots (Photo 8.). As compared to the storing roots, the starch contents of the thin primary cortex is minimal, and even this decreases as the roots get older. No ethereal oil was found in the cortical parenchyma of tensile roots and it was striking to see that the ethereal oil content of the hypodermis was also much lower than that of the storing roots. This can be partly explained by the fact that the rhizodermis of tensile roots is much more worn off than that of storing roots and thus the hypodermis plays rather the role of the exodermis. Consequently we have often observed the cells of the hypodermis to be damaged and torn. Adapting themselves to the function of storage or to that of transport and support, the roots of *Vale-*

riana are thus of a sharply contrasting structure. In case of the roots modified for the function of storage, a comparison of the roots of the three species (Photos 9, 10, 11, 12, 13, 14) has shown only the differences described for the primary roots. In the tensile roots there were — besides the above-mentioned differences in width — comparatively less tracheae and more lignified xylem parenchyma in *V. officinalis* ssp. *exaltata* than in the two other species.

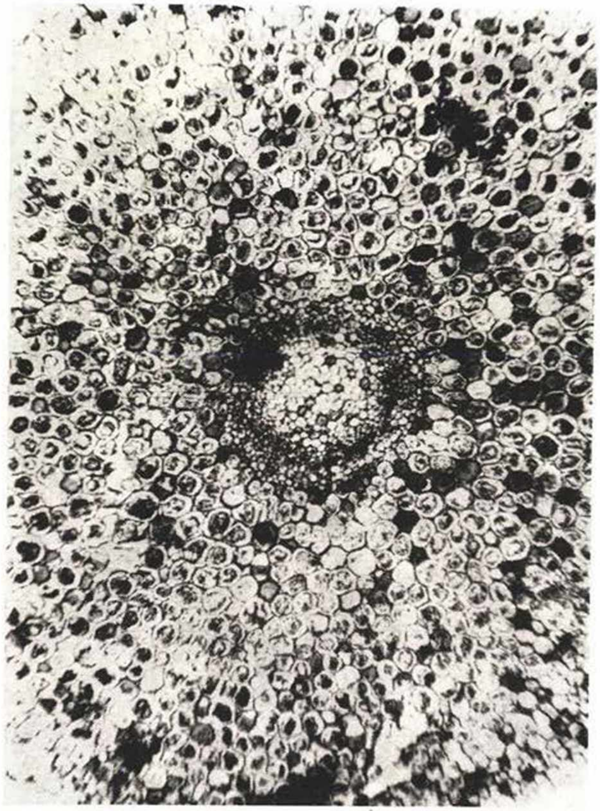

Photo 11. *V. officinalis* ssp. *exaltata*, transection of storing root. Oc. 2. 5, Obj. 6. 3

Our observations have lead us to the practical conclusion that the drug gathered from two-year-old plants is best suited for therapeutic purposes. The root yield of such plants is already adequate while the overwhelming majority of the roots is still of the storing type. The storing roots contain much oil in their hypodermis found to be intact. We were unable to detect any oil in the cortical parenchyma of tensile roots, whereas it was present in the storing roots of all three species examined. Although the pharmacological effect of this type of oil is not yet cleared up, it can be assumed that it is one of the many components amounting to the effect of *Valeriana*. Taking into

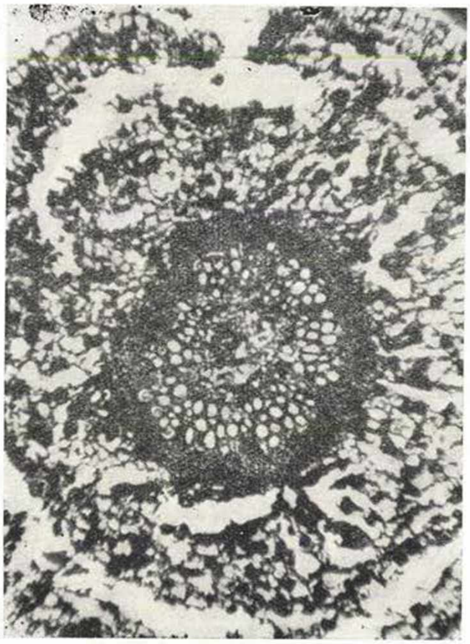

Photo 12. *V. officinalis* ssp. *exaltata*, transection of tensile root.
Oc. 2. 5, Obj. 6. 3

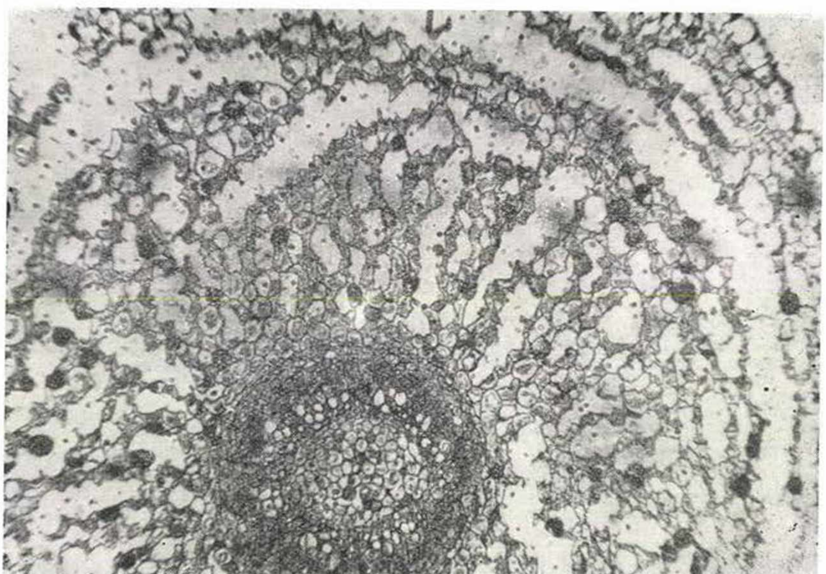

Photo 13. *V. sambucifolia*, transection of storing root.
Oc. 2. 5, Obj. 6. 3

account the choline and alkaloid contents of the *Valeriana* root (7) and considering the anatomical conditions of the two-type root, the storing root containing no supporting elements lends itself much more for development and obtention of these substances.

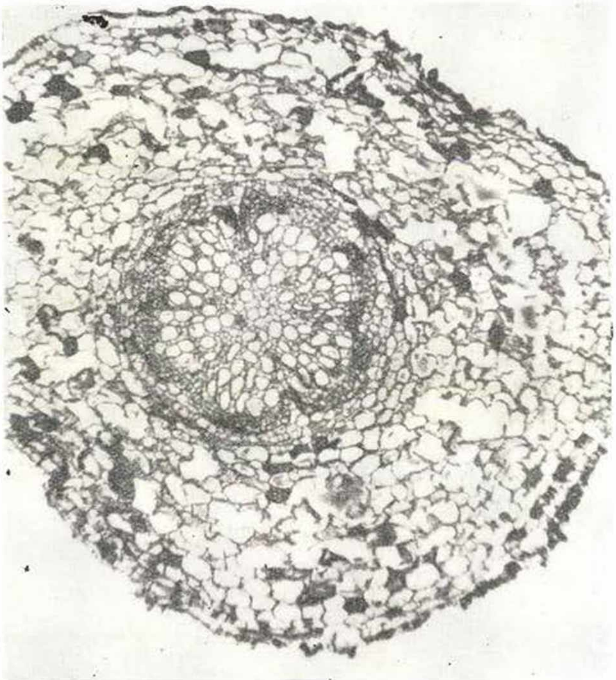

Photo 14. *V. sambucifolia*, transection of tensile root. Oc. 2. 5, Obj. 6. 3

Summary

Comparative anatomical examinations have been made on fully developed roots of three medicinal *Valerianae (V. officinalis* ssp. *collina, V. officinalis* ssp. *exaltata, V. sambucifolia)*. The main root is of diarch structure and persists often after the development of roots of shoot origin is accomplished; at such occasions, a slight secondary thickening can be observed in a short zone immediately under the stock. In the hypodermis of the main root there was no secretion of oil to be observed and the cortical parenchyma hardly contains stored starch.

The primary roots of shoot origin are of diarch structure above the root tip; proceeding upwards on the root, the number of xylem and phloem bundles increases and, depending on the species, the root can be 6 – 12 archic under the stock. Besides the number of bundles, we have observed differences among the three species in the width of the cortex and of the pith parenchyma.

During the secondary thickening of the roots of shoot origin, at the time the sinuous cambium takes shape, the initially Casparian endodermis undergoes a typical change and consists finally of big cells with suberized walls and of thin-walled cell chains linking them up.

The secondarily thickened roots can be divided into two groups according to function and structure. Functioning very slowly, the root cambium of the storing roots produces only a few secondary elements while supporting elements are cuissing. Storing roots are characterized by a very thick primary cortex containing much secondary starch and by the thin stele. The average width ratio of the two tissue regions is 4 : 1. The secondary thickening of the tensile roots is of a very high rate; at the same time, many tracheae of big lumen, less tracheidae and transitory configurations, as well as xylem parenchyma with lignified cell walls are organized in the xylem within the secondary phloem that consists of a few cells only. In the course of thickening the parenchyma of the secondary rays becomes also lignified. A thin stele can be seen within the thin primary cortex of the tensile roots; this cortex is often torn and contains hardly any secondary starch or none at all. The average width ratio of the two tissue regions is 1 : 2. A comparative examination of the secondary roots of the three species has shown that — in addition to the differences mentioned in connection with the primary roots — there are less tracheae and more xylem parenchyma with lignified walls in the tensile roots of *V. officinalis* ssp. *exaltata* than in the two other species. Taking into account the two-type roots of Valeriana, the rate and order of development of the roots as well as their anatomic conditions, we have found the roots of two-years-old plants to be best suited for drug-gathering.

РЕЗЮМЕ

На развившихся корнях трех видов лечебного действия Valeriana (V. officinalis ssp. collina, V. officinalis ssp. exaltata, V. sambucifolia) были нами проведены сравнительные анатомические исследования. Главный корень имеет двухлучевую структуру, в его гиподерме выделение масла нами не обнаружено, он чуть содержит уложенный крахмал.

Первичные корни побегового происхождения над верхушкой корня имеют двухлучевую структуру, число древесных или же оболонных пучков растет снизу кверх на корне, корень, во время его вмещения, может быть 6 – 12-лучевым, в зависимости от вида. Между тремя видами, кроме числа пучков, нами обнаружена разница в широте коры, и сердцевинной паренхимы.

Вторично утолщенные корни, на основе их функции и структуры, можно разбить на две группы. Для хранящих корней характерны очень широкая, содержащая много вторичного крахмала первичная кора, и узкий, чуть утолщающийся стель. Среднее отношение обеих тканевых областей равно 4 : 1. Внутри узкой, чуть хранящей вторичный крахмал первичной коры тяговых корней виден широкий, сильно утолщенный и одеревелый стель. Среднее отношение обеих областей тканей равно 1 : 2.

С учетом анатомической структуры двутипного корня валерианы, отношения развития и очереди корней, корни двухлетних особ нами считаются наиболее удобными для собирания этого лечебного растения.

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