Developmental History of the Abnormal Structure in the Geophilous Organ of *Aconitum*.

By

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With 1 Plate and 6 Text-figures.

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The genus *Aconitum* has now been classified systematically into two sections *Lycoctonum* and *Napellus*; the former has the perennial erect rhizome which usually divides later into several separate strands, while the latter has no rhizome, but has the thick biennial root-tuber which shows the abnormal thickening growth.

These abnormal features of the geophilous organs have hitherto been described in detail by numerous authors, such as IRMISCH (1873), SCHROFF (1861), WASOWICZ (1879), MEYER (1881), LANGGAARD (1881), JOST (1890), DROBNIG (1892), HARTWICH (1897), GORIS (1901), NEUBER (1904), SENFT (1904), STAPF (1905), HOLM (1907) and many others. The descriptions of these authors are chiefly concerned with the abnormal histological structure observed in the cross sections of nearly full-grown geophilous organ, and the developmental history of the abnormal structure in tuber seems to the writer to have hitherto been scarcely traced in detail by any author.

Regarding this, therefore, the detailed description will be given below. The present study was chiefly made with the tubers of *Aconitum mitakense* NAKAI found on Mt. Mitake, Musasi, not only because the tubers were easily obtained in quantities there, but also because the species is one of the representatives, among the Japanese species, which have the tuber showing the most abnormal features.

The large tubers of the species, more than 500 in all, were brought from Mt. Mitake from 1932 to 1936 and most of them were cultivated at Kanazawa for this study.

I. The Structure of the Tuber.

A. THE EXTERNAL FEATURE.

The external features of the tuber in this species are quite alike to those of other species of *Napellus*. The largest tuber examined by the writer was more than 40 mm. in the largest diameter and weighed more

than 60 grams. From the top of the tuber which is dormant in winter, some radical foliar leaves begin to sprout in March under the cultivated condition, and later an aerial stem therefrom. At the same time, one small axillary bud begins to develop at the subterranean base of the radical foliar leaf. The bud is provided with a few rudimentary scales at its end, its first internode being usually several millimetres long. Soon later, an adventitious root is formed on this axillary bud. It is, in March, still very small and short, about 1–1.5 mm. thick, and a few millimetres long, and can not be distinguished from the normal absorbing root, but it soon increases its diameter very rapidly and becomes a daughter tuber which is nearly one half in diameter of the mother tuber before the end of April. In early summer, the daughter tuber is nearly equal to the mother tuber in size.

The daughter tuber is laterally connected by the horizontal branch which corresponds to the first internode of the axillary bud, and the branch decays in autumn as well as the mother tuber itself.

Sometimes several daughter tubers are formed from one and the same mother tuber.

B. HISTOLOGY OF THE ABNORMAL STRUCTURE.

The occurrence of the irregular cambium in tuber is a well-known fact and was described in detail by HARTWICH, GORIS, STAPF, etc., the abnormalities being classified into several types. In the simplest case found in *Aconitum Napellus* L., the continuous cambium is stellate, the part near the primary xylem projecting outwards. In such extreme cases as found in *A. Anthora* L., *A. heterophyllum* WALL. and in some Japanese species, the continuous cambium is remarkably sinuous or is divided into numerous separate rings.

The abnormal histological structure was fully studied by the European authors not only in the European species but also in the Himalayan or Japanese species, for the dry tubers of these Oriental species have been imported into Europe for medical purposes.

Fig. 1 shows the cross sections of two full-grown tubers of *A. mitakense*, both 27 mm. in diameter. These represent the most irregular examples of the cambial topography, and not only the central large cambial layer is remarkably sinuous, but also several separate rings of cambium are observed. The exarch primary xylem (X) is situated either near the projecting part of the cambium or in the separate small ring of cambium. The primary (P) and secondary phloem strands are found scattered in the fundamental tissue. In the tubers drawn here, the cortex is perfectly lost, the outermost cell layers being represented by the endodermis.

Fig. 1. *Aconitum mitakense* NAKAI. Cross sections of two full-grown tubers, both 27 mm. in diameter, showing the sinuous or separated cambial layers. E: endodermal layer. X: primary xylem strands. X₂: secondary xylem strands. P: primary phloem strands. Secondary phloem strands are indicated by numerous black spots scattered in the fundamental tissue. The cortical tissue is destroyed and lost. $\times 2$.

Fig. 2 shows a part of a sinuous cambium which produces chiefly parenchymatous cells towards both sides; the secondary xylem (X₂) and most of the secondary phloem (P₂) are embedded in the secondary parenchyma as small blotches.

Fig. 2. A part of the sinuous cambium, more highly magnified than in fig. 1.

X₂: secondary xylem strands.

P₂, P₃: secondary phloem strands. $\times 50$.

Plate XVIII is a photograph of the successive cross sections of a tuber, showing the irregular construction of cambium. The separate small cambial strands anastomose with one another or with a large sinuous cambial layer, although the cambium becomes circular in the upper or lower parts of the tuber. Such abnormality is commonly found in the tuber whose diameter is more than 20 mm. and usually the larger the tuber is, the more irregular the cambium is. Such evident irregularity is not observed in the smaller tuber less than 15 mm. in diameter in the full-grown condition, though the cambium is often somewhat stellate.

The primary xylem strands in the tuber are usually 5–6 in number, as well as in the absorbing root, while those in the vigorous tuber are often 8–10 and are reduced also into 5–6 on the lower level of tuber by their mutual anastomosis. The lateral roots on the tuber are usually slender, but their basal parts often increase in diameter, growing 10–15 mm. thick. No cambial abnormalities are observed in such a thickened root of the secondary order. The trace bundles of the lateral root come out of the primary xylem of the tuber, as seen in the photograph.

II. The Developmental History of Abnormal Cambium.

As for the developmental history of the abnormal cambium, MEYER (1881) described that at first one circular secondary cambium is formed in the centre of the stele enclosed in the normal cambial ring, and then several strands of separate cambium are produced by the radial connection of both kinds of the cambial layer in several parts. This was referred to by SOLEREDER (1908), but, in fact, the description of MEYER's was made only in the dry tubers of *Aconitum heterophyllum* WALL., imported for medical purposes, so that it is not concerned with the developmental history. After that, no description in this respect seems to have been made.

From several years ago, the writer has cultivated hundreds of large mother tubers which are 20–40 mm. in diameter, and the daughter tubers which are destined to show the remarkable abnormality have been examined in order to trace the developmental process of the abnormal cambium.

The young daughter tuber in its earliest stage, observed in March 1937, was 1.5 mm. in diameter and less than 10 mm. long, and no differences were found between this and the normal absorbing root in external or histological structure except in its position of appearance. In this stage, all the cells are primary as shown in fig. 3, A. There are small intercellular spaces in the cortex and in the peripheral part of central cylinder. The pith consists of polygonal cells without any intercellular spaces and the endodermal cells are also thin-walled.

In the observation made on the 4th of April, the young tubers were

2—3 mm. in diameter, and about 20 mm. long; on the 14th, 4–6 mm. in diameter; on the 21st, 7–10 mm., i.e. nearly one third in diameter of the mother tuber; on the 28th, 10–15 mm., i.e. nearly one half in that of the mother tuber.

Fig. 3. Cross sections of the young daughter tubers. Only a part of the tuber is shown.

A, the earliest stage of the tuber which is 1.5 mm. in diameter, observed at the end of March. All the tissues are primary.

B, a little later stage of the tuber which is 2 mm. in diameter.

EP: Epidermis. H: root hairs.

E: endodermis. X: primary xylem.

P: primary phloem.

The left side of the figures corresponds to the centre of the pith. $\times 50$. Compare with fig. 4.

No indication of the abnormal cambium was observed in any tuber before the 14th of April; however, irregular layers of cambial cells had been generally formed on the 28th. In other words, the cambial abnormality, if it appears, is formed within two weeks of the end of April in the daughter tubers which are from one third to one half of the mother tuber in diameter. This may, of course, somewhat varies under different external factors; in the material cultivated by the writer, however, no irregular cambium was found before the 15th of April and it was formed early in May either in 1935 or in 1936, and in 1937 as well.

The further process of the abnormal thickening growth will be described below.

1. Very early in April, numerous cells inside the primary phloem become evidently meristematic and divide and elongate in radial direction, but the tangential row of initial cells, such as is usually called the cambium, is not yet clearly observed.

2. The cells in the peripheral parts of the central cylinder begin to divide by slow degrees in indefinite directions.

3. The fragments of the tangential cambium appear in the part outside the primary xylem.

4. The endodermal and cortical cells begin to divide tangentially by slow degrees.

Fig. 4. Cross section of young daughter tubers. Only a part of the tuber is shown.

The left side of the figures corresponds to the centre of the pith.

A, a tuber which is 4–6 mm. in diameter observed in the middle of April. Almost all the cells, except those of primary conducting tissue and epidermis, become meristematic. B, a little later stage of a tuber which is about 10 mm. in diameter. The secondary phloem strands appear scattered in the secondary meristem, but the sinuous cambium does not yet appear in the pith.

X₂: secondary xylem strand.

P₂: secondary phloem strands. Other abbreviations are as in fig. 3. × 50.

Fig. 3, B shows such a stage of the daughter tuber, which is about 2 mm. in diameter. The cells at the centre of the stele do not commence the secondary division in this stage.

5. The meristematic cells which are inside the phloem or are between the two strands of the primary xylem, divide most frequently in indefinite directions.

6. The pith becomes meristematic, its component cells beginning to divide successively in indefinite directions.

Such a stage of development was observed in the middle of April in the tuber 4–6 mm. thick, and is shown in fig. 4, A. In this stage, the cell divisions are successively progressing in all parts of the central cylinder, and the diameter of the tuber increases very rapidly. The tangential short row of initials, which cut off cells regularly towards both directions, are observed only in the part outside the primary xylem or in the middle part between two xylem strands; however, the continuous circular row of initials is never formed.

7. After that, blotches of the secondary phloem appear sparsely in the cortical parts outside the fragmental rows of initials or in the medullary part; and also small groups of the secondary vessels often appear in the several parts inside the fragmental rows of initials.

This stage of the tuber is shown in fig. 4, B.

8. Some fragmental rows of cambium appear in the secondary meristem of the centre of the tuber. These fragments often produce numerous small strands of secondary phloem in one direction, and a little later, a few small strands of secondary xylem in the other direction.

This stage observed at the end of April is shown diagrammatically in fig. 5, A. Fig. 5, B shows the fragmental cambium in its early stage found in the medullary part of the tuber.

9. All the fragments of cambium are extended laterally and connected with one another, thus the sinuous or separate cambial topography, characteristic of the tuber, is produced.

This process of development was finished in the course of April. In this stage the daughter tuber is nearly one half of the mother tuber in diameter.

10. After the irregular cambium has been formed nearly completely, all other cells of the central cylinder become permanent and increase remarkably in size. By the irregular cambium, some secondary parenchymatous cells are added inside and outside, and the small strands of secondary xylem and phloem appear scattered in these secondary parenchymatous tissues.

11. In the last stage, the epidermal and cortical tissues are destroyed

and the endodermal layer is exposed, thus the features found in the full-grown tuber are produced.

The strands of the secondary phloem are divided into two groups in their origin: the one is represented by the strands which are formed directly out of the secondary meristem of the stele before the fragments of cambium appear, and is indicated by P_3 in fig. 2 and fig. 5; the other, by the strands which are formed out of the tissue whose component cells are cut off by the rows of initials, as indicated by P_2 in fig. 2 and fig. 5; but the differences of these two are not always clear.

In short, the following facts are of great interest in the developmental process of the abnormal thickening growth: 1) In the earliest stage of development, a circular zone of meristematic tissue differentiates in the part inside the primary phloem and outside the primary xylem, but the typical tangential row of initial cells, usually called the cambium, is not observed inside the phloem, in contrast with the usual case of root. 2) The ends of the fragments of initials are extended laterally or radially, and are connected with one another, thus the sinuous or separate features of cambium are produced. 3)

The cambial fragments, which are formed in the central part of the stele,

Fig. 5. A, diagram showing the early stage of the sinuous cambium in the daughter tuber which is nearly one half of the mother tuber in diameter. A little later than the stage shown in fig. 4, B. The cambial fragments differentiate from the secondary meristem, $\times 6$.

B, a central part of the above figure more highly magnified, showing a cambial fragment in the early stage of its formation, $\times 50$.

EP: epidermis. E: endodermis. X: primary xylem strand. X_2 : secondary xylem strand. P : primary phloem strand. P_2, P_3 : secondary phloem strand. C: cambial fragment.

are derived not from the primary medullary tissue, but from the meristematic tissue of the secondary origin. 4) Most strands of the secondary phloem are derived from the cells cut off by the initials of sinuous cambium, but some of them directly from the secondary meristem mentioned above. 5) The formation of the abnormal cambium is completed in a rather short time.

III. Comparison of the Geophilous Organ between *Napellus* and *Lycotconum*.

In contrast with the case of the section *Napellus*, the geophilous organ of the section *Lycotconum* is represented by the erect rhizome which is often carelessly described as root. Although its morphological and anatomical abnormalities have been well-known, brief descriptions will be given below for comparison in *Aconitum gigas* LÉV. et VAN., a Japanese species of *Lycotconum*.

In this species, the subterranean part of the stem is perennial. In the early stage it has separate collateral vascular strands and the circular

Fig. 6. *Aconitum gigas* LÉV. et VAN. Diagrams showing the developmental history of the abnormal structure in the rhizome. A, the first stage of abnormality. D, the last stage. The rhizome is divided into pieces by the destruction of tissues. Xylem is shaded, phloem dotted. E: epidermis. B: fibrous cap. C: cambium. K₁: primary phellogen or cork layer. K₂: secondary phellogen or cork layer. $\times 10$.

normal cambium which cuts off secondary tissue inside and outside. Later a continuous ring of cork cambium K_1 appears outside the stele, as shown in fig. 6, A, and the cortical tissue outside is destroyed (fig. 6, B). Then some parts of the secondary xylem and phloem as well as the fragment of the normal cambium are enclosed in the separate rings of cork cambium K_2 more lately formed, as shown in fig. 6, C.

All the tissues enclosed in this cork cambium, i.e. the pith, some vascular strands and some parts of each strand, are destroyed sooner or later; thus the rhizome is separated into several fragmental strands, as shown in fig. 6, D. The both ends of the fragmental row of normal cambium are often extended in the later stage and become continuous by their connection.

In considering the characters of nectary, KRONFELD (1890) supposed that *Aconitum Lycoctonum* L. represents the derived form of *Aconitum*, while MEYER (1881) expressed his opinion based on the abnormal features of the geophilous organ, that *Aconitum Lycoctonum*, most abnormal in the structure of the geophilous organ, is the original form, from which the species less abnormal, such as *A. Napellus* L., are derived through the species more abnormal, such as *A. Anthora* L. and *A. heterophyllum* WALL. The view was supported by HARTWICH (1892).

As mentioned in the foregoing pages, the two cases of abnormal structure found in sections *Lycoctonum* and *Napellus*, are fundamentally different from each other, though they are observed in the geophilous organs.

In *Lycoctonum*, the geophilous organ which exhibits the abnormal structure is represented by the perennial rhizome, while in *Napellus* by the biennial adventitious root which is metamorphosed into the tuber. Further the abnormalities are also fundamentally different from each other in the developmental process and in the histological characters. From this fact it is quite unreasonable to compare the abnormal features of *Lycoctonum* with those of *Napellus*, or to assume that the one is more original than the other, and, in reality, the phyletic gap between *Lycoctonum* and *Napellus* may be considered to be fairly great, as FOURREAU (1868) recognized, who treated these as two separate genera.

IV. Summary.

1. The abnormal structure found in the root-tuber of *Aconitum mitakense* NAKAI has been described. This species is one of the representatives which exhibit the most remarkable abnormality, the tuber having both the very sinuous cambium and several separate strands of cambium. The remarkable abnormality of cambium is usually found in the tuber more than 2 cm. in diameter.

2. The development of the tuber has been traced. Under cultivation, the daughter tuber begins to appear in March, before the erect stem comes to sprout. In that time it is very small, 1.5 mm. in diameter, but in the middle of April it thickens to one third of the mother tuber in diameter, and at the end of the month, to nearly one half of it.

3. The developmental history of the abnormal cambium has been traced. In the earliest stage of development, a circular zone of meristematic tissue appears in the part inside the primary phloem and outside the primary xylem, though the tangential row of initial cells is not evident. Next some fragments of the tangential cambium appear in the zone. Soon later the pith begins to change into meristem, from which secondary phloem strands and fragments of the secondary cambium are differentiated. By the connection of these fragmental rows of cambium, the sinuous or separate cambial features, found in the full-grown tuber, are produced.

4. For comparison, the abnormal structure in the geophilous organ of *Aconitum gigas* LÉV. et VAN. (a species of *Lycoctonum*) has been examined.

5. The relationship between the two sections of *Aconitum*, i.e. *Lycoctonum* and *Napellus*, has been considered. In *Lycoctonum*, the geophilous organ, which exhibits the abnormal structure, is represented by the perennial rhizome, while in *Napellus* by the biennial root. Further their abnormalities are also fundamentally different from each other in the developmental process and in the histological characters. From these facts, it is quite unreasonable to assume that the one represents a more original type than the other; and the phyletic gap between *Lycoctonum* and *Napellus* seems to the writer to be fairly large.

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Explanation of Plate XVIII.

Photograph of successive cross sections of a fresh tuber of *Aconitum mitakense* NAKAI, showing the irregular construction of cambium. Follow the sections in each horizontal row from right to left, horizontal rows, from top to bottom. The first section in the photograph shows the upper part of the tuber, the last one the lower part. Slightly reduced.

M. KUMAZAWA—Abnormal Structure in *Aconitum*.