COMPARATIVE STRUCTURE OF THE NORMAL ROOT AND TUBERCLE OF LATHYRUS MARITIMUS, BIGEL.

By

GEORGE HERMANN KOPP.

A Thesis submitted for the Degree of

GRADUATE IN PHARMACY.

UNIVERSITY OF WISCONSIN.

1900.

INTRODUCTION.

Lythyrus maritimus, commonly known as Beach Pea, Sea or Seaside Pea, is mostly found along sea-beaches, hence its name. It occurs abundantly along the Atlantic sea shore from New Jersey to Artic America, on the shores of the Great Lakes and on the Pacific coast, as well as in northern Europe and Asia. it blooms from May to August sometimes again late in autumn.

This plant like many other leguminous plants possesses tubercles upon its roots. These root tubercles were considered as normal structures of the Leguminosae by old investigators, on account of their invariable occurance upon the roots of the plants of this order. But in more recent times it has been demenstrated that they are not, and only occur after infection by bacteria. Whether these bacteria are parasitic or not can not definitely be said? In the case of our common bean (phaseolus vulgaris), it acts as a parasite and therefore is injurious to the plant. On the other hand in the case of LUPINUS LUTEUS and PISUM SATIVUM the tubercle microbe acts as a stimulant and tends to strengthen the entire plant. Thus no general conclusions can be drawn, it has a different effect upon different plants. In the case of peas and lupins, tubercles take the place of humus when it is wanting.

In most cases it appears that the relation between the two plants is a symbiotic one; and that the higher form derives benifit from this symbiosis with bacteria, in that it uses them for its own nourishment. Ot the bacteria may support these

plants in other ways. About this very little is known as yet. Thus we have in the tubercle a peculiar form of symbiotic structure which is of use, both to the bacteria which forms it, as well as to the leguminous plants.

The host as we may designate the higher form, confines the bacteria to the center of the tubercle and surrounds them with other tissue. Root tubercles are not all caused by the same bacterial form, but rather by several, one in one locality another in another locality. The infection seems to take place through the root hairs. The organism which is generally present in the soil, enters the root hair at its extremity and makes its way into a surface cell after which infection is complete.

They then surround the nucleus and stimulate it to further growth whereupon the size of the cell also increases. How further infection goes on, that is, how the organism or rhizobia as they are called travel from one cell to another is not known It is supposed however that they have power to dissolve the cell wall of the host cell. The tubercles formed are abnormal growths produced by these organisms or rhizobia and generally grow out at right angles to the root. At first they are simply wart like, then they acquire a roundish appearance and finally become pear shaped. The tubercles may appear on the host root soon after germination of the seed or at the flowering stage or not at all, and are most frequently found on the roots near the surface of the soil. They are generally found on the un-

branched radicles and do not appear on old woody roots.

The tubercles are perishable, they seldom endure a year. The central cells become changed to a water colored blister containing homogenous nuclei. Lachman declares the tubercles to be not pathological but physiological organs. He states that they serve for gathering or storing up food when it is plenty and returning it to earth when there is a scarcity of it(especially nitrogen).

Most of the tubercle consists of parenchymatous tissue, very few vascular bundles (3, 4 or 5) connecting them with the central woody tissue of the root. The parenchyma consists of a central main mass. The peripheral region of the tubercle consists of several layers exhibiting an irregular ragged surface. Within these there are cells more or less flat containing granular matter. The parenchymatous cells are largest at the center of the tubercle and grow smaller as they approach the periphery. Between the two parenchymatous regions lay the reduced vascular bundles. The bundles do not extend to the very point of the tubercles but this region consists only of parenchyma cells which are continually being produced by the division of the cells of the meristem, here located. At the base of the tubercle the bundles unite and connect with the woody tissue of the root. The bundles differ somewhat from those of the root, the cells walls being much thinner. The parenchyma cells which surround these bundles contain a considerable number of starch grains.

Under the influence of reagents the bacteroids deport themselves as bacteria. They become colored yellow with iodine, ab-

sorbing with greatest facility the colors of aniline, particularly the fuchsine and the violet of methyl as well as haematoxylon.

SOURCE OF MATERIAL AND METHODS.

The material with which I worked was obtained from the shore of Lake Superior near the Cranberry River in the summer of 1897. The stains used were chloriodide of zinc, solution of iodine in water, haematoxylon and methyl violet. The sections were all cut by the free-hand method and mounted in glycerine. I used the camera lucida in making all drawings except that shown on Plate 5, which is more or less diagramatic and simply shows proportion and distribution of tissue.

RESULTS OF OBSERVATIONS.

In the normal root (Plate 1), we find a layer of epidermal cells (Epid) quite irregular in shape, projecting conversely? while some taper out into root hairs. They have a tangential diameter of 2.5 μ - 7 μ , and are 9 μ - 15 μ in radial diameter. The outer walls of these cells are somewhat thicker than the rest and this thickening extends about half way down its lateral walls. Directly beneath this layer of epidermal cells, we have the parenchymatous tissue (par) which constitutes most of the root structure. The size of these cells varies between 30 μ and 60 μ in tangential diameter and between 24 μ and 48 μ in radial diameter depending upon its position

in the tissue. The cells beneath the epidermis are comparatively small but gradually increase in size as the endodermis is approached; the cells about midway have the greatest size and then there is a gradual decrease in size toward the endodermis the cells here having about the same size as those next the epidermis.

The endodermis (End. Plate 1 - 111) consists of a single layer of cells having a radial diameter of 9μ - 15μ and a tangential of 9μ - 21μ. The cells are thin walled and very uniform in shape.

Within the endodermis is a tissue composed of cells with very thin walls (A, Plate 11), the cells having a diameter of from 6 - 15μ, the end walls of the same being very thin. Their longitudinal diameter is sevral times their transverse. Within this tissue lying in the neighborhood of the endodermis and about equidistant from each other are small strands of bast fibfe, (B, Plate 11), these take an intense yellow color when treated with chloriodide of zink and are very thick walled. There are generally five such areas of cells in the natural root, the external diameter of the cells being 6μ - 15μ, the internal 1μ - 4μ.

Midway between these bundles of bast fibres, we have the extremities of the vascular rays of the fibro-vascular bundles within the bundle. The larger vessels (C, Plate 11) are grouped about the center, while those composing the rays gradually decrease in diameter from the center outward. The internal diameter

of the largest being about 42μ and decreasing to those having an internal diameter of only 9μ and even less. The nuclei in cells of the paranchymatous tissue measure 6μ - 9μ.

In the abnormal root or tubercle as it is called, the structure has been changed entirely. It has increased in dimensions. Instead of having a centrally located fibro-vascular bundle as in the normal root, it has a number of reduced bundles scattered about its periphery (A, Plate VII), these are much smaller than the original, are composed of much smaller and fewer members. These smaller divided bundles are each surrounded by a tissue of thin walled cells which are very profusely filled with starch grains. The center of the tubercle or it might be said the bulk of the same consists of large thin walled cells (B, Plate VII) filled more or less with a granular substance which stains yellow to brown with chloriodide of zinc. These cells possess very large nuclei and nucleoli (X and Y Plate VIII) In the early stage of these cells they have somewhat the shape of polygons and are completely filled with granular material which as the tubercle grows older, changes partially to a colorless fluid due to some decomposition which probably takes place within the cells and consequently the cells take on more of a rounded shape. as the tubercle grows older, this change having gone on with the cell for some time, the cell walls become ruptured and the contents are set free. It is in this way that we finally have in the older tubercle simply a cavity filled with a colorless liquid containing a little granular material. The cortical

region of the tubercle does not possess the regularity of form and surface that is seen in that of the normal root (C, Plate VII), the cells on the outer surface having no regularity of shape or size. They are apparently empty and do not have their exposed wall thickened more than the other. It appears very much as though the tubercle during its development has gradually had the outer layers of the cells rubbed off by the resistance offered by the soil to the rapid growth of this part of the root.

The fibro vascular bundle as found in the tubercles is entirely changed (C, Plate II), it does not have the symmetry which is shown in the bundle of the normal root. Instead of one central bundle there are now a number of smaller ones located near the periphery. Each consists merely of a few tracheieds and bast fibres combined without any symmetry whatever (Plate IX). These cells are then surrounded by a layer of cells containing exceedingly large nuclei (B, Plate IX) and outside of this layer the surrounding cells are very thickly filled with starch grains (C, Plate IX).

These smaller bundles as found in the tubercles arise from the woody tissue of the root and envelope the parenchymatous tissue which is infected, they run up between the inner and outer parenchymatous regions through the starch region and extend almost to the distal end of the tubercle at which point it is hardly recognizable consisting here of but a few vessels. The relation which the tubercle bears to the root is well illustrated in a somewhat diagramatic drawing on Plate VI. This tubercle is just about half developed.

The lower part of the figure consists of a section through the root showing some of the epidermal cells (Epid, Plate V1) and their gradual transition from the regular closely packed to the loose and torn tissue which envelopes the tubercle. Then there are a few parenchyma cells shown (Par, Plate V1) and also a few of the larger vessels in the woody region (V, Plate V1) and the limit of the pith region (1, Plate V1).

Going over into the tubercle we get a longitudinal diagram of the same, the line x, outlines the position occupied by the deseased parenchymatous tissue. This region is entirely surrounded by such cells as are shown at A, Plate V1, which are somewhat elongated and flattened having their smallest diameter pass through the center of the tubercle. All of the tissue along the line x is densely filled with starchgrains. In B, C, D, Plate V1 we can notice the different stages of development through which these cells pass. B having been first formed and being the oldest tissue is farthest advanced while C comes next. The meristem or growing point is along F, by the continual division of this meristem new cells are always being furnished for the growth of bacteria, it is from this point that all further development takes place. At E we have one of the vascular bundles cut soemwhat obliquely, it does not extend much further up than is shown in the diagram.

The outermost cells as seen in section of the tubercle measure 18 - 30 μ radially and 57 - 114 μ in tangential section. The endodermis seems to have disappeared entirely in the abnormal

growth. The cells in the starch region measure 24 - 43 μ in tangential section and 21 - 36 μ in radial. The trachieds in the peripheral bundles measure 6 - 15 μ in diameter. The bundles themselves are about 90 μ in radial and 120 μ in tangential section.

The nuclei found in different tissues range between 12 and 21 μ in diameter thus showing a marked increase in size.

After having studied over the different characteristics of both the normal root and the tubercle we cannot help but see that the presence of the rhizobia has brought about great changes The entire structure of the plant where they are present is changed and changed radically.

BIBLIOGRAPHY.

Berichte d.botan.Gesell. 1889,p.332 (B.Frank)

Berichte d.botan. Gesell. 1892,p.242 (H.Moeller)

Landw.Jahrbuecher Bd.XIX, 1890,p.523

Landw. Jahrbuecher Bd.XXIII, 1894,p.649 (Gonnermann)

Landw.Versuchsstationen Bd.XXXVII 1890,p.161 (Adam Prazmowski)

" " Bd.XXXVIII 1890,p.1

Biedermann's Centralblatt Bd.XX 1891,p.837 (J.Lachermann)

Berichte d.Botan.Gesell. 1892,p.242

The Bot. Gazette 1893,p. 157; W, 4 pl.

Centralblatt f.Bakteriol Bd.X 1891, No.6 (Ferdinand Cohn.)

The American Naturalist Vol.XXVII 1893,p.782.

Berichte d.botan.Gesell. 1892,p.390.

Annales de l'Inst Pasteur t.V.1891, No.2 (Em.Laurent)

American Naturalist Vol.29,p.898

Berichte d.botan.Gesell. 1892,p.170 (B.Frank)

Comptes rendus de l'Acad (Paris) t 123,p.407 (D.Clos)

" " " " " t 111,p.754 (Em.Laurent)

" " " " " t 123,p.666 (C.Naudin)

EXPLANATION OF PLATES.

Plate 1.

General view of transverse section of normal root. (1 in. eye, low obj.)

Plate 2.

Pith region in transverse section of normal root taking in part of endodermis. (2 in. eye, high obj.)

Plate 3.

A portion of the tissue in the region of the endodermis, 1 in. eye, high obj.) transverse.

Plate 4.

A portion of the normal parenchymatous tissue showing intercellular spaces. (1 in. eye, high obj.)

Plate 5.

This is a diagramatic drawing to bring out proportion. (1 in. eye, low obj.) longitudinal section.

Plate 6.

A diagramatic drawing of tubercle and root to show distribution of different kinds of tissue. (2 in. eye, low obj.)

Plate 7.

Transverse section of tubercle. (2 in. eye, low obj.)

Plate 8.

The infected tissue of the tubercle enlarged.(2 in.eye, high obj.)

Plate 9.

Enlarged fibro-vascular bundle from tubercle, (1 in. eye, high obj.) transverse section.

Plate 10.

Fig.1, Outermost cells on tubercle.

Fig.11, Outermost cells on normal root.

(Both 2 in. eye, high obj.) Longitudinal section.

Plate 11.

Fig.1, Longitudinal section of endodermis region in normal root.

Fig.11, Longitudinal section of the same region as that of Fig.1, but in the tubercle. (2 in. eye, high obj.)

PLATE 1

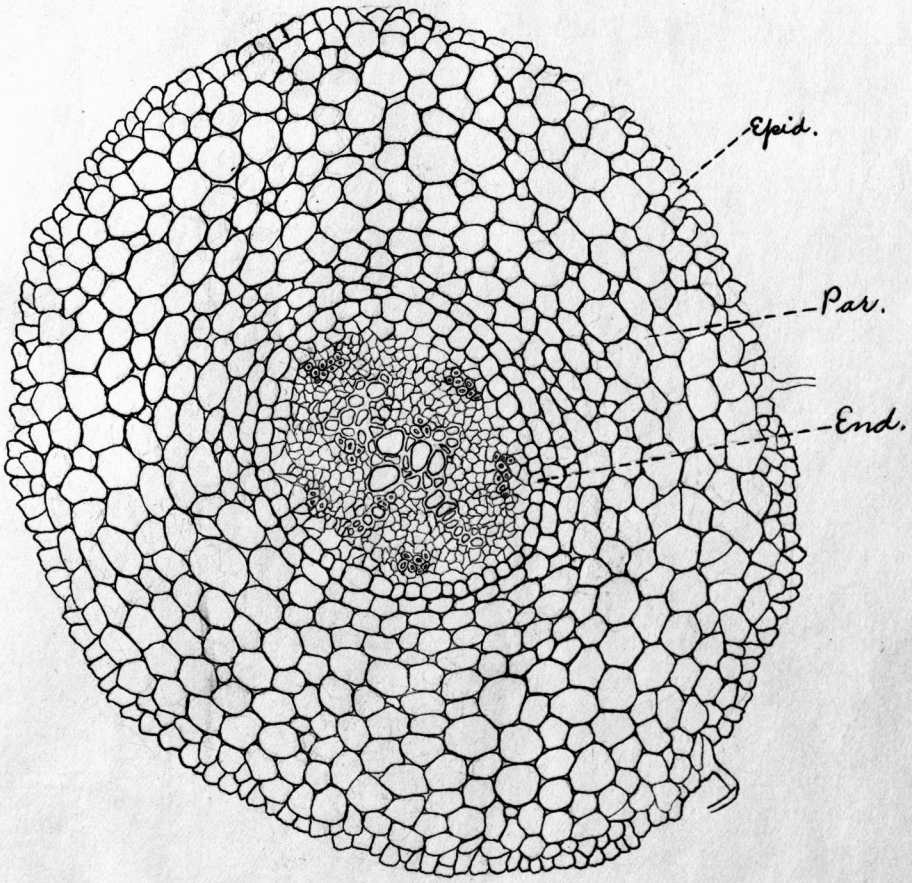


PLATE II,

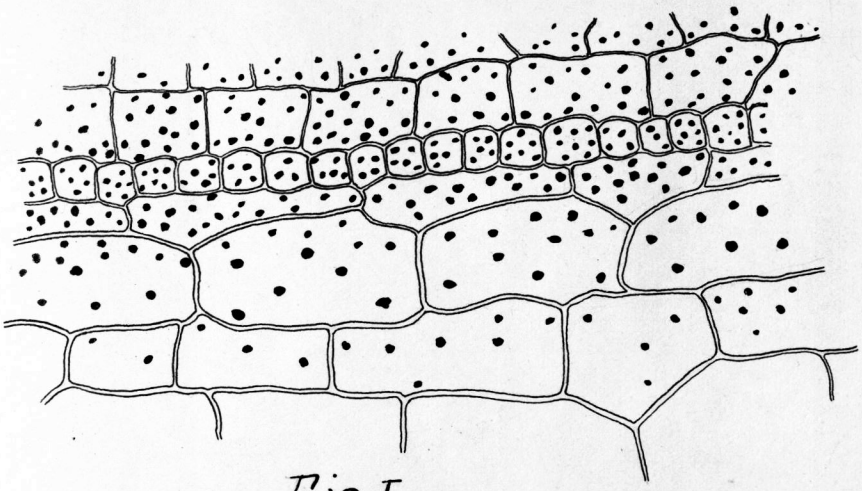


Fig I.

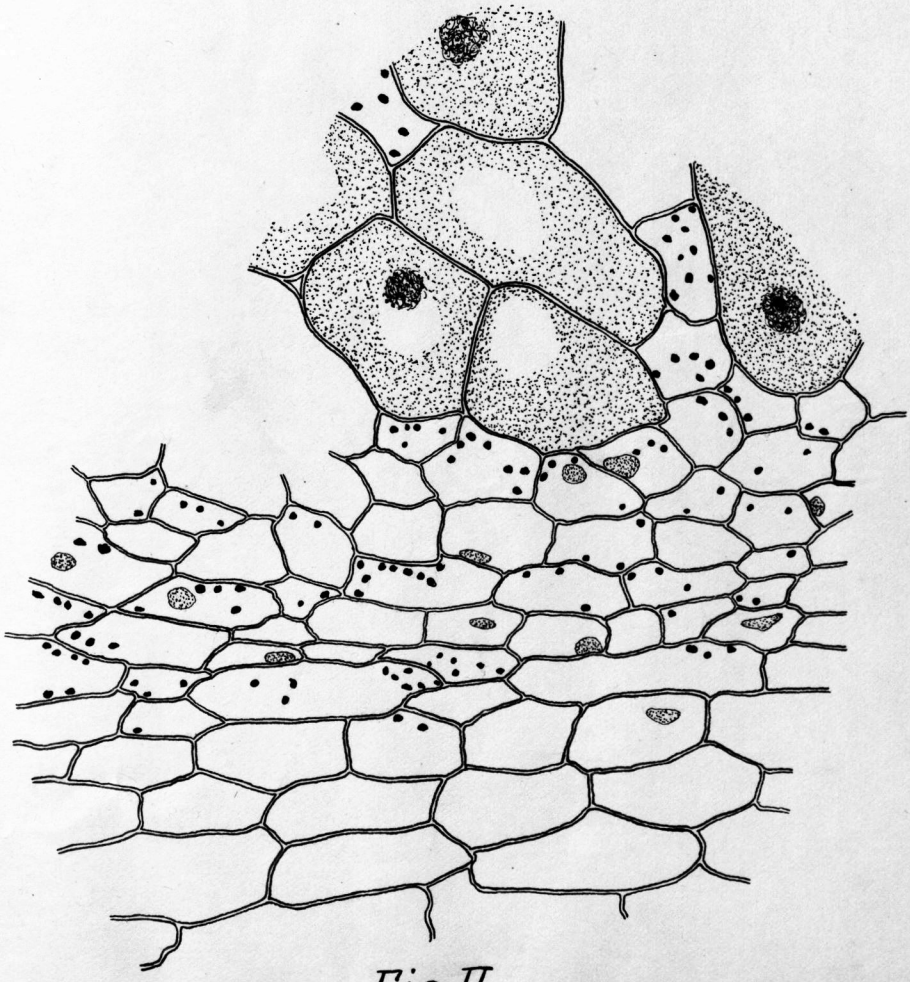


Fig II.

PLATE 3.

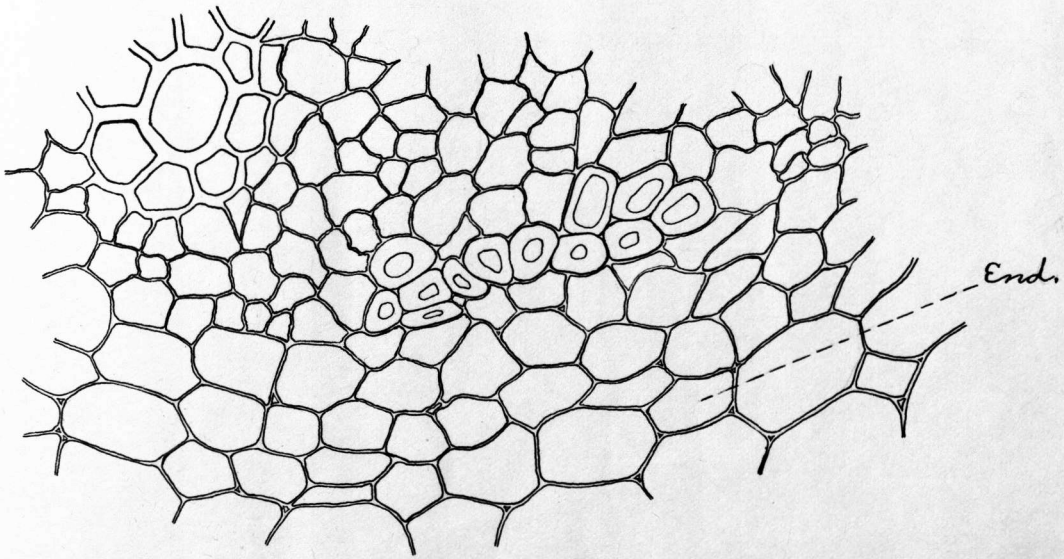


PLATE 2.

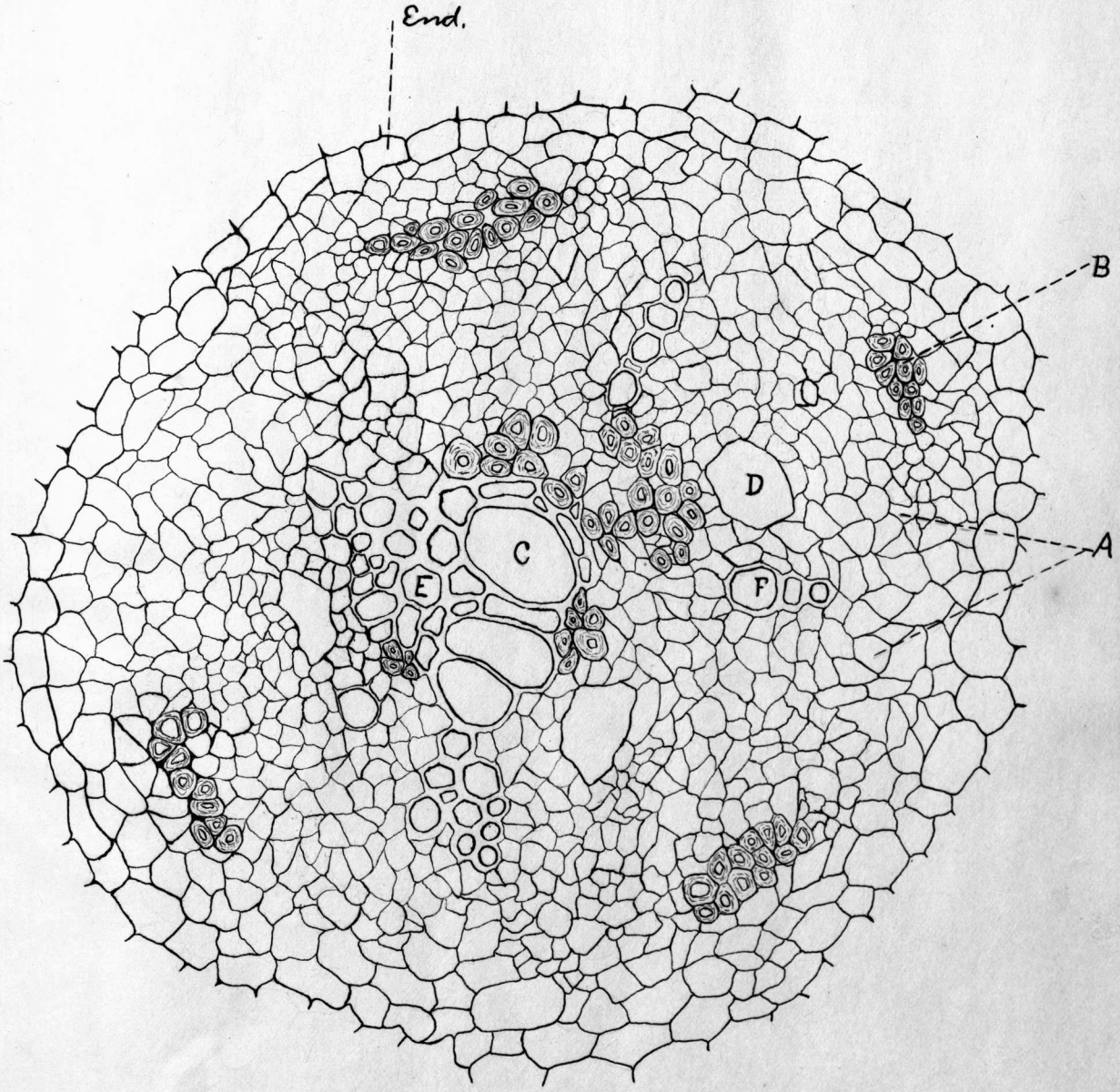


PLATE 4.

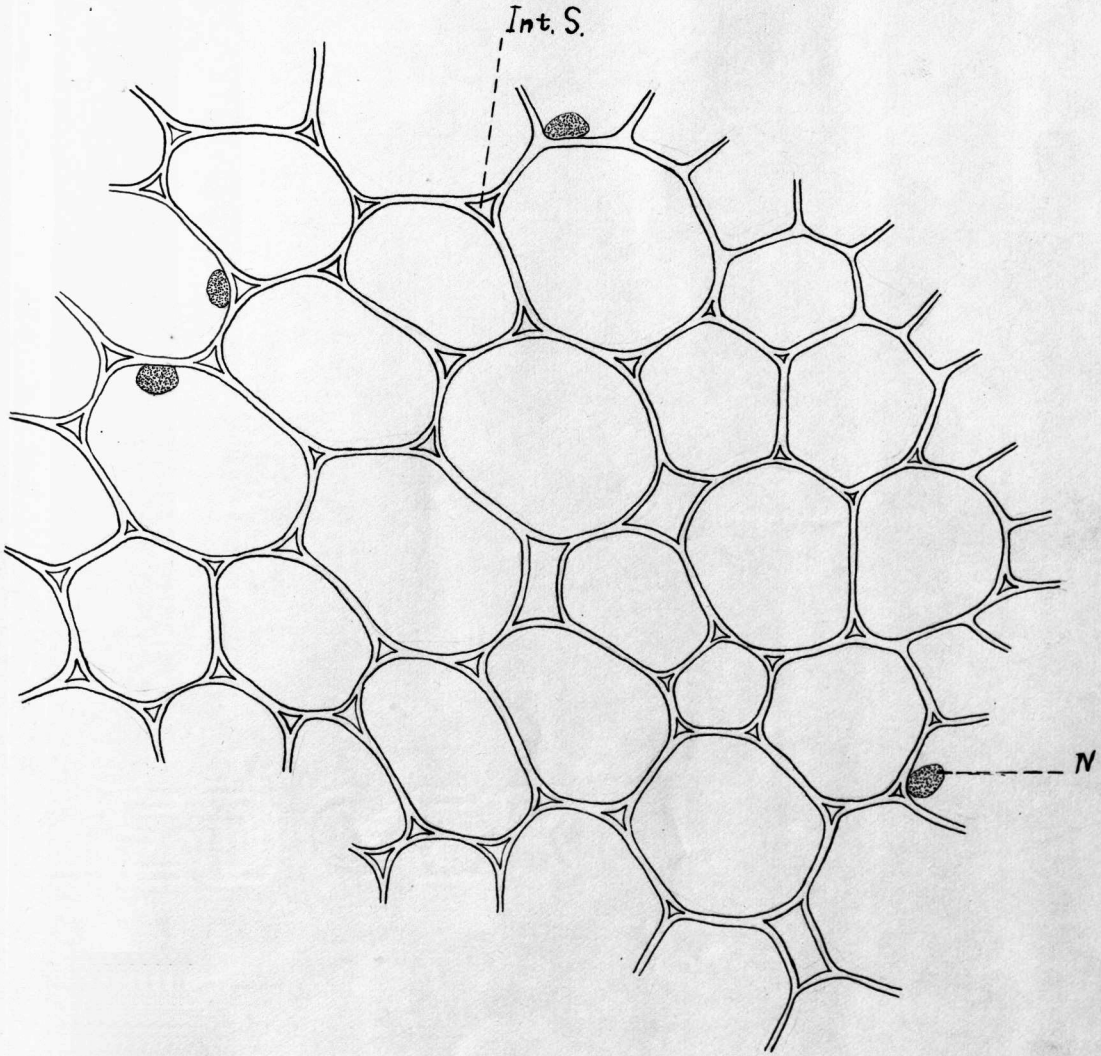


PLATE 5.

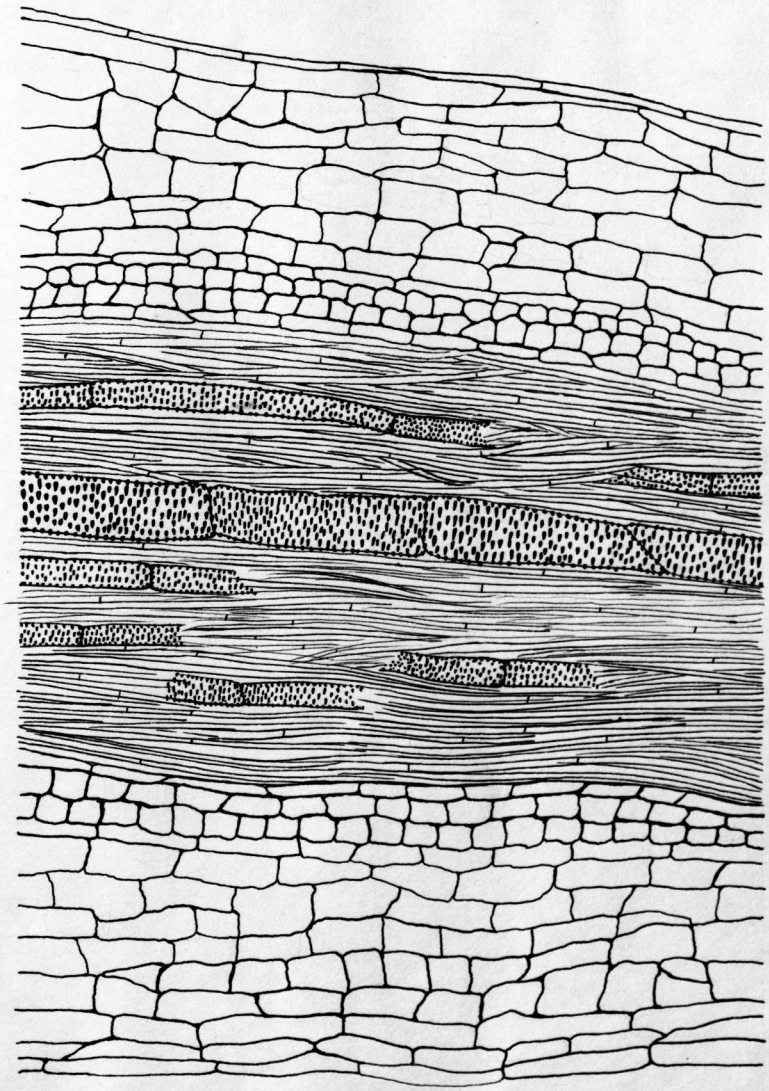


PLATE 6.

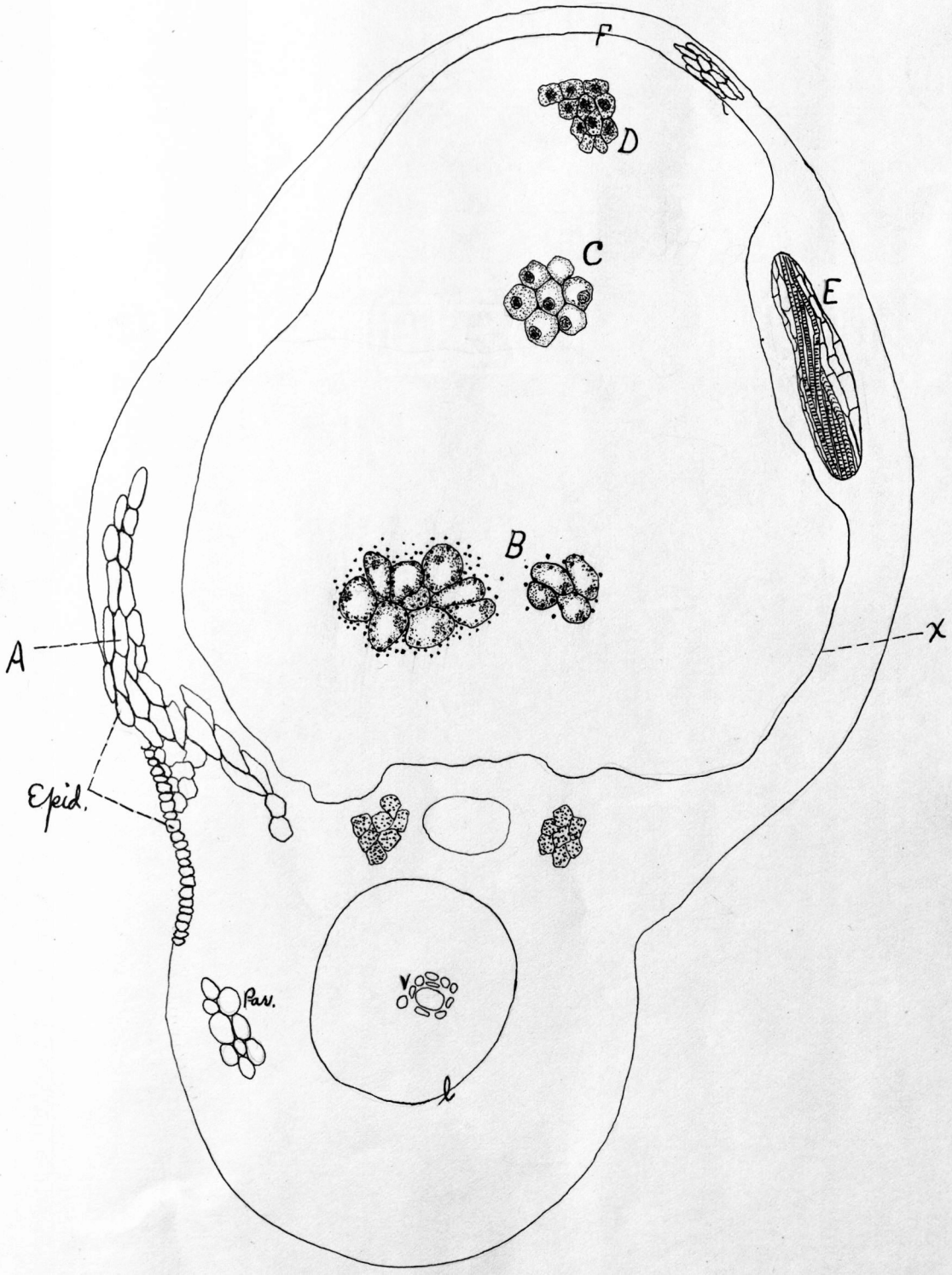


PLATE 7.

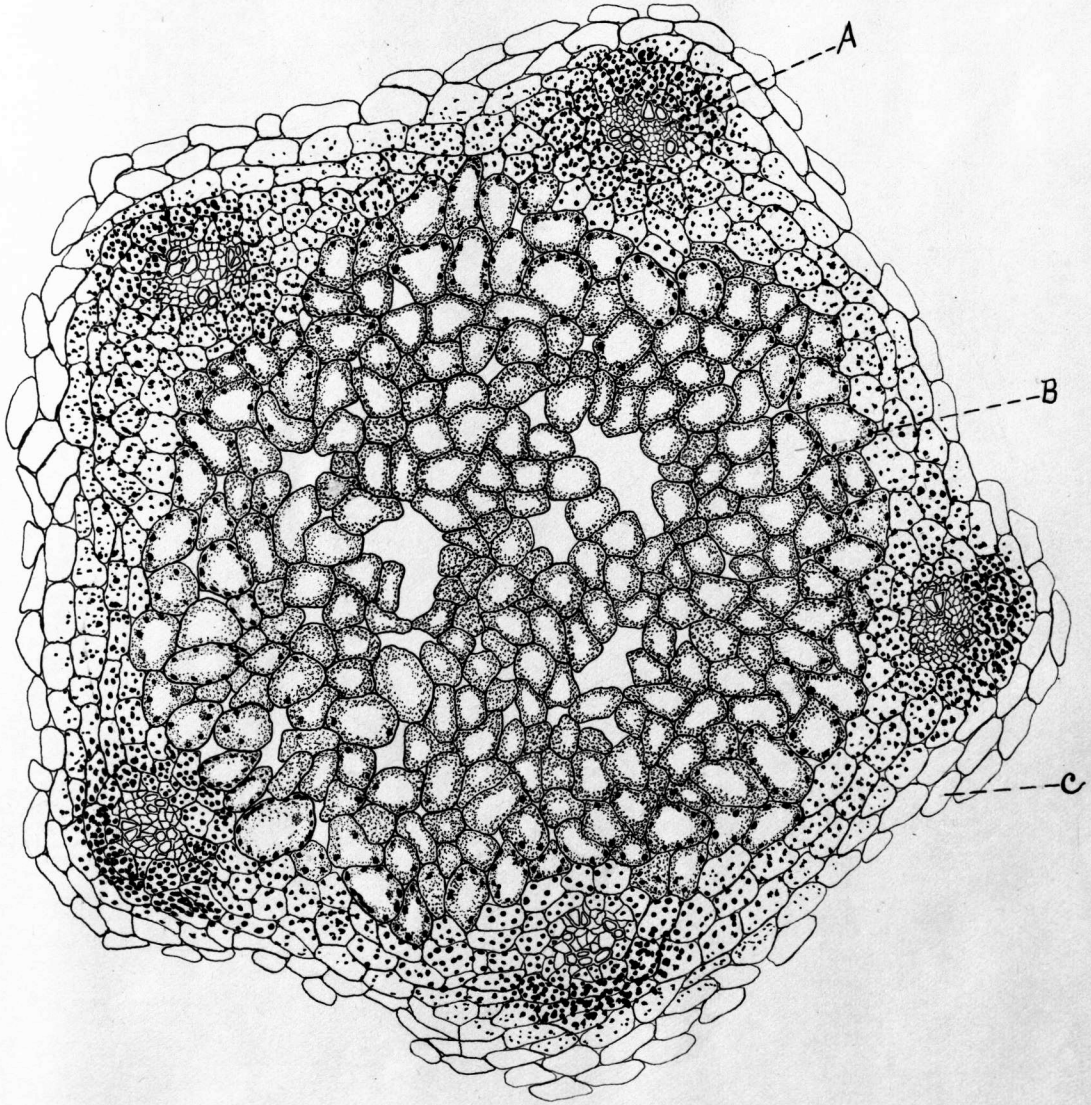


PLATE 8.

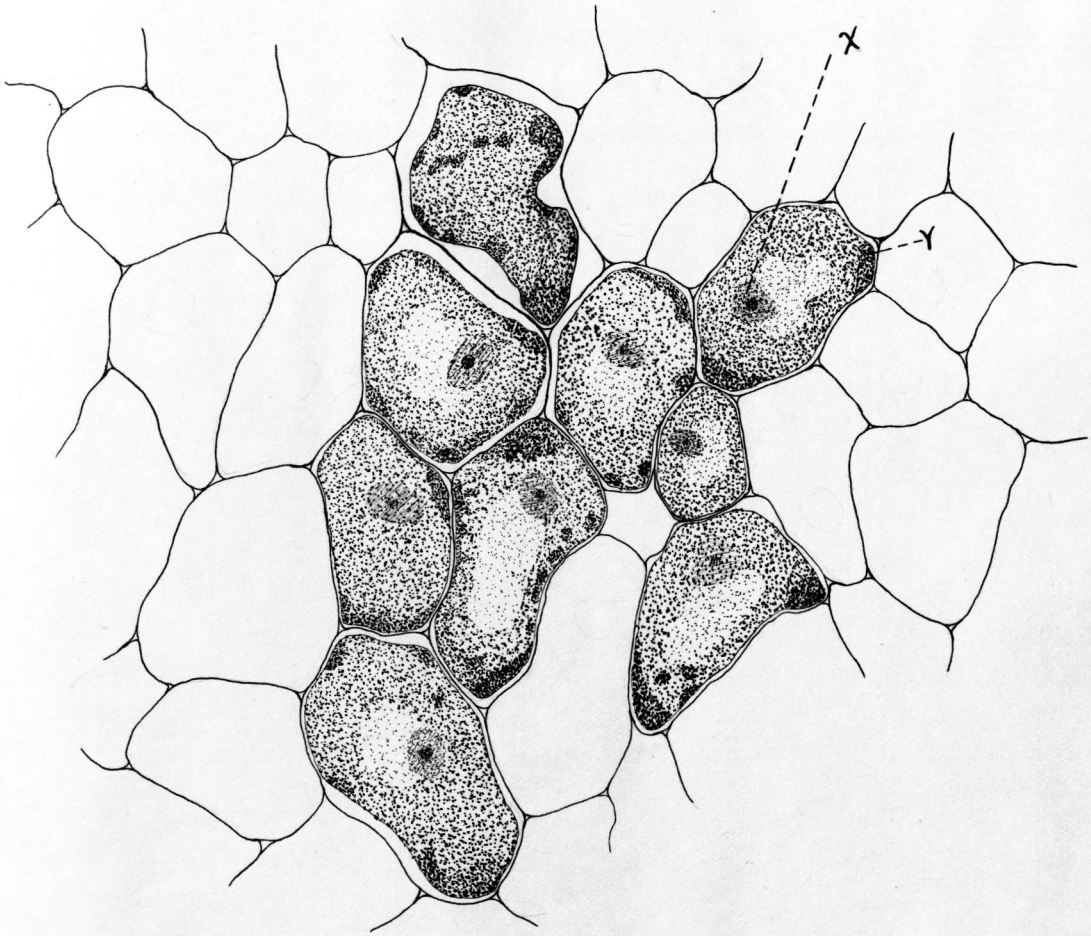


PLATE 9.

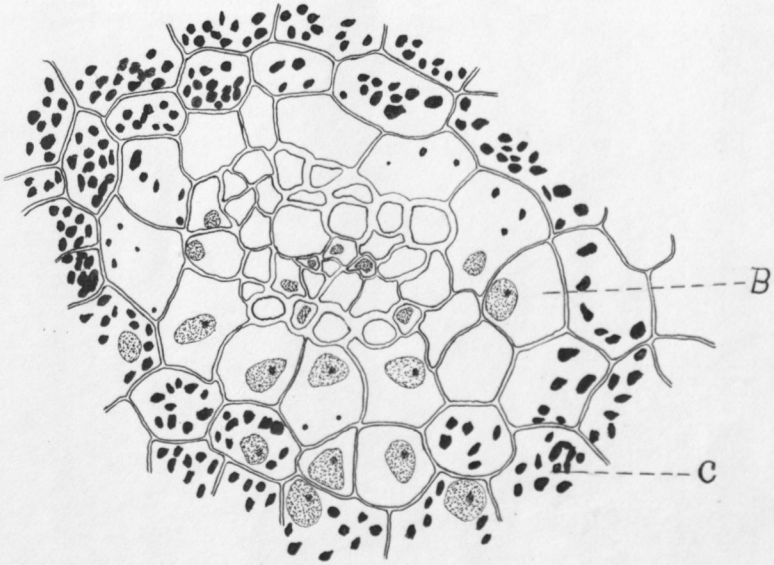


PLATE 10.

Fig. I.

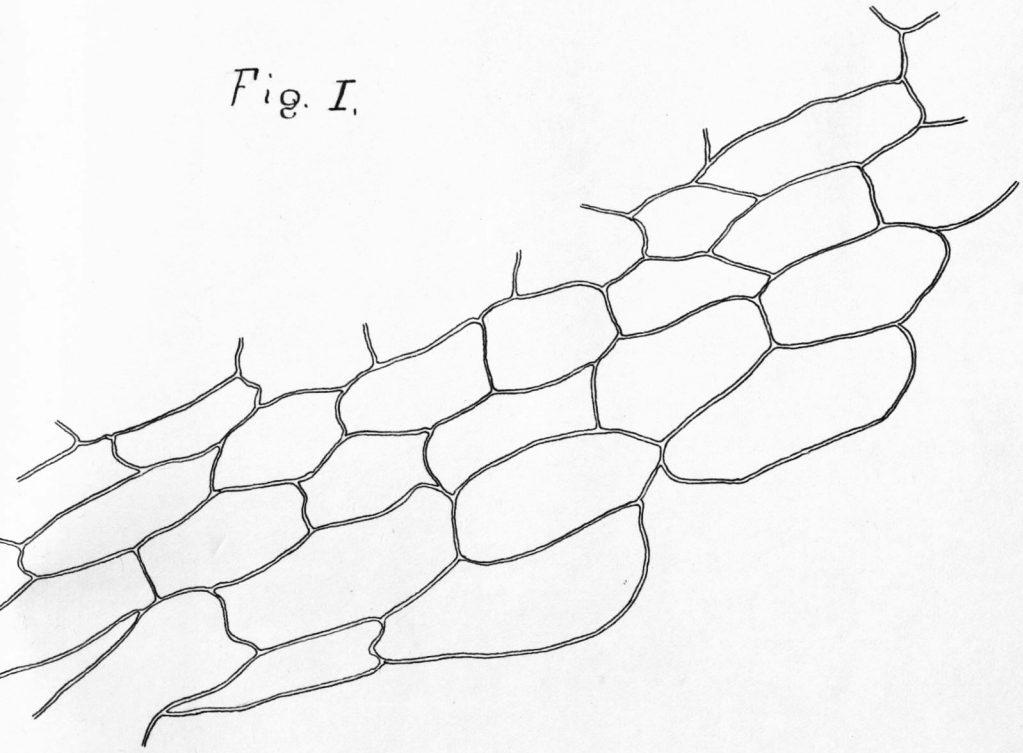
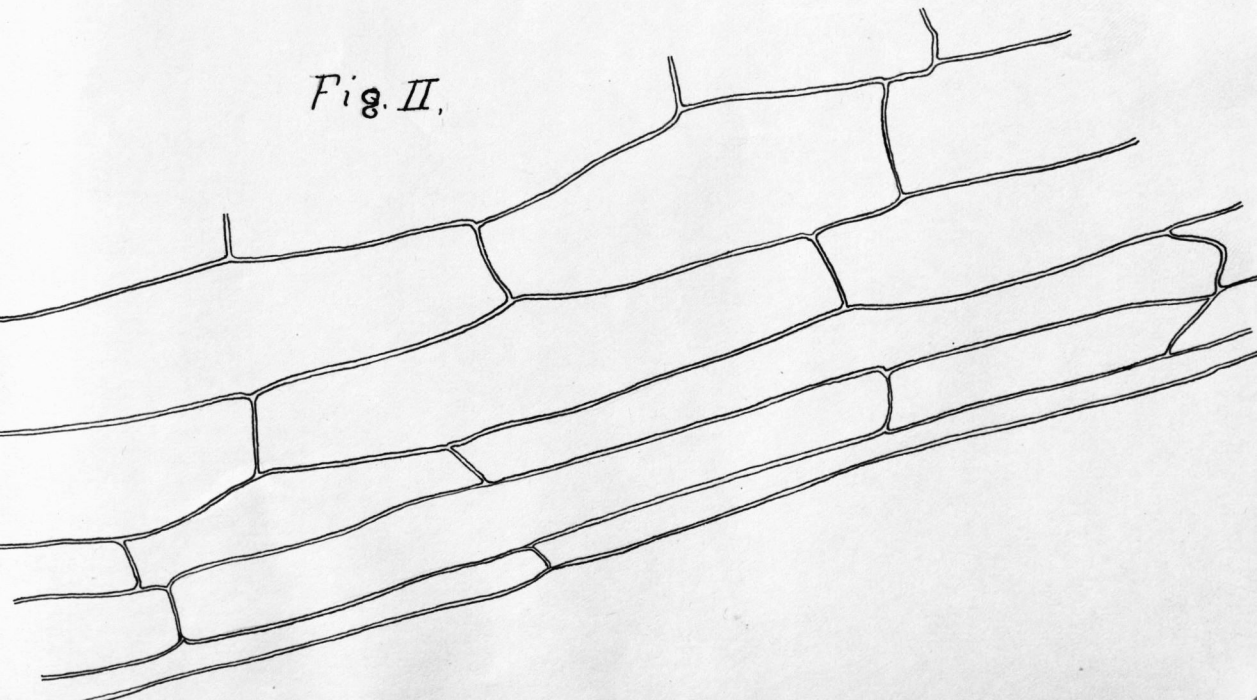


Fig. II.



APPROVED L. S. Cheney