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The Effects of Cigarette Smoke on the Seedlings of Vicia Sativa

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THE EFFECTS OF CIGARETTE SMOKE ON THE SEEDLINGS OF VICIA SATIVA

BY

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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ENTITLED The effects of Cigarette Smoke on the Seedlings of Vicia Sativa

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF Master Of Arts.

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In Charge of Major Work

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Recommendation concurred in:

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INTRODUCTION

In a recent number of the "Sitzungsberichte der Kaiserlichen Akadamie der Wissenshaften, zu wien Mathematische Naturwissenshaftliche Klasse", (1911), appeared an article by Hans Molisch entitled, "Uber den Einfluss des Tabakrauches auf die Pflanze".

In his introduction he states that he was led to investigate the effect of tobacco smoke on plants because they are so frequently subjected to its influence in our laboratories and dwellings. Since tobacco smoke is used for fumigation in our green-houses, it was thot worth while to study its effects on the plant in greater detail. The following studies were taken up with the view of learning more especially the complex effects of the smoke on growth and development.

In his experiments, Molisch used vetch seedlings, Vicia sativa, which he had grown on tulle net, stretched over the mouths of jars of water. Two such jars were then covered with bell jars, into one of which three puffs of the tobacco smoke were blown. The experiments were conducted in the dark at a temperature of 16° to 19° C. Twenty-four hours later the results were as follows: The stems of the seedlings in pure air had grown one and one-half centimeters, while those exposed to the tobacco smoke had not grown in length but showed a marked increase in thickness just below the growing point. After a period of six days the average length of the seedlings in pure

air was 13.9 cm., and that of the seedlings in the smoke 0.75 cm. Further differences were as follows:

Pure air.	Air with tobacco smoke.
Stems grew vertically	Stems grew horizontally
Stems slender	Stems greatly thickened
Roots long, 8 to 12 cm.	Roots short (1 to 3 cm.), ends curled, often bend at right angles.
Leaf buds red	Leaf buds pale yellow

In experiments similar to the above carried on in the light, the results were even more pronounced.

Seedlings somewhat older than the above subjected to tobacco smoke responded as follows: The stem in the region of elongation greatly thickened and grew horizontally or obliquely rather than vertically. Seedlings brought into pure air after two days treatment with tobacco smoke reassumed the vertical position and continued to development normally.

When vetch, grown in pots of earth, were subjected to the influence of tobacco smoke, the effects at first were the same as in water cultures. On the fourth day, however, the seedlings exposed to the smoke began to increase rapidly in length and continued to grow at a normal rate. The slender stem, the formation of the coloring matter, etc., showed that the effect of the smoke had disappeared and a normal growth was established. Molisch is inclined to believe that the porous earthen pots in four days sufficiently purified the air by the absorption of the harmful constituents of the smoke to allow normal

growth to take place.

Tobacco smoke is a mixture of complex chemical compounds, difficult of analysis. It is important that we know the constituents that so seriously interfere with the growth processes. Molisch upon analysing the smoke found the main constituents to be nicotine, pyridine, hydrogen sulfide, and carbon monoxide.

Seedlings exposed under bell jars to the fumes of five drops of nicotine for from twenty-four to forty-eight hours showed no ill effects. They could not be distinguished from the normal checks. Nicotine then can be eliminated from consideration.

Jars arranged as the above but furnished with three drops of pyridine in place of five drops of nicotine gave results as follows:

In pure air.	In pyridine air.
Stems 8 to 12 cm. long	Stems 2 to 3.5 cm. long
Stems upright	Stems oblique or horizontal
Buds with red coloring matter	No red coloring matter in buds

It is clear that pyridine has a decided effect. It inhibited the growth in length and caused the seedlings to bend over and grow horizontally as did those treated with tobacco smoke. They showed, however, this important difference, namely, there was no apparent thickening below the growing point of the stem.

Seedlings exposed to hydrogen sulfide wilted and died.

Seedlings treated with a quantity of carbon monoxide cor-

responding with that present in the tobacco smoke under a cultural vessel did not grow in length, but increased in thickness and the red coloring matter was absent. These results led Molisch to think that the carbon monoxide is the constituent of the tobacco smoke most harmful to plants. This belief was further confirmed by the fact that the smoke from filter paper, writing paper, wood shavings, etc., gave the same effects as did tobacco smoke. Beans and related plants gave the same results as were obtained with the vetch.

The experiments of Molisch were recently repeated by Crocker and Knight, of Chicago, and their results led them to very different conclusions. In a recent issue of Science (1913) they state their conclusions as follows: "In the burning of organic compounds the destructive distillation carbon bearing gases, CO, C_2H_2, C_2H_4 and CH_4 are not generally completely burned and may be the source of the injury in the smoke. Exact experiments on the delicate sweet pea seedlings, Early Cromer, shows that smoke from cigarets, cigars and cellulose-paper-cigarets does not contain sufficient CO, C_2H_2 or CH_4 to determine 1/200 of the toxicity of the smoke. This leaves C_2H_4, which we have already shown as extremely toxic to plants, as the substance probably determining the toxicity. They further maintain that the effect of ethylene on sweet pea seedlings is a triple response since it is marked by a reduction in the rate of the elongation, increased growth in diameter and diageotropism. They determined the effect of more than

fifty gases and vapors upon seedlings, including paint solvents, the possible impurities of laboratory air, the main constituents of illuminating gas, and the principal distillation products of coal tar, and find that sweet pea seedlings constitute a very reliable and extremely delicate test object for ethylene. Used as a test object for determining traces of injurious gases, they are from 2000 to 5000 times more delicate than the best chemical methods known.

I repeated these experiments once again with the view of studying the effect of injurious constituent or constituents of tobacco smoke on the cell, and to follow in greater detail the manner of response.

PHYSIOLOGICAL OBSERVATION

The seeds of the vetch, Vicia sativa, were soaked in water for twelve hours and then placed between moist filter paper for germination. In about two days, when the roots were approximately one inch long, the seedlings were placed on perforated paraffin disks, the roots extending through the holes into the water of the jar. Each jar was then covered with a bell jar of 1500 cc. capacity and placed in a dark room. The temperature of this room was practically constant at 27° C. The seedlings remained in the jars furnished with pure air for from twelve to eighteen hours, or until the stems were about one cm. high. Then three puffs of cigaret smoke were blown under the bell jars by means of a bent glass tube.

In from eight to ten hours a slight difference could be detected between the normal and the treated seedlings. The checks showed an average increase in length of one and one half centimeters; in the treated seedlings growth was entirely inhibited. The treated seedlings showed a slight tendency to thicken just below the recurved tip. Twenty-four hours after treatment the following differences were very marked:

In pure air.	In cigaret smoke.
Growth in length average 4.6 cm.	Growth in length average 2cm.
Stems straight and slender	Stems swollen below the flexure.
Leaves borne vertically except the growing point itself reflexed. (Fig. 1, Plate VIII)	Growing point grows at right angles to main axis (Fig. 2, Plate VIII)
Red pigment at base of growing point	No red pigment

After forty-eight hours a still greater difference was shown. The normal growth of the checks averaged 8-1/2 cm. as compared with 2-1/2 cm. in the treated. In the treated seedlings the enlargement under the flexure had markedly increased and had formed a double flexure (Fig. 2, Plate IX).

To determine the rate and manner of recovery, the above experiment was repeated and after an interval of two days the impure air was replaced by pure air. Four days from the beginning of the experiment or two days after admitting pure air, the growth in the normal averaged 8.87 cm. as compared to 6.67 cm. in the treated. This growth in the treated seedlings was distributed as follows: 1.47 cm. vertically, 1/2 cm. horizon-

tally, and then again 5.2 cm. vertically (Fig. 2, Plate X). The growth in pure air was normal.

One jar of the seedlings was exposed to the smoke for four days. The normal in this interval averaged 8.87 cm. as compared to 2.11 cm. in the treated. In the treated seedlings the growth in thickness was far greater on the inner side of the flexure and caused the stem to bend backward sufficiently to bring the growing point in a vertical position.

In experiments similar to the above but carried on in the light, the results were even more pronounced.

CYTOLOGICAL STUDY

In order to fix the protoplasmic constituents of the cells with their inclusions and to provide a means of comparing the effects of the different fixing agents, it seemed best to use both picric and chromic killing and fixing agents. The solutions used had the following composition:

A. 60 cc. of 1% chromic acid
   8 cc. of 2% osmic "
   4 cc. of glacial acetic acid
  72 cc. of distilled water

B. Picric acid, cold saturated solution - - 1 vol.
   Corrosive sublimate, " " - - 1 "
   Glacial acetic acid - - - - - - - - 1/2 vol.

One cannot obtain successful staining of all the cell constituents and cell inclusions by the use of a single stain.

Stems killed with the chromic acid were stained with Flemming's triple stain. This proved especially good for staining cell contents, more particularly in the swollen region. Stems killed with picric acid were stained with Heidenhaem's iron haemotoxylin or Delafield's haemotoxylin. Heidenhaem's iron haemotoxylin brought out nuclei and nucleoli especially well. Delafield's haemotoxylin stained the cell walls very distinctly and made it easy to follow the cell arrangement. The staining was done in the usual approved way.

We may distinguish the growing point of the vetch, as in stems in general, three phases of development. The formative is the first, and is characterized by comparatively small, regular cells with relatively large nuclei; large quantity of cytoplasm; thin cell walls, and frequent cell division. This group of cells makes up the primary meristem. The primary meristem is differentiated into three distinct layers, namely, the dermatogen, the periblem, and the plerome.

The rudiments of the leaves and new shoots first appear as small outgrowths from the periblem, due to a localized rapid growth of its cells.

The second phase of growth is the phase of elongation, caused by the rapid growth and development of the cells at the base of the growing point. The cells increase rapidly in size, and it is said by Davenport and others that this increase is due to vacuoles forming in the cytoplasm. Enlargement takes place in all the regions of the stem but in different degrees. The cells in the plerome, for example, greatly elongate, while

those of the periblem grow uniformly in every direction. When the cells of the second phase have attained their permanent size, internal differentiation takes place and the third phase of growth begins.

In the normal stem of the vetch seedling, Vicia sativa, (Fig.1, Plate VI), the growing tip is very small and low-dome-shaped. The leaves form as lateral outgrowths from the periblem. The cells of the tip (Fig.2, Plate VII), the primary meristem, are small and are arranged in regular rows about the elongated cells of the plerome. The nuclei are very large, occupying the greater part of the cell. Stems cut and fixed about eleven o'clock in the morning show a great many nuclei of the periblem cells in all stages of division. The chromosomes are relatively large, the nucleoli are very large and the hof is not distinct. The chromatin is distributed over the finely meshed reticulum in the form of granules of irregular size. The larger of the masses, karyosomes, nearly approach the nucleolus in size.

A little distance from the tip, the cells are somewhat enlarged, vacuolate, and with prominent nuclei. In the plerome the greatly elongated cells have become in part transformed into permanent vascular tissue. Still farther back from the tip, in a region below the strong flexure of the stem, the most marked cellular changes have occurred. The cells of the periblem have greatly elongated radially.

A cross section of the normal stem (Fig.1, Plate III)above

the flexure shows a very distinct central area of plerome cells in the process of forming the fibro-vascular elements. The cnetral cylinder is bounded by a well marked endodermis consisting of thick walled cells. The leaf traces are distinctly marked off in the cortex and lie near the surface. At intervals along the stem these connect with the central fibro-vascular bundles.

A cross section below the flexure (Fig.2, Plate III) is very similar to the one just described except that the parenchyma cells are larger and not so regular in form and the central xylem cells are more strongly developed.

The structure of the growing point of the seedling exposed to tobacco smoke (Fig.2, Plate VI and Fig.1, Plate VII) differs from the normal as follows: The periblem cells are very irregular in form and are not arranged in regular rows about the plerome. They give every appearance of sliding growth. The nuclei are relatively small and frequently are irregular in form. Mitotic division has ceased and consequently growth in length due to new cell formation does not take place. In a very few instances chromatin material was found deposited in the form of karyosomes on the reticulum. The nucleoli are very small and are surrounded by a large hof . The most striking difference between a normal stem and one exposed to tobacco smoke, as shown by Plates I and II, is to be found in the elongating region just below the flexure. Here the stem suddenly enlarges to form the characteristic swelling already de-

scribed. This enlargement is caused by a very marked localized growth of the cells of the periblem of this region.

It is to be noted that the cells on the inside of the flexure are radially elongated, thus differing markedly from the cells on the outside of the flexure. They are virtually free of all cell contents and their walls are much thinner than those of the normal.

Cross sections of treated and untreated stems (Figs.1 and 2, Plate III, and Plate IV) show well marked differences. The periblem cells of the stems exposed to the smoke are very much larger, irregular in outline and do not follow the normal arrangement. The leaf traces are located about half way between the cortical region and the plerome, while in the normal they are near the epidermis. The endodermis in treated stems is less clearly defined.

DISCUSSION

The irregular arrangement of the cells in the growing tips of the treated seedlings, as described above, is not an uncommon occurrence. Pfeffer (1903) says that normally the primitive arrangement of the new cell segments is only rarely maintained permanently. The arrangement undergoes a more or less marked secondary alterations and displacements as a result of subsequent growth in the elongating zones and still more owing to the heterogeneous tissue differentiation. Certain cells divide less frequently than others and hence become larger, although the rate of growth may be uniform. A growing tissue may

mould itself to the space available like so much plastic material. Frequently a cell or tissue makes room for its own growth by penetrating between other cells or by crushing other tissues which are unable to resist the pressure brought to bear upon them. It is, therefore, hardly surprising that certain cells should become larger than others and should penetrate between the latter by a process of sliding growth. It is this sliding growth which causes the irregular arrangement in the tip cells of the treated seedlings and for reasons as follows. The meristematic cells in different stages of development are differently affected by the smoke, and, since no mitotic division occurs, the cells least affected grow larger and force their way between the more affected and smaller cells.

The nucleolus in the resting stage of normal cells is very large. During mitotic division it grows smaller, often breaking up into granules before finally disappearing. There are four important theories concerning the function of the nucleolus. Hertwig believes it to be a reserve chromatin material: Haeker says it is waste material formed during the cell activity: Strasburger claims it is reserve food matter for kinoplasmic activity: Hottes claims it is reserve material for the general activities of the cell. In the treated seedlings the nucleoli are much reduced in size. Mitotic division is entirely inhibited by the smoke, but the cells continue to grow, and the general cell activities continue. The hof around the nucleolus is very large. The theory concerning the hof is that it is

composed of dissolved nucleolar matter which does not stain. These facts seemingly substantiate the last theory mentioned, namely, that the nucleolus is reserve food material for the activities of the cell.

It has been shown by Sachs (1882), Hofmeister, Hering (1896) and others that whenever growth of the tip cells is inhibited in any way, either chemically or mechanically, an enlargement always takes place in the elongating region. Hering (1896) proved this in his experiments by placing tips of the seedlings in plaster of paris casts, so they could not grow, and obtained the swelling similar to that caused by the cigaret smoke. In the growing tip of the treated seedlings, growth due to cell division is checked or entirely inhibited. But actual growth in the cells of the elongating region still continues although it is not a growth in length as in the normal but one in breadth. Jost (1903) speaks of this relationship as growth correlations. He says that when different organs of a plant body differ in structure and function, they necessarily influence each other in-asmuch as the performance of a definite function in one organ is essential to the carrying out of other functions in other organs. Harmonious development is possible only if correlations exist. The effect of the smoke on the tip cells causes the enlargement which is formed below the flexure because of the correlation which exists between the growing tip and the elongating region.

CONCLUSIONS

The effects of cigaret smoke on seedlings of Vicia sativa are as follows:

1. There is a marked reduction in the rate of elongation.

2. The growth in diameter is greatly increased throughout the whole seedling, and a large swelling forms in the elongating region.

3. Diageotropism replaces negative geotropism.

4. The smoke affects directly the cells of the growing tip. Some cells are affected more than others and this causes a sliding growth. Mitotic division is entirely inhibited. The nuclear matter is condensed, and the nucleoluar substance is reduced and at the same time the hof is increased.

Because of the correlations of growth which take place in the plant body, the checking of the growth of the growing tip causes the enlargement in the elongating region.

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EXPLANATION OF PLATES

All the figures were drawn by means of camera lucida.

Plate I. Longitudinal section of a normal seedling five days old.

Plate II. Longitudinal section of a seedling exposed to cigaret smoke three days.

Plates I and II made with two-inch eye-piece and two-inch objective. Camera lucida was used for general outline.

Plate III. Fig. 1. Cross section of a normal seedling above the flexure; (a) plerome; (b) leaf traces; (c) periblem; (d) cortex. Fig. 2. Cross section of a seedling below the flexure, esposed to smoke twelve hours; (a) pleurome; (b) leaf traces; (c) periblem; (d) cortex.

Plate IV. Cross section of seedling below the flexure exposed to smoke three days; (a) plerome; (b) leaf traces; (c) periblem; (d) cortex.

Plate V. Fig. 1. Cross section of a normal seedling taken above the flexure; (a) plerome; (b) leaf traces; (c) periblem; (d) cortex. Fig. 2. Cross section of seedling from above the flexure, exposed to the smoke three days.

Plates III, IV and V made with two-inch eye-piece and with 2/3 objective.

Plate VI. Fig. 1. Longitudinal section of growing tip of normal seedling. Fig. 2. Longitudinal section of growing tip of seedling exposed to smoke three days. These were

drawn with one-inch eye-piece and 1/6 objective.

Plate VII. Fig. 1. Cells from the growing tip of a seedling treated three days to cigarette smoke. Fig. 2. Cells from the growing tip of a normal seedling. These drawings were made with a one-inch eye-piece and 1/12 objective.

All the drawings were reduced one-third.

Photographs

Plate VIII.

Fig. 1. Normal seedlings.

Fig. 2. Seedlings exposed to cigarette smoke as soon as the stem appeared. Exposed three days.

Plate IX.

Fig. 1. Normal seedlings.

Fig. 2. Seedlings exposed to the smoke ten days.

Plate X. Seedlings the same as Fig. 2, Plate VIII, after they have recovered four days.

Plate XI.

Fig. 1. Normal seedlings.

Fig. 2. Seedlings exposed to cigarette smoke after they were one-half inch high. Exposed three days.

Plate I.

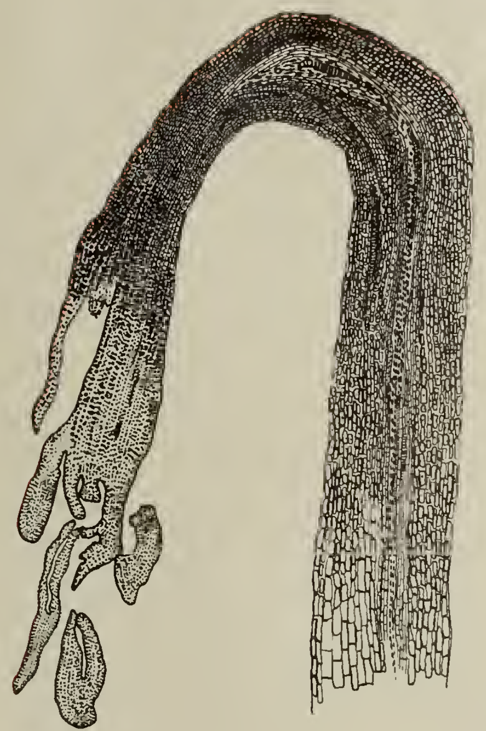


Plate II.

Plate III.

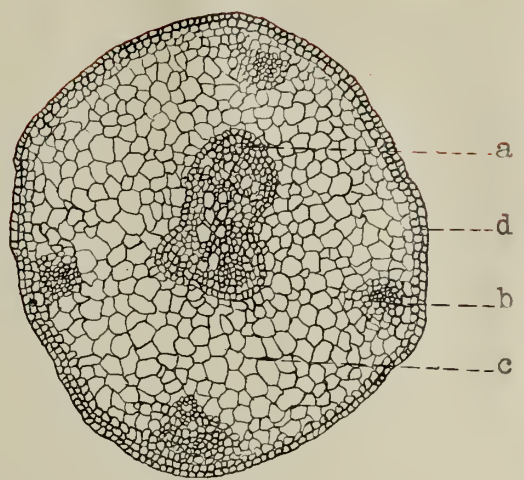


Fig 1

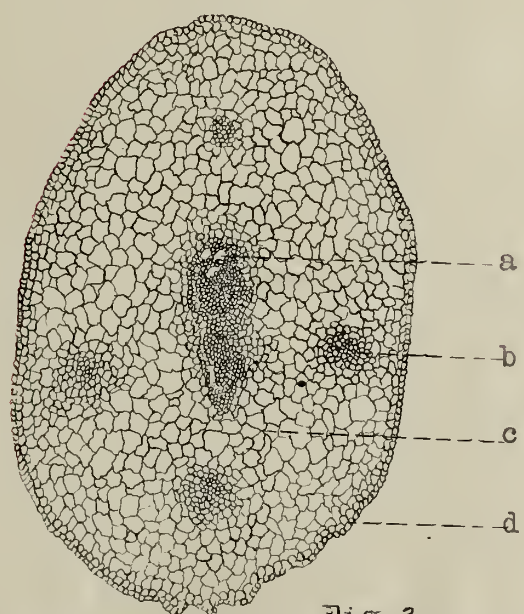


Fig 2

Plate IV.

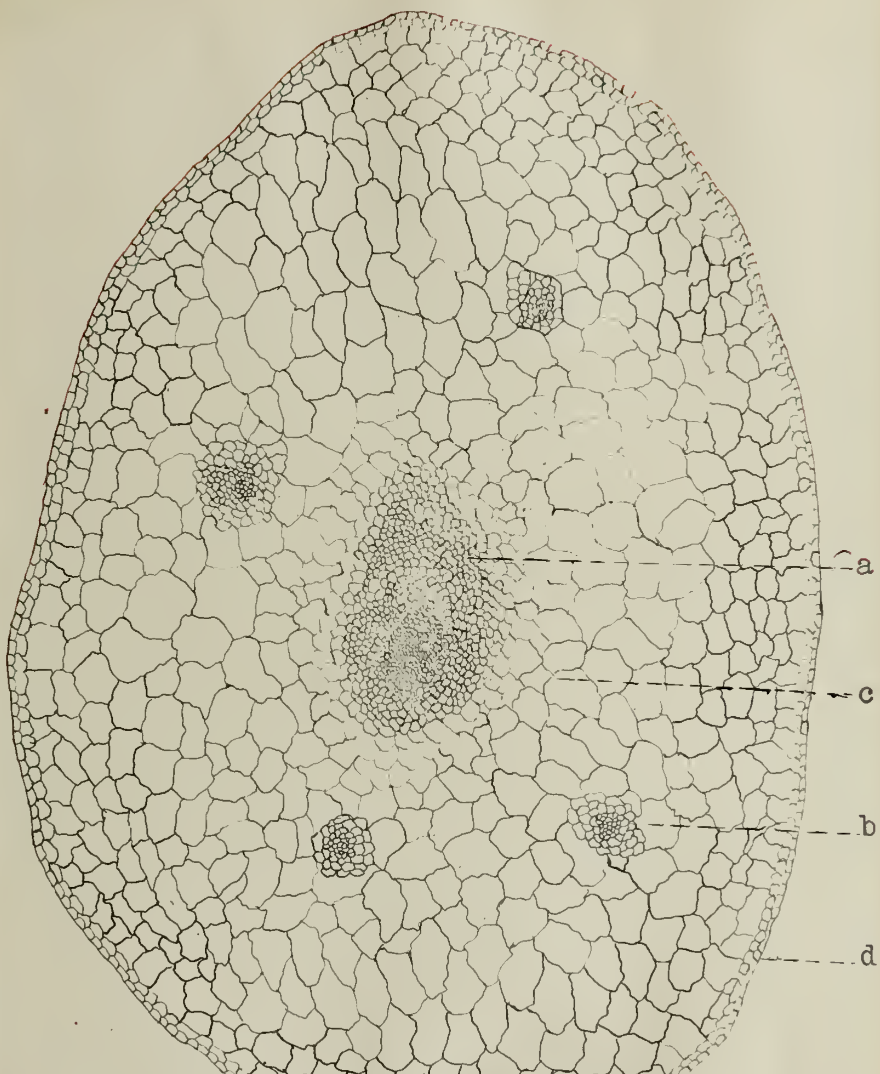


Plate V.

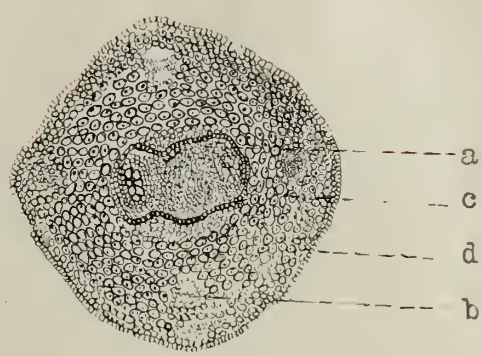


Fig 1

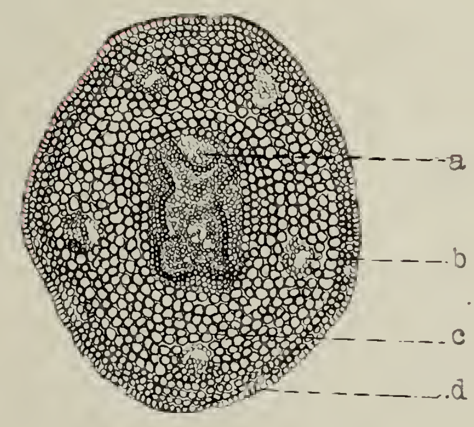


Fig 2

Plate VI.

Fig 1

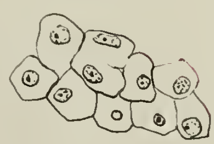


Fig 2

Plate VII.

Fig 2.

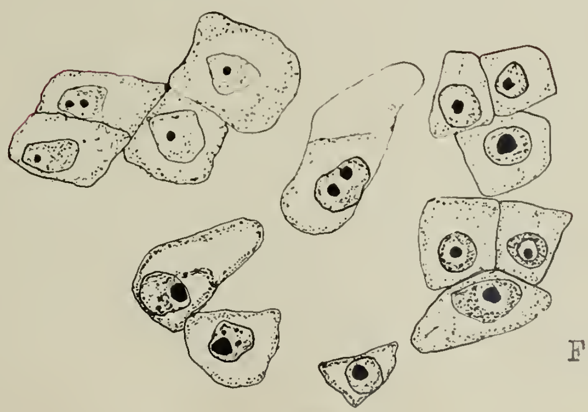
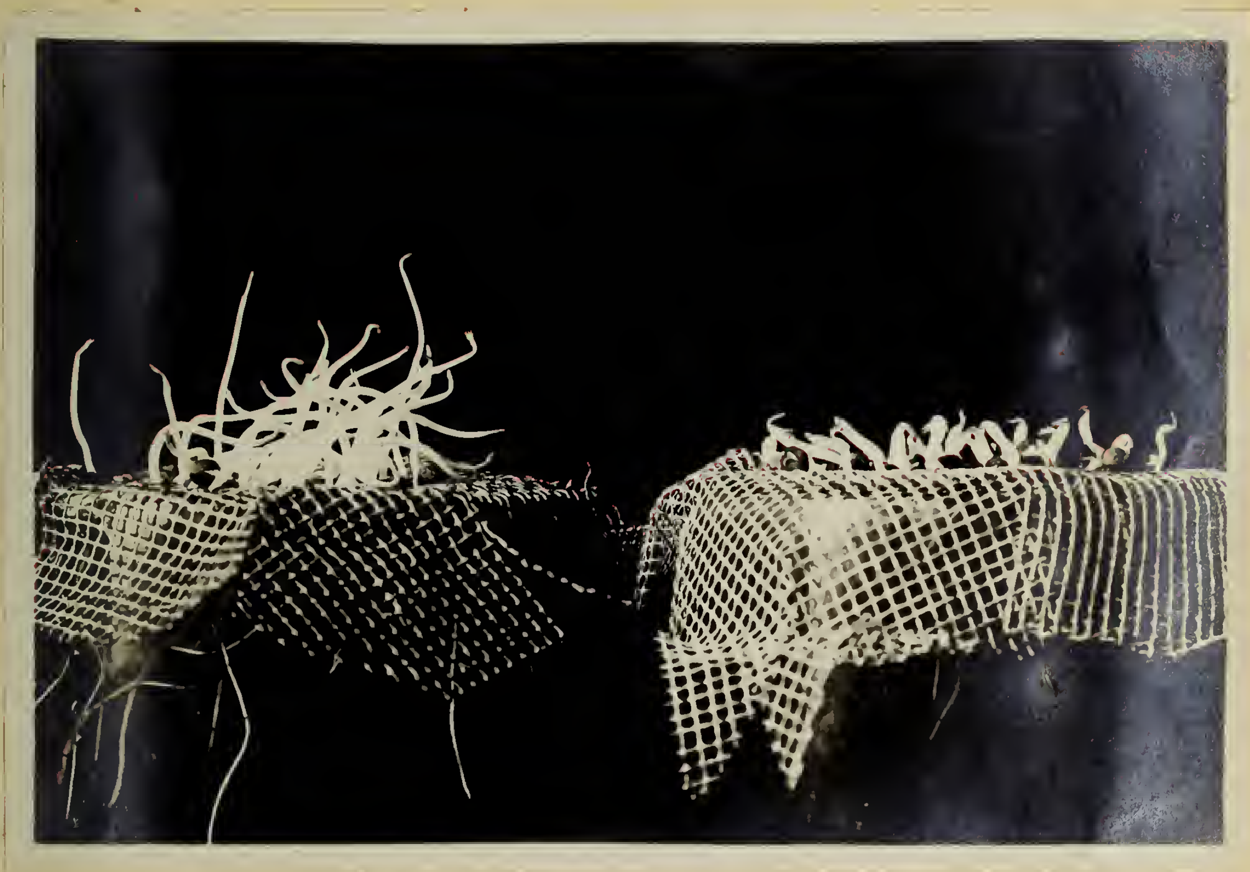


Fig 1

Plate VIII



I II

Plate IX

I II

Plate X

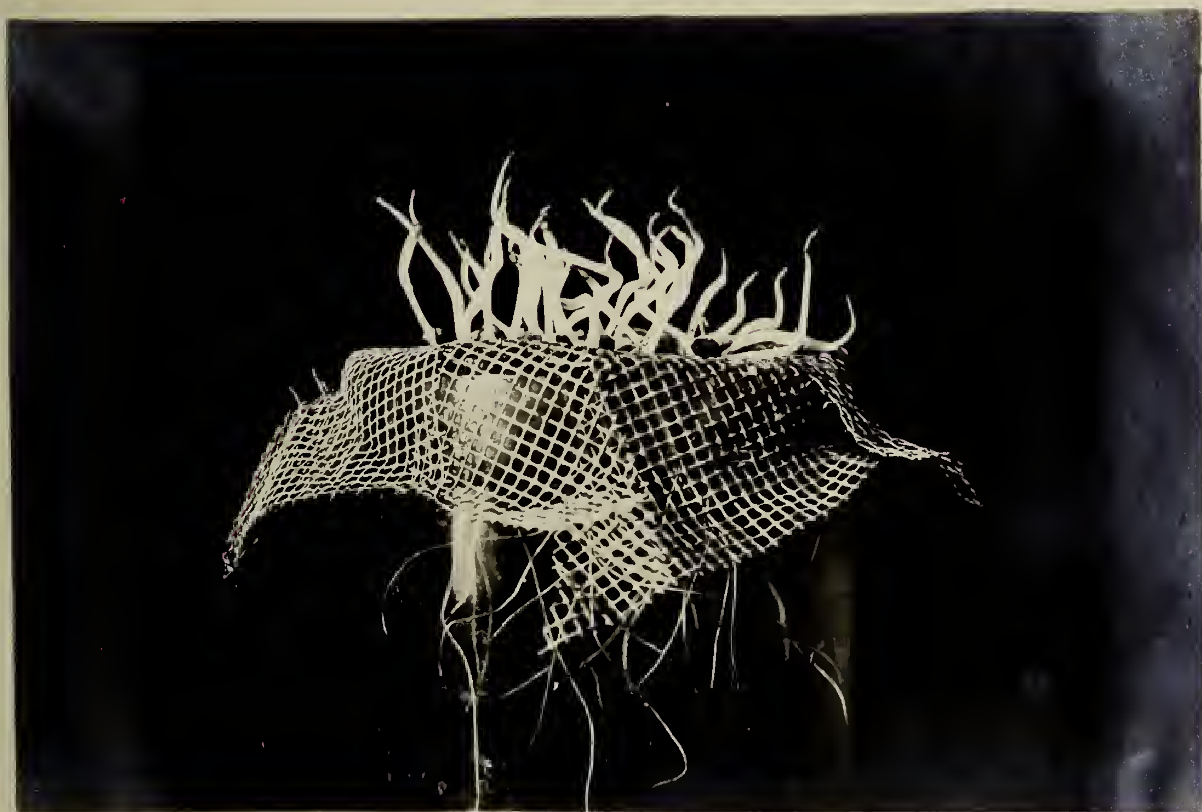


Plate XI

I II