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Further Experiments and Histological Investigations on Intumescences, with Some Observations on Nuclear Division in Pathological Tissues

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VII. *Further Experiments and Histological Investigations on Intumescences, with some Observations on Nuclear Division in Pathological Tissues.*

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[Plates 14–17.]

Introductory.

In a paper published in 1900* a description was given of certain pathological outgrowths, first called *intumescences* by Sorauer, but known as *œdemata* to American writers, occurring spontaneously on *Hibiscus vitifolius*, Linn. The paper is mainly *anatomical*, and the various modifications of intumescences found on the stems and leaves are described. On the stem the outgrowths originate by cell division, which is followed by the elongation of the cells concerned. At a later stage the stem intumescence is cut off by cork, so that the cells above the layer of cork die and collapse.

In leaf intumescences there is less cell division and no formation of cork. Preliminary experiments were made to determine the conditions under which intumescences arise, and showed the importance of changes in the external conditions in connection with the question of the formation of outgrowths. Strong and healthy plants, *grown in the open air*, did not develop intumescences, nor did very weakly plants grown in a cool and poorly lighted, though damp, greenhouse. On the other hand, plants grown in warm, damp, well-lighted greenhouses produced intumescences in varying number and degree.

A second paper published in 1901† deals chiefly with the *external conditions*, and records the results of a series of experiments made with *Hibiscus vitifolius* to test the influence of *moisture*, *light*, and *heat* upon the formation of intumescences. The result of these experiments showed that while *moist air* is essential, moist soil has no effect if the aerial parts of the plant are in dry air. But outgrowths are not formed when a shoot is submerged in water. In warm dry air no intumescences are formed, and the rapid healthy growth of the plant is promoted. If a shoot of such a healthy plant, while still attached to the main stem, is isolated in a damp

* Dale (1).

† Dale (2).

 15.1.06.

atmosphere, well lighted and heated, a great number of large intumescences are formed in a couple of days.

The importance of *illumination* in *Hibiscus* is shown by the fact that intumescences will form under clear glass, red, yellow, and white-washed glass, but not under blue or green glass, nor in a poor light, nor in darkness. But in some later experiments with *Populus tremula*, KÜSTER'S* results seem to show that light is not necessary in all cases: these exceptional results will be examined subsequently.

Again, a *high temperature* is necessary in the case of *Hibiscus*, but not in that of *Populus tremula*. Still, my experiments with various plants, including *Populus tremula*, show that, *ceteris paribus*, intumescences form more rapidly at a high than a low temperature, though the minimum temperature varies in different plants.

The present paper is partly experimental, and is partly concerned with a consideration of the *biological factors*—*i.e.*, the state of the plant, and especially with the *internal* conditions which influence the formation of intumescences. It contains the results of work done during the last three years, and includes some anatomical and cytological investigations.

1. *Experimental Results.*

Intumescences have been observed occurring spontaneously in the following plants by previous investigators: by SORAUER,† in *Dracena angustifolia* and other species, in *Cassia tomentosa*, several species of *Acacia*, *Vitis* (two kinds of outgrowths), *Lavatera trimestris*, *Malope grandiflora*, several species of *Eucalyptus*, in *Hedera helix*, *Impatiens Sultani*, *Solanum Warscewiczii*, *Ficus elastica*; by FRANK,‡ in *Ribes aureum*, *Rosa*, *Phaseolus*, and *Yucca*; by MASTERS,§ in *Solanum tuberosum*; and by TOMASCHEK,|| in *Ampelopsis hederacea*.

During 1901, such intumescences were observed in the Cambridge Botanic Garden occurring spontaneously on about 20 species of Dicotyledons, but on no Monocotyledon.

With the exception of *Vitis* and *Ampelopsis*, the plants were all growing under glass. The outgrowths were nearly all formed during the Spring (most of them were noticed in May) on young and actively-growing leaves. The following is a list of the plants affected:—

Pavonia arabica, HOCHST (*Hibiscus flavus*).
Hibiscus esculentus, LINN.
,, *Abelmoschus*, LINN.
,, *Manihot*, LINN.
,, *vitifolius*, LINN.
Theobroma Cacao, LINN.
Acalypha marginata, SPRENG.
Piper ornatum, N.E. Br.
Gmelina hystrix, SCHULT.
Ampelopsis inconstans, MIG.
,, *hederacea*, DC.

* KÜSTER (9). † SORAUER (3, 4, and 5). ‡ FRANK (6). § MASTERS (7). || TOMASCHEK (8).

Eucalyptus diversicolor, F. MUELL.	*Vitis rotundifolia*, MICHX.
,, *melliodora*, A. CUNN.	,, *heterophylla*, THUNB.
,, *botryoides*, SM.	*Kendrickia Walkeri*, HOOK.
,, *resinifera*, SM.	*Ipomea Woodii*, N.E. Br.
,, *saligna*, SM.	*Cassia floribunda*, CAV.

In addition to the spontaneous occurrence of outgrowths, they had, as previously described, been artificially induced on *Hibiscus vitifolius* and *Ipomea Woodii.* Earlier but unsuccessful experiments had also been made with *Solanum tuberosum* and *Vitis vinifera.* Later and successful experiments showed that the previous failures were due to the fact that the plants used were not in the right condition because they were too old. Artificial outgrowths were formed during the Spring of 1901 in the following plants :—

Solanum tuberosum, LINN.
,, *aculeatissimum*, JACQ.
,, *pyracanthum*, JACQ.
Vitis vinifera, LINN.
Cassia floribunda, CAV. DESC.

The results obtained with the Potato were even more striking than those previously obtained with *Hibiscus vitifolius,* and effectually demonstrate the general truth of the conclusions previously put forward as to the conditions necessary for the production of intumescences in *Hibiscus.*

The Potato was chosen as a plant for investigation on account of a reference in the 'Gardeners' Chronicle'* to "warty" potatoes. Though no description or figure was given, the "warts" seemed as if they might be intumescences.

The method of experiment which was used was most simple. Potato tubers were planted in pots, and the shoots springing from them were allowed to grow for about a week or a fortnight in a cool greenhouse. They were then still quite young and growing vigorously, though some of the leaves were almost mature. One of the pots was removed on the morning of May 25, which happened to be a Saturday, to another greenhouse with an average temperature of about 85° F. The plant, in its pot, was simply placed under a bell-jar, in as strong a light as possible, while another plant, to serve as a control, was left by its side uncovered. On the following Monday morning, about 48 hours after the experiment was begun, almost every leaf and stem was covered with a mass of pale green intumescences, like watery blisters. On the leaves they were almost exclusively produced on the upper side, generally in the middle of the lamina. In many cases, owing to the number of the outgrowths and their proximity to one another, their individuality was lost, the greater part of the leaf surface being covered with a continuous mass of soft, watery tissue. Most of the affected leaves were curled downwards and inwards, because the outgrowths

* MASTERS (7).

were on the upper surface, and evidently distended it. In the case of large intumescences the hypertrophy of the leaf surface was so great that it was raised up into wrinkles on the upper side, with corresponding depressions on the lower side, owing to the extension of the leaf surface where the outgrowths had arisen. The whole aspect of the plant was very striking, and distinctly pathological, as may be seen in the photograph, fig. 1A, and in 1B (Plate 14).

If such a plant be removed from under the bell-jar to a drier atmosphere, as soon as the intumescences are formed, they dry up into black dead spots; or, in the case of large intumescences, actual holes appear in the leaves, though to a certain extent the rest of the plant recovers itself. If, on the other hand, the plant be left for some days longer under the bell-jar, the intumescences become disorganised by breaking down into a brownish watery mass, the leaves drop off, and ultimately the plant dies. It is noteworthy that after the fall of a leaf a large cushion of intumescences is formed on the leaf-scar, and bears a striking resemblance to wound-callus.

Older plants placed under similar conditions do not form so many outgrowths, nor do they develop so rapidly, as in the case of young shoots. On quite old leaves no intumescences are formed.

Seedlings produce outgrowths in two or three days, but they are small and not very numerous. Otherwise they resemble those on shoots grown from tubers. Subsequent experiments showed that intumescences will form in about 24 hours after a suitable plant has been placed under the necessary conditions.

Intumescences were also induced on *Solanum aculeatissimum*. A young plant with several leaves was transferred, on May 28, from a cold frame in the garden, to the hottest greenhouse, where it was placed under a bell-jar. By the 1st of June it had developed abundant outgrowths, which were restricted to the *under* sides of the *older* leaves, and were especially numerous along the sides of the large out-standing ribs near the base of the lamina. The absence of outgrowths from the upper side of the leaf may possibly be correlated with the absence of stomata (fig. 2).

At a later stage the intumescences break down, and, in many cases, give rise to irregular holes which may fuse, and thus lead to the destruction of the greater part of the lamina.

In *Solanum pyracanthum* intumescences did not arise spontaneously. The species has leaves with orange-yellow spines and reddish woolly hairs. A young plant which had been growing in a cold frame was removed to the hottest greenhouse and covered with a bell-jar. After two or three weeks outgrowths were sparingly developed along the sides of the veins under the leaf.

Cassia floribunda.—A plant placed under the same conditions as in the preceding experiments produced very small but numerous intumescences, causing marked leaf-curl.

Vitis vinifera.—A Vine in a pot was removed from the temperate house to the hottest greenhouse. The plant was not growing quickly. One branch was isolated under a bell-jar, as in previous experiments with *Hibiscus vitifolius.* After about a week, intumescences of two types (to be described later, see p. 234) were formed. One type consisted of colourless spheres, as in *Ampelopsis*, and the other of small green outgrowths giving a rough warty look to the under surface of the leaves, and making the green colour slightly paler. All the leaves on the branch in the bell-jar formed outgrowths of this type, and some were developed on the leaves outside the bell-jar. The older as well as the younger leaves were affected.

In November of the same year some diseased leaves of the Ivy-leaved Geranium (*Pelargonium*) were sent to the laboratory for investigation. They had previously heen examined by other observers to determine the presence or absence of a vegetable or animal parasite, but none had been found.

Sections through the leaves showed that the diseased spots were due either to young green outgrowths, or to older ones which had become brown. The plants from which the leaves had been taken had been removed from the open air to a greenhouse, where they remained healthy until the temperature and at the same time the moisture in the air were increased, in order to force them. They then became diseased. The conditions were, therefore, exactly those which had been used to induce the artificial formation of intumescences.

As previous experiments had shown that intumescences could be formed on single branches placed under the necessary conditions while *still attached* to the parent plant, it was thought desirable to try to obtain outgrowths on shoots, leaves, and portions of leaves which had been *cut off* from the plant. These experiments were made in the spring of 1903, before the publication of Dr. KÜSTER'S* paper on *Populus tremula*, which did not appear until November of that year.

A shoot from a potato plant which had been grown for about 10 days in a cool greenhouse, was placed in water, under a bell-jar, in the hottest greenhouse, at a temperature of about 85° F., in as bright a light as possible. By the next day numerous intumescences had been produced.

Two potato *leaves* from a plant grown under similar conditions were treated in the same way as the shoot, and the same results were obtained.

Two separate *leaflets* were next taken, the stalk of one was placed in water and the other was simply laid upon cotton wool soaked in distilled water and placed in a saucer covered with a beaker in the same hothouse. By the next day some intumescences had already been formed, and on the second day the leaflets were thickly covered with them.

Finally, *a lamina was cut up into seven fragments*, some with cut edges on every side, and less than a centimetre across. The pieces were laid on wet cotton wool as in the preceding experiment. *In two days, six out of the seven pieces had produced well developed, and in most cases numerous, intumescences,* all upon the upper side.

These experiments show conclusively that *root pressure has nothing whatever to do*

* KÜSTER (9).

with the formation of outgrowths, and also that the conditions determining the production of outgrowths are extremely local; and that the substances concerned in their formation are made in the affected cells themselves, and are not brought from a distance by conducting tissues (*cp.* p. 247).

In November, 1903, KÜSTER* published the paper already referred to, in which he gives the results of some experiments he made on the artificial formation of intumescences in the leaves of *Populus tremula*.

KÜSTER'S method was to float leaves, or portions of leaves, on distilled water, on KNOP'S nutritive solution, or on a 1-per-cent. to 3-per-cent. solution of sugar, in Petri dishes. Some of the dishes were placed in the dark, others in a laboratory with a north aspect, with a south aspect, and in the *Victoria regia* house, so that different degrees of illumination and temperature were obtained. Under all these conditions, even in *darkness*, intumescences were formed.

The author came to the conclusion that intumescences form more readily in a poor than in a strong light. They were developed on the side of the leaf in contact with the fluid as well as on the upper side, and that whether the morphologically upper or lower side was physically uppermost; but in a poor light the outgrowths were on the physically upper side, while in a bright light they were on the physically lower one, whether the affected side were in contact with the fluid or not.

The author also considers the relation between leaf-galls and intumescences.

In the course of his paper, KÜSTER refers to my previous work on intumescences in *Hibiscus vitifolius*, and compares my results with his own. But since, in each case, different plants and different methods were used, the results obtained can hardly be strictly comparable, and generalisation is impossible until experiments have been made with a large number of species, more or less nearly allied. In the meanwhile the results which are being obtained, will make these experiments easier to carry out.

For the sake of comparison I have repeated KÜSTER'S experiments with *Populus tremula*, and I have also used his methods for potato leaves, and my own methods for *Populus tremula* and other species of *Populus*. Next summer I hope to apply the same or similar methods to a larger number of plants with a view to obtaining data for further generalisation.

The accompanying table gives a list of the experiments which were made to test the effects of KNOP'S solution, of a 2-per-cent. solution, and of distilled water on leaves of Potato and of *Populus tremula* in damp air under varying conditions of illumination and temperature.

* KÜSTER (9).

TABLE I.

I. Nutritive solution ...... (KNOP'S)	1.	Tropical pit in light.
	2.	,, ,, darkness.
	3.	Garden laboratory in north light.
	4.	,, ,, darkness.
	5.	Shade greenhouse in light without sun.
	6.	Sterilising room in south aspect.
		Controls—
		a. On wet cotton wool in Petri dish.
		b. With stems in water, bell-jar over.
		c. ,, ,, uncovered.
II. Sugar solution (2 per cent.)	7.	Tropical pit in light.
	8.	,, ,, darkness.
	9.	Garden laboratory in north light.
	10.	,, ,, darkness.
	11.	Shade greenhouse in light without sun.
	12.	Sterilising room in south aspect.
		Control—
		One control on cotton wool in Petri dish.
III. Distilled water	13.	Tropical pit in light.
	14.	,, ,, darkness.
	15.	Garden laboratory in north light.
	16.	,, darkness.
	17.	Shade greenhouse in light without sun.
	18.	Sterilising room in south aspect.

Additional experiments were made by submerging leaves, wholly or partially, under the three fluids, in the tropical pit.

In the above table the leaves in the first experiment in each series (1, 7, and 13) were exposed to as much light as possible, including all available sunlight, in a greenhouse, with a temperature of about 80° F. In the second experiment in each series (2, 8, and 14) leaves were placed in the same greenhouse in darkness.

The 3rd, 9th, and 15th were placed in a cool laboratory (about 60° F.) with a north aspect, so that they had light, but no sun. Three others (4, 10, and 16) were put in the dark in the same laboratory.

The 5th, 11th, and 17th were also exposed to light without sun, but in a higher temperature, in a greenhouse specially built so that the sun never shines into it.

Numbers 6, 12, and 18 were placed in a laboratory with a south aspect at a temperature of about 60° F.

Therefore six experiments were at a high temperature (about 80° F.), three in light and three in darkness; six more were in a comparatively cool room without sun, three in light and three in darkness. Of the remaining six, three were put under the same conditions of illumination as the last three, which were exposed to light, but at a higher temperature, while the remaining three were exposed to sunlight at an ordinary temperature. By way of control experiments, leaves, laid on wet cotton wool in closed glass dishes, were placed under the same conditions as the floating leaves.

The first experiments were begun on February 18, and examined on the 26th of the same month, *i.e.*, after eight days. The results are given in the accompanying table (Table II.).

TABLE II.—Potato, Feb. 18 to Feb. 26.

No. 7.	In tropical pit	Sugar	U $\overline{\text{L}}$*
			L U
,, 13.	,, ...	Water	U L
			L U
,, 11.	In shade greenhouse...	Sugar	U $\overline{\text{L}}$
			$\underline{\text{L}}$ $\underline{\text{U}}$

* "U" denotes the upper, "L" the lower side of a leaf. A line indicates intumescences, *e.g.*, $\frac{\text{U}}{\underline{\text{L}}}$ denotes a leaf or piece of leaf with its lower surface in contact with the fluid, and with intumescences on the same side.

On the controls there were only traces on the leaves exposed to light in the tropical pit.

The table shows that intumescences were only formed on three sets of leaves out of the eighteen. The reason why so few of these experiments were successful is because (1) the potato plants had grown very slowly, at first in the tropical pit and later in the intermediate pit, and were not in a sufficiently responsive condition; and (2) because the temperature of the tropical pit at this time of the year is subject to considerable variations which are dependent on cold nights, and the presence or absence of sunlight during the day. Consequently, the external conditions are not so favourable as in summer weather.

But the results even when negative, are instructive, and show the importance of a high temperature, and of food material in an easily assimilable condition. For the best intumescences were formed at a high temperature on leaves floating on sugar. Only a few were formed on leaves floating on water if the temperature was sufficiently high, or at a lower temperature if the leaves were supplied with sugar. These facts support the conclusions to which my former experiments pointed, viz., that assimilation is involved in the formation of intumescences.

The results of these earlier experiments showed that they were worth repeating under more favourable external conditions with leaves in a more responsive state.

On June 9 the experiments were repeated with (*a*) potato leaves from plants grown quickly in pots, first in the intermediate, and then in the cool pit; and (*b*) leaves from potato grown slowly in a garden under ordinary conditions. In every case young leaves, or pieces of leaves, were used, and those from the indoor plants were placed in the same dishes as those from the plants grown out of doors, in order to ensure identity of conditions. By way of control experiments, some shoots were cut off and placed in water, uncovered, in the tropical pit; others were similarly treated, but covered with a bell-glass; while yet others were placed on wet cotton wool in a covered dish.

The leaves were examined on the next day, June 10, when intumescences were already beginning to form on those covered with a bell-jar, *on the leaves from indoor plants only.*

The *indoor* leaves, but not the outdoor leaves, lying on wet cotton wool, were also *thickly* covered with intumescences.

Table III. shows the condition of the floating leaves exposed to light.

TABLE III.—Potato, June 9 to 10.

			Indoor.	Outdoor.
No. 7......	Tropical pit	Sugar...........	$\overline{\mathrm{U}}$ L L $\underline{\mathrm{U}}$	U L L U
„ 13......	„	Water	$\overline{\mathrm{U}}$ L L $\underline{\mathrm{U}}$	U L L U
„ 11......	Shade greenhouse	Sugar...........	× ×	× –*
„ 17......	„	Water	× –	× ×
Control	Tropical pit	Cotton wool ...	$\overline{\mathrm{U}}$ L L $\underline{\mathrm{U}}$	U L L U

* *Remarks.*— × denotes intumescence.
– denotes no intumescence.
The position of the outgrowths was not noted.

No intumescences were formed on any other leaves, including all those in the dark.

The above results confirm and extend those of the earlier experiments, the best results being obtained with the leaves which had been quickly grown indoors. Under the more favourable conditions, both internal and external, the leaves are not so dependent upon sugar as a food supply. It is noteworthy that in no case were the intumescences formed on leaves floating in KNOP'S solution, but only on those in sugar solution or in distilled water.

Both series of experiments show that *in every particular the Potato behaves like Hibiscus.* In both plants intumescences form most readily on the leaves of plants grown quickly and then placed under conditions in which they obtain, in a *saturated atmosphere*, most *heat* and *light*, while intumescences are never formed if the leaves are placed in a cool and shady position, even if the atmosphere is saturated with moisture, nor will they form in darkness or under water.

As will appear later, the results obtained with the Potato and *Hibiscus* differ in some details from those given by experiments with *Populus tremula.*

An exactly similar series of experiments was made with *Populus tremula*, at the end of May, while the leaves were still young and actively assimilating. The experiments were started on May 25 in the tropical pit at a temperature of about 85° F., and in a laboratory with a north aspect with an average temperature of 65° F., and on the following day in two other greenhouses, one the shade house and the other exposed to sunlight, both having a temperature of about 65° F., but (especially the sunny one) subject to considerable variations. Four leaves or pieces of leaves were placed in each dish, two with their lower surfaces, and two with their upper surfaces, in contact with the fluid on which the leaves were floating.

By May 28, *i.e.*, in three days, there were traces of intumescences on the branches placed with their stems in water and covered with a bell-jar, in the tropical pit. By May 30, *i.e.*, in five days, the results noted in Table IV. were observed.

TABLE IV.—*Populus tremula*, May 25 and 26 to 30.

						Notes.
No. 7	Tropical pit...........	Sugar	Light ...	$U\ \overline{U}$ $L\ L$	$\overline{L}\ L$ $U\ U$	
Control	,, ...	Cotton wool ..	,, ...	$\overline{U}\ \overline{U}$ $L\ L$	$L\ L$ $\underline{U}\ \underline{U}$	Very abundant.
No. 9	North laboratory ...	Sugar	,, ...	$\overline{U}\ \overline{U}$ $L\ L$	$L\ L$ $U\ U$	The affected leaves tightly curled up.
,, 15	,, ...	Water	,, ...	$\overline{U}\ \overline{U}$ $L\ L$	$L\ L$ $\underline{U}\ \underline{U}$	Very numerous.
,, 3	,, ...	Knop	,, ...	$\overline{U}\ \overline{U}$ $L\ L$	$L\ L$ $U\ U$	Like 9, but not so many.
,, 10	,, ...	Sugar	Dark ...	$U\ U$ $\underline{L}\ \underline{L}$	$L\ L$ $\underline{U}\ \underline{U}$	Only a few on leaves with upper side down. The other pieces tightly rolled.
,, 16	,, ...	Water	,, ...	$U\ U$ $\underline{L}\ \underline{L}$	$\overline{L}\ \overline{L}$ $U\ U$	Not so many on leaf with the lower side down.
,, 4	,, ...	Knop	,, ...	$U\ U$ $L\ L$	$\overline{L}\ \overline{L}$ $U\ U$	Only a *very* few.
Control	,, ...	Cotton wool ..	,, ...	$U\ U$ $L\ L$	$L\ L$ $\underline{U}\ \underline{U}$	Most on a mature leaf.

On this day (May 30) leaves of *Populus tremula* were submerged, wholly or partially, in KNOP'S solution, sugar solution, and distilled water, respectively, and placed in the light in the tropical pit.

Two series of leaves floating in the three liquids, and others laid on cotton wool, were also placed in the roof greenhouse, one series in the light and the other in darkness. By May 31, *i.e.*, in six days, all the pieces of leaf in the sugar solution (No. 9) were curling up because they were so thickly covered with intumescences.

In four days no outgrowths had been formed on the submerged leaves, but on a leaf only partially submerged, in KNOP'S solution, there were outgrowths on the parts which projected beyond the surface of the liquid. After five days the protruding parts of the partially submerged leaves were *thickly* covered with outgrowths on both sides, but, except for very doubtful traces on the leaf immersed in sugar solution, there were none on the submerged parts.

The floating leaves in the tropical pit soon began to decay, so that the experiments came to an end, but those in the cooler houses kept fresh much longer. The leaves on cotton wool in the cool houses retained their freshness longest of all.

On June 6, *i.e.*, 12 days after the first experiments were started, the leaves were again all carefully examined, and the following results noted (see Table V.):—

TABLE V.—*Populus tremula*, May 25 to June 6.

No. 9	North laboratory ...	Sugar	Light ...	U U $\underline{L}$ $\underline{L}$	$\overline{L}$ $\overline{L}$ U U	Very tightly curled up.
„ 15	„ ...	Water	„ ...	$\overline{U}$ $\overline{U}$ $\underline{L}$ $\underline{L}$	L L $\underline{U}$ $\underline{U}$	Tightly curled. A few only on $\frac{L}{U}$.
„ 3	„ ...	Knop	„ ...	$\overline{U}$ $\overline{U}$ L L	L L $\underline{U}$ $\underline{U}$	Only a few. Leaves going black.
„ 10	„ ...	Sugar	Dark ...	U U $\underline{L}$ $\underline{L}$	L L $\underline{U}$ $\underline{U}$	$\frac{U}{L}$ both tightly curled. The other two slightly curled.
„ 16	„ ...	Water	„ ...	U U $\underline{L}$ $\underline{L}$	L L U U	One $\frac{L}{U}$ exuding dark brown drops, the other going black in patches.
„ 4	„ ...	Knop	„	UU LL	LL UU	A very few on $\frac{L}{U}$. Traces only on $\frac{U}{L}$, which were going black.

On the series which had been put in the shade greenhouse on May 26, only a few intumescences had been developed by June 6 on those in sugar solution in the light, and not on any of the other leaves.

The series placed in the greenhouse on the roof on May 30 were also examined on June 6, *i.e.*, after a week, and the results given in Table VI. were noted.

TABLE VI.—*Populus tremula* in Roof Greenhouse, May 30 to June 6.

Sugar	Light	U U	$\overline{L}$ $\overline{L}$	Most on U.
		$\underline{L}$ $\underline{L}$	U U	,, L.
Water	,, ...	U U	L L	
		$\underline{L}$ $\underline{L}$	U U	
Knop	,, ...	U U	$\overline{L}$ $\overline{L}$	A very few.
		L L	U U	

In the dark there were no intumescences, and on the controls only slight and doubtful traces.

The results obtained with *Populus tremula* differ considerably from those obtained with *Hibiscus vitifolius* and the Potato, especially in that *Populus* can form intumescences in the dark while the other two plants cannot. But, according to my results, in *Populus*, as in *Hibiscus* and Potato, the formation of intumescences is promoted by heat, and by the presence of easily assimilable food material, such as sugar. Up to the present time *Populus tremula* is the only plant in which intumescences have been formed in darkness, and we seem therefore to be justified in assuming, until further experimental evidence is forthcoming, that *Populus* is in this respect exceptional. It is abundantly clear from all the experiments that assimilation is a most important factor in the formation of intumescences, and yet it is one to which little attention has been paid by most of those working at this subject. The fact which, more than any other, shows that assimilation is an *essential* factor is that intumescences are never formed under water because land plants cannot assimilate under water. The fact that the leaves of *Populus tremula* alone, so far as is known, form intumescences in the absence of light, may probably prove to be due to the presence of some special substance in the leaves. This question is referred to again on p. 252.

The experiments made with other plants, chiefly other specimens of *Populus*, may now be considered.

In most cases the leaves or branches were placed with their ends in water, or they were simply laid on wet cotton wool, and the vessel containing them was covered with a bell-jar. In a few cases the leaves were floated on liquids in covered Petri dishes.

In *Populus alba* intumescences formed very thickly along the veins on the *top* of the leaf, especially near its base. Particularly large intumescences were produced on the stem along a wound which had been formed by stripping off the bark in breaking off a piece of the branch to use for the experiment.

A dense white mass of tissue forming a thick frill about 3 millims. deep and several millims. across, ran along the whole length of the wound and was fully formed six days after the experiment had been started in the hottest greenhouse at the end of May. Masses of outgrowths were also formed on the cut end of the stem and closely resembled callus. Still more like callus were the outgrowths formed on the cut end of a twig of *Populus nigra*. After some days the leaves began to drop off the twigs used for these experiments.

In *P. deltoides*, only traces of intumescences were formed on the leaves, a few small ones also in *P. nigra*.

On account of the strong external resemblance between the intumescences formed on the wounded poplar stems and normal wound-callus, a series of preliminary experiments were made with a view to comparing stem-intumescences with callus, both as to conditions of formation and also as to histological and cytological characters. As I hope to go into this question more fully next summer, the details of the results obtained may be left for consideration in a subsequent paper. It may, however, be said that they bear out the opinion that there is a close connection between intumescences and wound-callus, both with regard to conditions of formation and internal structure, even to cytological details.

2. *Anatomical Investigations.*

Although intumescences have been described by various authors on different plants, their development, structure, and especially their cell-contents and nuclei, have in most cases not been considered in any detail, nor has any attempt at a systematic classification of intumescences, or a comparative account of this disease been made. Though, perhaps, not of very great practical value, it has a special interest to the botanist because it involves, and throws light upon, so many questions of physiology and pathology.

In his recent most interesting and important work on "Pathologische Pflanzenanatomie," KÜSTER* devotes a section to the consideration of intumescences. He places these outgrowths amongst a group of diseases characterised by abnormally enlarged cells. Such cells or tissues he calls "*hypertrophies*," whether the increase in size is due to excess of water or of food material.

In such hypertrophic cells or tissues the chief characteristic is *the increase of size of pre-existing cells with little or no cell division.* When cell division occurs to any extent he distinguishes both the process and the resulting group of cells as "*hyperplasy.*" Intumescences he places in a special group of hypertrophic tissues, which he calls *hyperhydric hypertrophies—hyperhydric* because they arise through excess of water in the cells affected, and *hypertrophies* because he regards all cell division in intumescences as the exception rather than the rule. But at the same time he admits that no sharp line can be drawn between hypertrophy and hyperplasy. The three figures of intumescences reproduced by KÜSTER, including one of my own drawings, are all cases of hypertrophy and not hyperplasy, but in the description of my drawing I expressly stated that the intumescences figured were

* KÜSTER (10).

young, and the figure immediately below the one reproduced is of a stem intumescence with marked hyperplasy. Also in a previous paper I had figured hyperplastic intumescences both in stems and leaves, and other examples will be given in the present paper (see figs. 7, 8, 11, 18). Some intumescences, especially those on leaves (as for example the Potato, figs. 20–22), are certainly exclusively hypertrophic, but in the leaves on many plants, and almost always in stems, hypertrophy is followed by hyperplasy. Most authors seem only to have examined intumescences at one stage of their development and not to have followed their complete life history. I have, therefore, ventured to attempt a classification of intumescences based upon macroscopical and microscopical characters as well as upon development.

Classification of Intumescences.

1. *Spherical* type (*Perldrüsen*).
   - (*a*) Purely epidermal.
   - (*b*) Epidermal and sub-epidermal.
2. The *Hemispherical* type.
   - (*a*) Cells in uninterrupted connection and no rupture of tissues.
   - (*b*) Mesophyll cells separating from one another and breaking through the epidermis.

The mode of occurrence and general macroscopic characters may first be considered, and then certain microscopic features, with special reference to the cell contents and the nuclear phenomena.

A. *Macroscopic Characters.*—Intumescences are of two well-marked types: (1) colourless *spherical* outgrowths (the "Perldrüsen" of German writers) which are about a millimetre in diameter and very slightly attached to the plant; and (2) outgrowths with a broader insertion and often more or less *hemispherical* in form. These latter may be either (*a*) colourless, or (*b*) pale green. In some cases both types occur on the same plant.

1. The *colourless spherical outgrowths*, or *Perldrüsen*, which were first described in detail by TOMASCHEK* and later noted by SORAUER,† were noticed in the spring of 1901 on the following plants:—

Vitis vinifera, LINN.
Vitis rotundifolia, MICHX.
Pavonia arabica, HOCHST. *ex* STEND. (*Hibiscus flavus*).
Hibiscus esculentus, LINN.
,, *Abelmoschus*, LINN.
,, *Manihot*, LINN.

* TOMASCHEK, *loc. cit.*, p. 2 (8).

† SORAUER (3), p. 224.

Piper ornatum, N.E., Br.
Theobroma Cacao, LINN.
Kendrickia Walkeri, HOOK.

In most of these plants the spherical intumescences occur almost exclusively on the under sides of the leaves, and chiefly along the course of the larger veins of the young leaves. A few are generally formed also on young stems.

The most striking examples of spherical outgrowths occurred on a young plant of *Vitis rotundifolia*, raised from seed brought from Somaliland, and growing in a rather cool greenhouse. The leaves are succulent, smooth, and covered with wax, so that the glistening outgrowths stand out conspicuously upon them like drops of dew, either clustered together or scattered irregularly on both sides of the leaves, as well as on the stems and petioles. The dead intumescences appear as black specks, which at a distance resemble aphides or mites. It is noteworthy that an old plant, growing in the palm-house, though in a much hotter and damper atmosphere, was quite devoid of outgrowths. Perhaps this old plant had become able to adapt itself to its environment, for, apparently, the plant, being a succulent, is one which would naturally grow in a dry habitat.

Kendrickia Walkeri, also a succulent, had outgrowths on the leaves.

Spherical outgrowths, similar to those on *Vitis rotundifolia*, but smaller in size, occurred on the young leaves of two other species of *Vitis*, *V. vinifera* and *V. heterophylla* and also on *Ampelopsis Veitchii* and *A. hederacea*, all growing in the open. These are the only cases which have been observed by the present writer of the occurrence of intumescences of any kind out of doors.

They were first noticed in May, when the plants were coming into leaf, and were abundant on all the leaves and on the young stems. But numerous outgrowths were subsequently observed as late as the end of July.

On the three species of *Vitis*, and on *Pavonia arabica*, these outgrowths were fairly numerous on the leaves and stems of seedlings, which continued to produce them as they grew. On *Piper ornatum* and *Theobroma Cacao* the outgrowths were only produced while the fresh leaves were developing, and their formation ceased when the foliage was mature.

KÜSTER* considers that these "Perldrüsen" may perhaps be normal structures and not pathological, but the evidence seems to be in favour of the view that they are to be regarded as intumescences, under which group KÜSTER indeed places these structures, and TOMASCHEK considers them as pathological. But, as will be seen later, they differ in various points from the more usual type of intumescence.

2. The *hemispherical type* of intumescences is more frequent than the spherical type, and in many cases such outgrowths cause serious injury to the parts affected, and may even prove fatal. They may be arranged in two groups (*a*) one in which the outgrowths are *colourless*, and (*b*) another in which they are *pale green*.

* KÜSTER, *loc. cit.* (10), p. 90.

(*a*) *Colourless* outgrowths of the type now under consideration were observed in the following plants :—

Ipomea Woodii, N.E. Br.
Acalypha marginata, Spreng.
Hibiscus vitifolius, Linn.

In the last-named plant colourless outgrowths are often associated with those that are green. In *Ipomea* and *Acalypha* the outgrowths are exclusively confined to the under sides of the leaves, possibly because in these plants there are no stomata on the upper side. In *Acalypha*, the leaves are red. Another species of *Acalypha*, *A. Mackayana*, also growing in the stove, was free from outgrowths.

(*b*) Pale *green* intumescences appeared spontaneously on *Solanum aculeatissimum*.

Gmelina hystrix, Schult. *ex* Kurs.
Hibiscus vitifolius, Linn.
Cassia floribunda, Cav. Desc.
Eucalyptus diversicolor, F. Muell. Fragm.
,, *melliodora*, A. Cunn. *ex* Schau.
,, *botryoides*, Sm.
,, *resinifera*, Sm.
,, *saligna*, Sm.

In *Solanum aculeatissimum* the outgrowths occur on the under side of the leaves, along the larger veins, especially near the base of the lamina. The older outgrowths often eventually give rise to irregular perforations in the leaf.

In *Gmelina hystrix* and *Cassia floribunda* the individual outgrowths were very small, but so numerous as to give to the surface of the leaves a warty roughness, especially near the veins.

Owing to the large number and small size of these outgrowths, and to the fact that they were all on one side of the leaf, they caused the lamina to curl, or even to roll up, as if they had been attacked by a parasitic animal or leaf-rolling caterpillar. In other respects the leaves did not appear to be injuriously affected.

The species of *Eucalyptus* had rather small and somewhat irregularly disposed outgrowths, which, when old, give rise to brown, slightly raised projections on the leaves. Growing under identical conditions with the affected species were the following, which were devoid of any trace of outgrowths :—

Eucalyptus paniculata, Sm.
,, *leucoxylon*, F. Muell.
,, *irrigata.*
Eucalyptus pilularis, Sm.
,, *citriodora*, Hook.
,, *tereticornis*, Sm.

A plant of *E. globulus* growing in a cool greenhouse, and free from outgrowths, was removed to the stove, but developed no outgrowths. This was probably due to the fact that the leaves were old, as the plant was hardly growing at all when the experiments were made.

Outgrowths have been described by SORAUER in *E. globulus* and *E. rostrata*. The same observer has also noted the occurrence of outgrowths in *E. Stuartiana*, *E. coccifera*, and *E. saligna*.*

With regard to the pathological effects of outgrowths, some observations have already been made which may here be summarised. The colourless globular outgrowths are very transitory, and apparently have no injurious effect upon the leaves and stems which bear them. But the remains of the old intumescences which have gone black somewhat disfigure the plants.

With regard to the second type, if the outgrowths are small and numerous, especially if they are confined to one side of the lamina, the result is usually the occurrence of leaf-curl, but the outgrowths do not seem to otherwise injure the leaf. They are not such transitory structures, but often persist as long as the leaf on which they arise.

In some cases, however, the affected leaves apparently do not live as long as those which are quite healthy. Larger outgrowths are generally more quickly formed and shorter lived. They affect the leaf in two different ways. If they break through the epidermis as in the Potato (figs. 21–24) their constituent cells usually fall apart and a perforation of the lamina results. In cases in which the constituent cells are in uninterrupted contact, and in which the walls are often thickened, the upper part of the outgrowth is usually cut off by cork, leaving a brown scar, as, *e.g.*, in the stem of *Hibiscus vitifolius*. In other cases no cork is formed, but the upper part of the outgrowth dries up and shrivels, sometimes leaving an open wound. Such wounds, and also those due to leaf perforations, may give entrance to parasitic fungi which could not injure sound tissues.

But, apart from wounds, intumescences may have fatal results, or, rather, the conditions under which intumescences arise may cause the death of the plant, by so disturbing its metabolism as to lead to starvation. Thus intumescences are a symptom rather than a cause of disease.

B. *Microscopic Characters.*—Some account of the structure of intumescences as they occur in *Hibiscus vitifolius* has already been given in previous papers;† but further descriptions, chiefly relating to other plants, may now be added.

1. The *spherical type* (*Perldrüsen*).—Outgrowths of this type have been examined in three species of *Vitis*, two of *Ampelopsis*, and also in *Pavonia arabica* and *Kendrickia Walkeri*. Though similar, two well-marked varieties may be distinguished.

(A) In *Pavonia* and *Kendrickia* the outgrowths consist only of a few large epidermal cells, and *have no stomata*. The inner, common walls are straight, but

* SORAUER (4).

† DALE (1 and 2). In these papers references are given to earlier literature on the subject.

the outer walls are strongly curved owing to great turgescence (fig. 3). Near the base of the outgrowth is a group of small cells attached to the leaf by a few tabular stalk-cells (fig. 4). Each cell contains a large nucleus and fairly abundant parietal protoplasm, as well as strands crossing the central vacuole. The protoplasm shows active streaming.

There is an abundance of oil occurring in large and small drops. The small drops stream round in the protoplasm and come into contact with one another without fusing. The larger drops are most abundant round the nucleus, close to the walls common to two cells.

(B) In *Vitis* and in *Ampelopsis* the outgrowths are larger than in *Pavonia* and *Kendrickia*, but the constituent cells are much smaller and therefore more numerous. The outer walls of the cells do not project above the surface of the sphere, which is accordingly even, so that the outgrowth has an almost spherical form (fig. 5). The mode of origin and structure of these outgrowths, as they occur in *Ampelopsis*, have been shortly described, but not figured, in the paper already referred to, by TOMASCHEK, who notes that each has an apical stoma. According to this author the outgrowths arise as follows:—The parenchymatous cells of the mesophyll, lying below the epidermis and next to the stoma, first elongate and block up the respiratory cavity. Later they increase in number, and lift up the epidermis.

The parenchymatous cells fill up the outgrowth which, while it is being lifted up, becomes constricted at the base. The outgrowth therefore consists of both mesophyll and epidermal cells. TOMASCHEK notes the fluid contents, the protoplasm, and the nuclei. It would therefore appear that the outgrowths in *Pavonia* and *Kendrickia* are exclusively epidermal, but that in *Vitis* and *Ampelopsis* they consist of a central mass of mesophyll cells surrounded by a spherical wall of epidermal cells. In the case of globular outgrowths consisting partly of mesophyll and partly of epidermis, their development shows that there is a close connection between the formation of the outgrowth and the distribution of the stomata.

The cells of all the outgrowths, in any of the species under consideration, contained both oil and starch (figs. 3 and 6). The oil occurs in large and small drops which stain black with osmic acid, and red with tincture of Alkanna. The abundant starch is contained in spherical leucoplasts, either in the form of minute granules showing active molecular movement, or as larger granules (fig. 6). In rather old outgrowths of *Vitis heterophylla* the leucoplasts had a greenish hue, but in every other case no sign of chlorophyll was seen; the whole structure was absolutely colourless. Nevertheless, in the internal cells of the type of outgrowth occurring in *Vitis*, the leucoplasts must have arisen from chloroplasts which have degenerated. In some cases the leucoplasts were swollen and watery—another sign of degeneration. They are most numerous round the nucleus, which contains a very distinct nucleolus. The starch granules stain purplish or reddish-brown with iodine. This starch is very persistent, as the following experiment shows. A shoot bearing intumescences (while still attached to the growing plant) was darkened for four days and five nights, and at the end of that time the oil and starch were as abundant as at the beginning of the experiment.

2. *Hemispherical Outgrowths.*—By far the greater number of outgrowths belong to this type, which has accordingly been already described by various authors in a considerable number of species.

Some new facts have, however, been observed in the course of the present investigations. Intumescences of this type consist of both epidermal and mesophyll cells, and are of two kinds, each kind having a different pathological effect upon the plant which bears it. In the one kind (*a*) the cells of the outgrowth, both the mesophyll cells and those of the epidermis, are in uninterrupted connection, and no rupture of the tissues occurs. In the other kind (*b*) the mesophyll cells soon break through the epidermis, and the whole intumescence separates into its component cells, so that a mechanical injury is inflicted upon the organ. The two kinds may be considered separately.

(*a*) *Outgrowths in which there is no Rupture of the Tissues.* In some cases as in *Ipomea Woodii* (fig. 7) the intumescence is made up almost exclusively of epidermal cells, which contain large quantities of *oil* but no starch. This oil is almost confined to the cells of the outgrowths, but extends a little way into the adjacent tissues of the leaf, occurring chiefly in the palisade cells near the veins. It occurs in normal cells which also contain starch in the chloroplasts or, in the case of epidermal cells, leucoplasts.

In *Acalypha marginata* (fig. 8) the intumescences are very similar both in structure and cell contents to those in *Ipomea Woodii*, but more of the underlying mesophyll takes part in their formation.

In *Hibiscus*, also, oil is most abundant in those outgrowths which are chiefly, or exclusively, made up of epidermal cells, and occur for the most part in cells which have been derived from the epidermis (figs. 17–19).

Intumescences made up wholly or chiefly of epidermal cells resemble the spherical type in consisting entirely of thin-walled cells (mostly colourless and of epidermal origin) which contain oil, but differ from them by their broad insertion, which involves greater injury to the leaf.

In *Gmelina hystrix* (fig. 9) sections of the small outgrowths mentioned above (see p. 236) show that they also consist chiefly of epidermal cells which have become enlarged, with little or no cell division.

No oil or starch was observed in the enlarged cells. This fact seems to indicate that the disturbance of the normal metabolism caused by the formation of these small intumescences was only slight, but it is possible that, under other conditions, the outgrowths might become larger and their contents different. In the formation of most intumescences the mesophyll plays the chief part.

Eucalyptus.—In this genus either one or both sides of a leaf were affected, and the mesophyll and epidermis attacked in varying degrees. In *E. botryoides* (fig. 10) the epidermis and *palisade* were both affected, the latter only slightly, the cells being a little more elongated and divided by transverse walls. The epidermal cells were enlarged but not divided. Oil was present in the epidermal cells and in the outermost of those of the hypertrophied palisade. In older intumescences, as noted by SORAUER, cork is formed. The cork arises immediately below the epidermis and causes the death of the cells external to it.

In *E. divisicolor* (fig. 11) the outgrowths consisted chiefly of hypertrophied *spongy parenchyma*. The cells were in uninterrupted connection, and therefore formed a marked contrast to the particularly loose structure of the normal spongy parenchyma.

In *Vitis vinifera* spontaneously occurring outgrowths have been described by SORAUER. In the intumescences induced artificially, in my experiments, the tissue affected was the spongy parenchyma, which grew into a kind of pseudo-palisade (fig. 12). The epidermal cells only became very slightly enlarged, and were not broken through by the underlying tissues. Probably this fact is to be explained by the small size of the outgrowths formed, as SORAUER* notes that, in the cases he noticed, the epidermis was ruptured. Again, the small size of the intumescences appears to be due to the fact that they were developed slowly, on leaves which were not growing actively. No oil, starch, nor any abnormal substance was observed in the cells. Later their walls became brownish.

Pelargonium (*Dibrachya*). The outgrowths in the ivy-leaved geranium were found to consist chiefly of hypertrophied mesophyll. The young intumescences were pale green; in the older ones the outer parts were corky. The cells contained little or no oil.

Not only are there differences between the intumescences of the same type in different plants, but on the same species of plant grown under different conditions. This was shown by an examination and comparison of the outgrowths artificially induced on the leaves of *Hibiscus vitifolius* grown under the different conditions described in a previous paper. These intumescences differ both in structure and cell contents, and even the normal parts of the leaves which bear them show considerable differences. The leaves vary greatly in thickness. That grown under green glass, though small in area, is thicker than any, but it formed no outgrowths. The normal leaf, grown out of doors, and also devoid of outgrowths, is similar in structure, but considerably thinner. The other leaves are all thinner than these two.

No outgrowths were formed under blue or green glass, those under red glass were small, while those under the yellow glass and under whitewashed glass were large and numerous, as were also those rapidly produced under clear glass. Though both epidermis and mesophyll are involved in the formation of outgrowths, *in no case* do the mesophyll cells break through the epidermis, the cells of which increase in

* SORAUER (4).

number and size or in both, to as great or to a greater extent than the green underlying cells.

The various cases may be considered separately. Under *red* glass the intumescences were rather small, and more or less distinct from one another, but fairly numerous (fig. 13). The parts affected were the palisade parenchyma and the epidermis, but chiefly the latter. The intumescences are often conical or almost tubular in form.

Under *yellow* glass the *upper* surface is most affected, and is so densely covered with outgrowths that hardly any part of the leaf is free from them (fig. 14). They also occur in considerable numbers on parts of the under surface of certain leaves, where they form a kind of pseudo-parenchyma. Cells containing mucilage are abundant. These cells *never swell up*, perhaps because all or most of the normal cell-contents are replaced by mucilage, so that assimilation, and therefore growth, cannot take place. For this reason epidermal mucilage cells often lie at the base of a depression caused by the swelling and lifting up of the surrounding epidermal cells (fig. 14).

Under *whitewashed glass*, on the leaf from which sections were made, many large outgrowths had developed on the *upper* side. Some were so large that the under side of the leaf below the outgrowth was pitted. There were hardly any outgrowths on the under side (fig. 15).

Under *clear glass* the outgrowths had formed with great rapidity and in large numbers *exclusively on the under side*, whereas under whitewashed glass they were almost all confined to the *upper* side. They begin in the spongy parenchyma, where the cells elongate until they become longer than the palisade cells, so that they form a kind of pseudo-palisade (figs. 16 and 17). The epidermal cells also enlarge but not so rapidly as those of the mesophyll, though they grow fast enough to keep pace with the mesophyll cells and prevent them from rupturing the epidermis. Later the cells all swell up and lose their chlorophyll, so that the colour of the outgrowth changes from pale green to glistening white. At first (fig. 17) all the cells are thin-walled. In an older outgrowth (figs. 18 and 19) the tubular cells both of the mesophyll and epidermis, but especially the former, have become divided by transverse walls into shorter segments and the whole thickness of the leaf becomes involved in the intumescence. Though the cells of the palisade and of the upper epidermis do not become enlarged, or only slightly, their walls become considerably thickened, as do those of the cells adjoining the palisade until the inter-cellular spaces are entirely occluded.

The cells of the intumescences, especially those derived from the epidermis, contain a large quantity of oil (fig. 18). In an old outgrowth (fig. 19) the cell-walls of the basal part of the outgrowth become much more thickened, in some cases so much so that the cell lumen is almost obliterated and the contents have degenerated into amorphous masses, though some of the oil still persists. The walls of the uppermost cells, which remained thin, have collapsed and shrivelled. There is a gradual transition from these thin-walled cells to those which are thick-walled.

As regards cell contents in *Hibiscus*, *calcium oxalate* is formed abundantly in all the leaves, especially under whitewashed glass, yellow and green glass. It is not so plentiful in the normal leaf and there is comparatively little formed under red glass. The crystals are of two kinds. One kind consists of small stellate masses, apparently formed by a number of small crystals of imperfect shape, united together. These masses occur chiefly along the course of the veins. The other kind occurs in the areas between the vascular net-work and consist of large tabular crystals.

Mucilage is also abundant, and it is noteworthy that it is most plentiful in leaves grown under red glass, where there is less calcium oxalate than in the leaves of plants grown under other conditions. The distribution of the mucilage is very irregular. Some parts of the same leaf may be nearly free from mucilage cells, while in other parts they are present in greater or less numbers.

The distribution of *oil* has already been mentioned, and also, in an earlier paper, the abundant formation of *starch*.

(*b*) *Intumescences in which the enlarged mesophyll cells break through the epidermis and separate from one another.*

Solanum. In all three species examined, the mesophyll ruptured the epidermis. Transverse sections of a leaf of *Solanum tuberosum* bearing rapidly formed outgrowths on its upper surface show that the tissue chiefly affected is the palisade parenchyma (fig. 20).

The formation begins either in the mesophyll alone, or the epidermis may at once be involved. The epidermal cells, however, never divide, but each swells up into an isolated papilla. The mesophyll cells rarely divide, but elongate into club-shaped structures which soon break through the epidermis, which falls off in isolated cells (fig. 21). Tho club-shaped mesophyll cells first separate from one another at their free ends, and ultimately they also fall apart into their constituent elements (figs. 22 and 23). The underlying tissues may also be affected down to, but (as SORAUER observed in other cases) not including, the epidermis of the lower side. The lower epidermis, however, ruptures, and the result is a perforation in the leaf. The club shape of the hypertrophied cells is apparently due to the fact that after the cells have broken through the epidermis, the pressure is removed from their free ends, which consequently swell, while the lower ends are still pressed upon by one another. The elongated cells are extremely thin-walled. They contain nuclei with highly refractive, degenerating nucleoli. They also contain degenerating chloroplasts, which, on account of the enlargement of the cells which contain them, become widely separated from one another. There is no oil in these intumescences, oil, as noted above, being most abundant in outgrowths formed wholly or in part of epidermal cells. But the fact that the normal course of metabolism has been disturbed is shown by the occurrence in the cells of the affected tissues of numerous *crytalloids of proteid,* usually one or more in each cell (figs. 20 and 21).

The structure of the outgrowths on the stem of the Potato is essentially the same as that of the intumescences on the leaves. The normal cortical tissues consist of an epidermis, below which is a single layer of cells containing chlorophyll.

Internal to this layer is collenchyma, passing gradually into the parenchyma surrounding the bundles. The chlorophyll-containing cells are chiefly, if not exclusively, the part which forms the outgrowth. The cells, elongate, occasionally divide, and break through the epidermis, of which the cells sometimes dilate into vesicles. In some cases the collenchyma is slightly affected.

In *Solanum aculeatissimum* the structure of the outgrowths is very like that in *Solanum tuberosum,* but here the intumescences only occur on the *under* side of the leaf, possibly on account of the distribution of the stomata. The formation of the intumescences begins with the hypertrophy of the *epidermal* cells, and of the spongy parenchyma, and extends to the more internal layers, until it includes the palisade. The tissues affected are cast off as isolated cells until only the epidermis of the upper side is left (fig. 24). This ruptures, and a ragged hole is made in the leaf.

Solanum pyracanthum resembles the other two species.

The different pathological effects referred to above (p. 237) as being produced by the two different kinds of intumescences which have been described, are evidently due to the structural differences between the two kinds of outgrowth.

For the sake of comparison we may consider the rapidly-formed outgrowths in *Hibiscus vitifolius* and in *Solanum tuberosum.* In the former the epidermis is not ruptured, and the walls at the base of the outgrowth become thickened, so that the older intumescences do not become disorganised as is the case in all the three species of *Solanum* examined, in which also the cells all remained thin-walled. In *Hibiscus* the cells are all in uninterrupted connection, while in *Solanum* they become isolated. In *Hibiscus* the rapidly-formed outgrowths all occur on the *under* side, in *Solanum* they are exclusively on the *upper* side of the leaves. Again, in *Hibiscus* the index of disturbed metabolism is *oil,* and perhaps also *calcium oxalate,* whereas in *Solanum* the disturbance is indicated by *proteid crystals.*

In connection with the question of cell contents, some experiments may be mentioned which were made in order to discover whether the amount of acids or of salts were the same in plants with intumescences as in those which were healthy. The methods adopted were those described by ASTRUC* in a paper entitled "Recherches sur l'acidité végétale."

Leaves and young stems of *Solanum tuberosum* were used and were obtained from plants grown under different conditions.

(A) A potato plant grown from tubers, in a pot, in a cool greenhouse, in May.

(B) A potato plant grown under the same conditions as (A), and then removed to a

* ASTRUC (11).

hot greenhouse and placed under a bell-glass for four days, when it was so covered with intumescences that it was in a dying condition. Some of the leaves had dropped off, and all were covered with bursting intumescences. The stalks and all the remaining leaves were cut off and weighed. The diseased plant had few leaves and these were small, but both the leaves and stems were very thick and their tissues watery. The leaves of the plant from the cool greenhouse were larger and darker in colour.

(c) A potato plant which had grown more slowly out of doors, and was, therefore, rather older than those from the greenhouse, was used for purposes of comparison.

A rough estimate of the quantity of acids and of salts in each plant, according to ASTRUC'S methods, showed that in this respect there was little or no difference in the three plants. Further experiments confirmed these results, so that we may conclude that there is no important difference in the quantity of either acids or salts in potato plants grown under different conditions, whether the plants are healthy or covered with outgrowths.

Nuclear Phenomena.—As might be expected from the pathological nature of intumescences, their nuclear phenomena are peculiar. The behaviour of the nuclei has been specially examined in the intumescences on the *stems* of *Hibiscus vitifolius* and *Populus alba*, and in the outgrowths on the wounded stems of *Populus nigra*. As it is difficult, if indeed it is not impossible, to distinguish certain stem intumescences from wound-callus, some callus grown under various conditions has also been examined for purposes of comparison.

In many leaf intumescences, *e.g.*, in the Potato, there is little or no true growth, *i.e.*, cell division, the increase in size being due almost exclusively to the enlargement of pre-existing cells. In such cases the nuclei are at first more or less nearly normal, but later they become distended and watery and lose their definite outlines. The stem outgrowths are always larger, and their size is generally due to cell division. Their constituent cells, with their contained nuclei, are also larger. It is specially noteworthy that in the intumescences on the stem of *Hibiscus* and in the more or less callus-like outgrowths on the stems of *Populus alba* and *P. nigra*, as well as in true callus, the *nuclear phenomena are identical*. The usual form of the nucleus is rounded or oval (fig. 25, *a–f*), though spindle-shaped nuclei are very abundant (fig. 25, *g*). Each nucleus has a definite membrane, and a large nucleolus surrounded by a clear zone.

The nucleoplasm is granular, or more or less clearly reticulate (fig. 25, *l*, *m*). In many respects these nuclei suggest those of certain fungi. Many of the nuclei have two or more nucleoli (figs. 25, *e*, *f*, *g*, *h*, *i*, *m*, *n*, 26, *e*, *f*, *h–m*, etc.), or the single nucleolus may be of enormous size (fig. 25, *d*). Not infrequently there are two or sometimes even three or four nuclei in a single cell (fig. 25, *m*). This is the case in the cells of the pith even where the tissue is not directly affected by the intumescences.

Further examination shows that the additional nuclei arise almost exclusively by a process of direct division, or amitosis (figs. 25, *h–n*, 26, *a*, *b*, *l–m*, etc.). Only in one or two cases in each section of a large intumescence or piece of callus were any signs of indirect (mitotic) division to be seen.

In most cases these indirect divisions were curious forms which may be regarded as transitional between mitosis and amitosis (figs. 25, *o* and *p*, 26, *o*, *p*, and *q*).

The most usual type of direct division seems to be that the nucleus divides directly, for nuclei with two nucleoli are very abundant, the nucleoli being either close together (as if they had just separated from one another), or at varying distances apart and of the same or different sizes. Usually each is surrounded by a clear zone. There is apparently no connection between the shape of the nucleus and the number of nucleoli. The nucleoplasm becomes constricted in the middle, and the two halves thus formed gradually move away from one another, sometimes sliding over one another (26, *i*). The resulting nuclei may be of the same or different sizes, and they may have a definite membrane or, more usually, no definite outline, but extend into processes resembling pseudo-podia (25, *i*, *l*, 26, *a*, *b*). In fact, the nuclei very often closely resemble amœboid organisms. In many cases the nuclei of the two daughter cells begin to divide again before any cell division takes place, so that in one cell there may be (as in fig. 25, *m*) one nucleus with two nucleoli while the other has partly or completely divided into two grand-daughter nuclei.

In some cases a nucleus with four nucleoli may divide into two halves, each with a couple of nucleoli of the same or different sizes (26, *h*, *l*, *n*). Other cases occur in which there are three or more nucleoli, up to as many as six or eight or even more, but the higher numbers are comparatively infrequent (26, *m*). When a number of nucleoli are arranged along the middle of an elongated nucleus (as in fig. 25, *o*, *p*) the appearance suggests the spindle of a nucleus which is dividing karyokinetically.

A certain number of examples occur of what seem to be intermediate forms between direct and indirect division, as if the nucleus were attempting mitosis with a greater or less measure of success.

In the cases which most nearly resemble direct division the nucleus divides into two parts, in each of which the nucleolus has broken up into fragments which, in different examples, vary considerably in number and size. The two halves ultimately separate from one another (figs. 25, *o*, *p*, 26, *o*, *p*, *q*).

In some cases the division of the nucleolus is carried so far that the appearance of the di-aster stage of karyokinesis is produced. In all such nuclei the nucleoplasm is very dense and stains deeply (26, *o* and *p*). In some nucleoli there are one or more granules which stain still more deeply than the surrounding nucleolin (26, *f*).

In some cases the nuclei present a stage resembling the spindle phase of karyokinesis, but with granules of nucleolin in place of chromosomes (26, *q*). Only in a single nucleus (in the callus of a cutting of *Chrysanthemum Broussonetii* struck in a greenhouse) were true and characteristic chromosomes seen (fig. 28, *a*), but in two other cases (one from the callus of the same stem and one in an intumescence on *Hibiscus*) were nuclei seen with structures which appeared to be nuclear plates seen in polar view (*cp.* JUEL, in 'Pringsheim's Jahrbuch,' vol. xxxv., Plate 16, fig. 32, of the first division in the pollen mother cell of *Carex acuta*), but which might be cases of nucleli in which the nucleoli had fragmented before division (figs. 27, *d*, 28, *a*).

Only one or two cases were seen of the nuclear division called by TISCHLER* budding ("Knospung" or "Sprossung") (25, *n*, 26, *l*).

There were several cases observed in which a nucleus with several nucleoli seemed to be breaking up and rounding off into several daughter nuclei, like the segmented nuclei described by PRILLIEUX† in the giant cells formed in seedlings grown at high temperatures (26, *k*, *m*).

General Considerations and Conclusions.

The results of the experiments recorded above, confirmed, on the whole, the conclusions to which the earlier investigations pointed, viz., that the essential external conditions are *damp air* and *warmth* and *light*. They also seem to indicate that an abundant supply of *oxygen* is required (p. 248). The experiments, especially those with the Potato, bring out very clearly the great importance of a biological factor—*the condition of the plant at the time of experiment*. This biological factor is very complex, and therefore difficult to analyse, but the experiments with the Potato (the negative results as clearly as the positive) show that, generally speaking, unless an organ is young and *actively assimilating* it cannot produce outgrowths.

This is true of the green parts of plants but not of woody stems in which food material is stored up. Apparent exceptions occurred in the case of *Vitis vinifera* and *Solanum aculeatissimum*, in which outgrowths were formed on older leaves and not on those which were younger (p. 224). It is possible that in these cases the younger leaves were still incompletely developed and had not yet acquired their full power of carbon dioxide assimilation.

The importance of the state of the plant is also shown by the outgrowths which occurred spontaneously on the various species of *Eucalyptus* (p. 236). Of 11 species of *Eucalyptus*, all growing under the same external conditions, five produced outgrowths while six had none.

In the case of the Potato and of *Hibiscus* we are led to the somewhat paradoxical conclusion that, given the necessary external conditions, the healthier the plant the more liable is it to assume the pathological condition indicated by the formation of outgrowths, owing to abnormal metabolism set up by these external conditions.

* TISCHLER (12).

† PRILLIEUX (13) cited by KÜSTER, 'Pflanzenanatomie,' p. 130.

Some clue as to the changes in metabolism (which may in extreme cases lead to the death of the plant) are furnished by an examination and comparison of the contents of the cells in the normal and in the hypertrophied tissues, and by a consideration of their physiological *rôle*. But the subject is so vast and complex that it is only possible to note a few somewhat isolated considerations which have been suggested by the experiments carried out during the last four years. The results of earlier experiments showed that active assimilation must have been going on in a plant which had developed outgrowths, doubtless because the products of assimilation are necessary for the sudden increase in size in the cells concerned, and for the cell division which frequently accompanies this increase in volume in the cells.

The largest outgrowths, the most rapidly formed, and those which show the greatest histological and cytological changes, are developed when, given the necessary physiological condition of the plant, the external conditions are changed *suddenly*. The cause of this appears to be that the changed conditions alter the course of metabolism too quickly for the plant to accommodate itself to them. As was pointed out above, in the leaf intumescences of the Potato there is little cell division and formation of new tissue, but merely a distension of already existing cells. In *Hibiscus*, on the other hand, there is considerable actual growth, in the sense of the formation of new tissue elements by cell division. In stem outgrowths there is still more division, so that large masses of tissue are formed, which in some cases are almost indistinguishable from wound-callus.

It, therefore, seems probable that the fact that the outgrowths usually begin in the green cells is to be explained by the relation between assimilation, turgescence, and growth. In this connection it would be interesting to know if the intumescences described by SORAUER* in the *flowers* of the orchid *Cymbidium Lowi* occurred in tissues containing either chloroplasts or leucoplasts. Neither the description nor the figure elucidates this point.

The experiments on the estimation of acids and salts in potato shoots (p. 243) show that the substance causing the excessive turgescence, which is the first visible stage in the formation of intumescences, is not an acid or salt which is confined to the affected organs, nor, indeed, anything peculiar to plants bearing intumescences.

Again, the experiments with small fragments of potato leaves (p. 225) show that the *internal causes of the formation of intumescences are extremely local*, and that the osmotic substance must be sought *in the actively-assimilating cells* which may themselves take part in the formation of the hypertrophied tissues.

These conclusions are opposed to those reached by COPELAND† and ATKINSON‡ as a result of some experiments with *Lycopersicum esculentum*.

COPELAND, after criticising adversely HABERLANDT'S§ view that in the liane,

* SORAUER (14). † COPELAND (15).
‡ ATKINSON, cited by COPELAND (15). § HABERLANDT (25).

Conocephalus ovatus, the "compensating hydathodes" are "new organs," whose function is to excrete drops of water, describes some experiments made by himself, and in his laboratory, by JOHNSON, and also, previously, by ATKINSON. These experiments were made in connection with the excretion of drops of water from leaves under pressure, and, in the course of the experiments, intumescences (or, as COPELAND calls them, œdemata) arose on the stems and leaves, as these investigators think, on account of the increased pressure. But it seems to me that we have here to deal with two distinct and independent phenomena: (1) the formation of intumescences, and (2) the excretion of drops of water under pressure. In my earlier work, one of the experiments made in COPELAND'S laboratory with *Lycopersicum*, was made with *Hibiscus*, and with the same results, viz., the excretion of drops of water and the formation of intumescences. I then thought, as COPELAND and ATKINSON do, that there was a connection between these two phenomena. But the later experiments with fragments of leaves show clearly that the intumescences arise *quite independently of root pressure*. In the case of HABERLANDT'S experiments with *Conocephalus*, I agree with COPELAND that the water was excreted at the points where the normal organs had been cut, because those were the places of least resistance.

For the same reason the masses of new tissue, which, with KÜSTER and COPELAND, I regard as intumescences, were formed over the injured parts of the leaves. The intumescences formed on the leafscars of potatoes when the leaves have fallen off appear to be of a similar nature (p. 224). In the case of *Lycopersicum*, COPELAND and ATKINSON find that the drops of water are excreted round the edges of the leaves and *not* through the outgrowths—a fact which seems to me to show conclusively that intumescences have no connection with root-pressure, and that the internal causes must be sought in the assimilating cells of the parts affected.

In considering the contents of the assimilating cells we may first take the case of the normal cells, especially those which have been most fully examined by the present writer, viz., in *Hibiscus vitifolius and Solanum tuberosum*. It has been shown that starch is abundantly formed in *Hibiscus*, particularly in plants grown under conditions which promote the development of outgrowths. The Potato is also a plant which produces large quantities of starch. According to PFEFFER* and DETMER† carbohydrates give rise to organic acids, by oxidation or combustion. These organic acids decompose nitrates,‡ and, with the non-nitrogenous organic materials resulting from carbon dioxide assimilation, proteids are formed. In this process calcium oxalate is deposited in the cells as a bye-product. Calcium oxalate may therefore be regarded as an index of proteid metabolism. Proteid metabolism, accordingly, depends upon the presence of salts and of the products of carbon

* PFEFFER (16). † DETMER (17), pp. 324 and 327.

‡ DETMER, *loc. cit.*, pp. 73 and 74.

dioxide assimilation. In *Hibiscus*, under normal conditions, the salts are brought by the transpiration current, which has been experimentally shown to be very strong, as it also doubtless is in the Potato, while in both plants assimilation is very active.

Proteid metabolism is also promoted by light. SCHIMPER regards the formation of proteids as directly dependent on light, and as taking place only in green cells. DETMER,* on the other hand, considers the relation between proteid formation and light as only indirect.

The large quantity of calcium oxalate in *Hibiscus* shows that oxalic acid is, or has been, abundant. Now organic acids† intensify turgescence, and turgescence, due to some osmotically active substance, is the immediate cause of the increase in size of the cells which form the outgrowths. What, then, is this osmotically active substance? In a previous paper I suggested that this substance might be mucilage, but later experiments show that this is impossible, because, in the case of the Potato and most of the other plants examined, mucilage is absent, and because in *Hibiscus* the mucilage-containing cells are just the ones which do *not* swell (p. 241). It now seems probable that the osmotically active substance is an acid, because an alkali would saponify the fat in the fat-containing cells. Again, the abundance of calcium oxalate, especially in and near the outgrowths, suggests that the osmotically active substance is *oxalic acid*.

The further question now suggests itself—Does this osmotically active substance (or some product of abnormal metabolism) act as a stimulus in the formation of outgrowths in some such way as the stimulus given by the bite of an insect gives rise to a gall, or the irritation caused by a fungus originates the formation of hypertrophied tissues, or a wound to callus? To this question the present investigation seems to indicate that the answer is affirmative.

Attention has been drawn to the occurrence of *proteid crystals* in potato shoots which have developed intumescences. These crystals do not occur in normal shoots, nor have they before been produced experimentally. Similar crystals have been described by HEINRICHER‡ in diseased potato shoots grown in a field during a very wet summer. The disease in question appears to be due to intumescences, for he observes that the leaves were rolled up and no fungus was seen, and that the disease was not epidemic, because healthy and unhealthy plants were growing side by side. He regards the damp situation of the potatoes as the cause of the mischief.§ Here, again, is seen the importance of the condition of the plant itself as a factor in determining the formation of outgrowths, for the external conditions were identical.

Previous to HEINRICHER's observation, crystalloids had only once been recorded, by SORAUER, in *young potato shoots*, though they had been described by COHN in potato

* *Loc. cit.*, p. 75. † DETMER (17), p. 324. ‡ HEINRICHER (18).

§ Intumescences sometimes occur spontaneously on early potatoes grown under glass.

tubers. Proteid crystals have also been described by MARSHALL WARD* in the *young* shoots of potato plants attacked by a parasitic fungus.

They are regarded by this author as probably due to an accumulation of proteids in the leaves, from which the passages of transference at the nodes of the stem had afterwards been cut off by the fungus.

The presence of crystalloids in potato shoots bearing intumescences may be due to the sudden check to assimilation, to growth, and to the transport of plastic and other substances, so that proteids are stored up in the cells in the form of crystals.

With regard to the formation of *oil*, in *Hibiscus*, *Ipomea*, and *Acalypha*, as well as in the spherical outgrowths of the various species of *Vitis* and *Ampelopsis*, there are two possible sources—(1) it may arise directly from the carbohydrates, or (2) it may be a product of the degeneration of the protoplasm. Of these two possible sources there is most evidence in favour of the latter.

Little seems to be known with certainty either as to the place or manner of the formation of oil.

A good deal of the work on the subject has been done with oily seeds, in which the question is apparently restricted to the conversion of other reserve materials into oil.

PFEFFER and DETMER both agree that oil and starch are physiologically equivalent, but while, on the one hand, PFEFFER† maintains that oils can pass unaltered through the cell wall and protoplasm, on the other hand DETMER‡ states that the migration of oil from cell to cell has not been observed. In the present case, also, it is not certain that the substance in question is merely a simple oil, more especially as it is not destroyed by the various fixing, hardening, and staining re-agents through which the sections were passed in making permanent preparations.

As a general rule no definite organs seem to be concerned in the formation of fats, which may arise in any part of the protoplasm. But, in certain plants, structures, supposed to be oil-forming organs, and which have therefore been called *elaioplasts*, have been discovered by various authors. WAKKER§ has described them in *Vanilla planifolia*, and ZIMMERMANN‖ in several other plants including *Ornithogalum*, where, for the sake of comparison, they have also been examined by the present writer. They occur chiefly in the epidermis. Structures of a different form, also regarded as oil-producing bodies and known as "colourless oil plastids," have been described by LUNDSTRÖM¶ in various species of *Potamogeton*, where they chiefly occur in the *epidermis* of *submerged* leaves. As they arise *before the chlorophyll is formed*, the production of oil is here obviously independent of carbon dioxide assimilation.

In the case of the oil in the epidermal cells of *Hibiscus*, etc., it seemed at first to be possible that the leucoplasts in these cells might be regarded as oil-forming

* MARSHALL WARD (19). † PFEFFER (16), p. 470, p. 114. ‡ DETMER (17), p. 308.
§ WAKKER (20). ‖ ZIMMERMANN (21). ¶ LUNDSTRÖM (22).

organs, but the improbability of this is shown by the fact that they sometimes contain starch in minute granules.

In the higher plants oil has not yet been proved to be a direct product of carbon dioxide assimilation.*

On the contrary, WAKKER states that the oil in the chlorophyll bodies of Monocotyledons which form no starch is not a product of assimilation, but rather indicates the beginning of degeneration. He also notes that the oil was chiefly found in plants grown in greenhouses. This statement is interesting in connection with the fact that all the outgrowths developed during the present experiments were formed in plants grown under glass.

The formation of the oil in the outgrowths appears to be closely connected with *respiration.* In certain other cases the production of oil is known to be due to imperfect respiration. For example, a fungus growing in air may contain comparatively little oil, whereas the same fungus when submerged in water or a nutritive fluid becomes crowded with oil in large and small drops. Here, of course, there can be no question of carbon dioxide assimilation.

In *Potamogeton*, as noted above, oil is formed in *submerged* leaves. Again, in the Crassulacæ there is a distinct relation between feeble respiration and the formation of oil.

In the case of intumescences, oil occurs only in the type in which the cells are in uninterrupted connection, *e.g.*, in *Hibiscus*, but not in the type in which the cells at an early stage separate from one another as in the various species of *Solanum*. The obliteration of the intercellular spaces in the neighbourhood of the outgrowths in the leaves of *Ipomea*, *Eucalyptus*, *Hibiscus*, and *Acalypha*, and in all stem outgrowths, as well as in wound-callus, would prevent to a considerable extent the passage of carbon dioxide and of oxygen, so that both assimilation and respiration would be checked. Carbon dioxide assimilation is stopped if the stomata are blocked,† as they are in the outgrowths of the plants in question. But, on the other hand, a certain amount of cuticular respiration may in some cases take place through the very thin walls of the overgrown cells. The occurrence of oil, first and chiefly, in the epidermal cells, may possibly be due to a cessation of respiration first in the colourless cells, because, in the green cells, the oxygen set free by carbon dioxide assimilation would be available for respiration, and because, in the green cells, there is (at least, at the beginning of the formation of outgrowths) an excess of carbohydrates. In other words, the green cells are for a time, so to speak, more self-supporting than the colourless cells, in which the protoplasm sooner begins to degenerate. The absence of oil in *Solanum* may be occasioned by the early separation of the cells from one another, before there has been any check to respiration. The formation of oil by the degeneration of protoplasm is comparable to the fatty degeneration of muscle which

* PFEFFER (16), p. 320.

† PFEFFER (16), p. 186; DETMER (17), pp. 56 and 57.

takes place in animals. The available evidence, therefore, points to the origin of the oil in outgrowths as a product of degeneration, and to the importance in this process of diminished respiration.*

The succession of events may be stated briefly as follows: carbohydrates form organic acids, organic acids cause turgescence, turgescence gives rise to hypertrophy of the cells, so that an intumescence is formed.

Three more questions may be considered in relation to assimilation, namely, (1) the *age of leaves* forming intumescences, (2) the effect of *illumination*, and (3) of *temperature*.

(1) As before stated, intumescences form most readily in young but mature leaves, not only because in such leaves the powers of assimilation are greater than in developing or degenerating leaves, but also because old tissues lose their irritability, and therefore their capability of response to stimuli. There are, however, some exceptions to this rule, as, for example, in *Vitis* and *Solanum* in my own experiments, in the *Pelargonium* brought to the laboratory in November, in the case of the vine observed by SORAUER,† and of the pinks described by PRILLIEUX.‡

SORAUER, in fact, regards the formation of intumescences as a sign of *depressed* vitality, and therefore of *reduced* powers of assimilation, and PRILLIEUX is inclined to agree with him.

The case of the pinks cited by PRILLIEUX is peculiar because the disease appeared in plants in the open, towards the end of summer, in August and September. The author gives no figures and does not say whether the disease appeared in wet or dry weather, though he notes that the plants had been freely watered.

When formed experimentally, intumescences are never developed *rapidly* on older leaves, but only quite slowly, nor are they so large as those on younger leaves, even under the same external conditions.

These facts are evidence in favour of the view, which is confirmed by most of the experiments made, that the leaf must be *actively assimilating and in an irritable condition*. Even in a young leaf, if the external conditions are changed gradually, the response is gradual; on the other hand sudden changes call forth an immediate response.

In some cases if the conditions are changed very slowly the plant is able to accommodate itself to them without any formation of intumescences, *i.e.*, without any considerable change in metabolism.

(2) Again, the presence or absence of *light* in the formation of intumescences is, doubtless, connected with assimilation. Some plants, *e.g.*, *Hibiscus*, do not form intumescences in darkness, while others, *e.g.*, *Populus tremula*, do so with some readiness. Probably this depends partly upon the presence or absence of the

* For an account of the passage of gases into and out of leaves, see BLACKMAN (23).

† SORAUER (3), p. 225. ‡ PRILLIEUX (24).

products of assimilation in the leaf at the time of experiment. It might be tested by trying whether an aspen leaf which has been emptied of its carbohydrates would form intumescences in darkness. Some light is thrown on the question by the fact that intumescences will form on leaves floating on a solution of sugar under conditions in which they are not produced in leaves floating on water or on Knop's solution. Under the conditions favourable to the formation of intumescences assimilation is *gradually* checked by the lack of salts due to the stoppage of the transpiration current owing to the damp air. In darkness carbon dioxide assimilation is stopped *at once*, so that only pre-existing carbohydrates are available.

In this connection it is instructive to note that some stem intumescences and all wound-callus form more rapidly and extensively in darkness than in light. But in the case, *e.g.*, of callus, the starch stored in the stem is used up in the formation of new tissue, as this gradually disappears towards the neighbourhood of the callus.

(3) With regard to the influence of *temperature* as a factor in the formation of intumescences, there is evidence that the optimum temperature for the formation of intumescences may be related to the optimum temperature for normal growth, which is known to vary in different plants. In the case of the Poplar, a native of a temperate region, intumescences form at a lower temperature than is the case in *Hibiscus* or *Solanum tuberosum*, which are natives of much warmer countries. But even in *Populus* intumescences form more rapidly, and attain to a much larger size, and are more numerous at a temperature of say 80° F. than at 60°.

Temperature also exerts an extremely important influence in the formation of *callus*. In *Populus* enormous masses of callus are formed at a temperature of 70°–80° F., in a saturated atmosphere and in darkness, while a not insignificant quantity is developed in daylight.

The experiments recorded in the present paper also throw some light on the relation between stomata and the distribution of intumescences. In some cases, *e.g.*, those spherical intumescences (Perldrüsen) which contain mesophyll cells, there is a close and direct connection dependent upon development (p. 238). On the other hand, in cases of purely epidermal growths, whether spherical or hemispherical, stomata have obviously no influence, since the outgrowth arises by dilatation (which may or may not be accompanied by division) of the epidermal cells, whether stomata are present or absent. In the remaining types (which include the great majority of intumescences) experiments seem to show that in most cases *stomata have no direct connection with the formation of outgrowths*, especially in those cases in which the mesophyll cells swell up and break through the epidermis. They may, however, have an indirect effect in the development of intumescences like those found in *Hibiscus* and *Eucalyptus*.

Reference has several times been made to the similarity between intumescences, especially stem intumescences, and, though in a less degree, leaf intumescences also, and wound-callus. These tissues may here be compared in more detail, as the resemblances seem to be more than superficial and to point to an identity in all essential points.

As has been said, stem intumescences, and wound-callus on stems, have the same structure, and the experiments which have so far been made show that they arise under the same external conditions. In fact, in many cases it is impossible to draw any line between stem intumescences and wound-callus. Much work has been already done on callus, which KÜSTER summarises in his 'Pflanzenanatomie,' also giving an account of his own researches on this question, and showing the importance of damp air and of warmth.

I have repeated some of his experiments, and made some of my own, which I propose to extend next summer with a view to comparing intumescences and callus, both in leaves and in stems.

At present very few experiments have been made with wounded leaves. Those made by HABERLANDT* have been discussed above (p. 247).

In discussing intumescences in his 'Pflanzenanatomie,' KÜSTER treats chiefly of those on leaves, passing with a few words over those on stems (which, however, he notes are formed on the *sunny* side of the stem), because he regards intumescences as typical "hypertrophies," *i.e.*, enlarged tissues in which the increase in size is due to dilatation of pre-existing cells and not to cell-division. Consequently, he regards the cases recorded by SORAUER,† in which cell-division takes place, as an exception to the rule. Apparently he assumes the structure of intumescences in leaves and on stems to be always the same. But it seems to me that the difference between hypertrophy and hyperplasy is one of degree only, and therefore non-essential. In fact, KÜSTER himself‡ says that in higher plants the cells of any tissue which can form a hypertrophy can also form a hyperplasy, and that no distinct line can be drawn between the two. Internal and external factors, he says, determine whether a hypertrophy or a hyperplasy will be formed. Other hypertrophic (or hyperplastic) tissues also show similarities in structure to intumescences and callus, notably galls, whether due to vegetable or animal parasites.

Another tissue closely related to intumescences and to wound-callus is the abnormal growth which takes place in lenticels, and which is formed under the same conditions. This also I hope to consider further in a subsequent paper.

The function of wound-callus is twofold—it heals the wound by covering it with new cells, and it provides a meristemmatic tissue from which adventitious roots and shoots are formed. But callus is only formed in damp air; in dry air, as MASSART§

* HABERLANDT (25).

† The diseases described by SORAUER as *aurigo* and that known as *orchid spot* seem to be related to intumescences and to be due to disturbances in metabolism.

‡ 'Pflanzenanatomie,' p. 92.

§ MASSART (26).

has shown, the formation of cork is the chief phenomenon in the healing of wounds, and cicatrisation takes place with a very slight development of new tissues.

It seems, therefore, as if intumescences, callus and cicatrisation by cork formation represented a series of tissues of which the production is directly dependent on external conditions, of which by far the most important determining factor is the amount of *moisture* in the air, but in which temperature, oxygen and occasionally illumination, are also to be considered.

Given an unwounded stem in a suitable physiological condition, exposure to a saturated atmosphere causes the formation of *intumescences*, *i.e.*, of a large increase in size of the parenchymatous cells usually accompanied by cell-division. Owing to the excessive dampness of the air, which causes abnormal metabolism, this tissue soon degenerates while it is still quite homogeneous. If cork is formed it appears in the later stages.

But in a wounded stem in damp air, if other conditions are favourable, *callus* is formed, varying in extent both with the state of the tissues, with the food supply available, and with the external conditions. The initial step in the formation of callus is generally the formation of cork, the drier the air the more cork is formed, and this is followed by the formation of a tissue indistinguishable from certain forms of intumescence.

Sooner or later this tissue loses its homogeneity by the formation of tracheides, and also by the establishment of groups of meristemmatic tissue from which adventitious roots and shoots are developed.

This tissue, then, is not so markedly and destructively pathological as intumescences. Lastly, if the air is very dry, dilatation of the cells does not occur at all, but a cork cambium is formed, and the wound is healed by cicatrisation.

The truth of this view seems to be supported by an experiment made by MASSART, who cut in a hollow stem of *Ricinus* two internal longitudinal slits and two external. The external slits which were exposed to dry air healed up by cork formation, while the cells round the internal slits grew out into thin-walled vesicles, like the cells of an intumescence or of callus.

If the above conclusions are correct, we may say that in damp air wounds are healed by the formation of a mass of homogeneous tissue of thin-walled cells with watery contents and little or no cork, *i.e.*, by *callus*. If the air is dry the wounds are healed almost exclusively by *cork*. Varying degrees of dampness give rise to the production of varying proportions of cork and protoplasmic cells.

The formation of cork, of course, prevents the underlying tissues from the desiccation which would otherwise occur in dry air, while the formation of masses of soft tissues is caused by the excess of water in the tissues due to the check to transpiration by the damp air.

In connection with the effects of heat and moisture on the metabolic activity of plants, the researches of PRILLIEUX* on seedlings grown in hot soil are instructive.

* PRILLIEUX (13).

Seeds of bean and pumpkin were sown in an earthen pot in a damp and cold part of the laboratory. The earth was heated by a piece of brass plunged vertically into the pot and heated on the outer side by gas. The stems of the seedlings which came up were extremely thick, and much shorter than in normal cases. The thickness was caused by swelling of the internal cells, by which the tension was so increased that deep cracks were formed in the outer tissues.

Apparently the internal cells did not grow out as intumescences, perhaps because the air was too cold to be very damp. Presumably the wounds, if healed at all, were healed by cork, as they were exposed to the open air, but PRILLIEUX does not give any account of their fate, nor does he give much explanation of the phenomena he describes. It seems to me that the swelling is, so to speak, a kind of internal intumescence. The internal tissues swell up because transpiration is checked by the damp and cold air, which cannot contain so much moisture as warm air, while at the same time the warm soil encourages the active absorption of water.

Nuclear Phenomena.

In conclusion, we may consider the *nuclear phenomena* in intumescences and in other pathological tissues in plants. *Amitosis* has been observed in a considerable number of plant tissues, all of which are more or less pathological.

It has already been stated that *amitosis* is the usual type of division in both intumescences and wound-callus. It has also been observed in galls (whether caused by parasitic plants or animals) chiefly in so-called "giant cells" by TOUMEY,* NATHANSOHN,† TISCHLER,‡ KÜSTER§, and others. Its occurrence in wound-tissues—viz., in callus, has been shown by MASSART‖ and NATHANSOHN,† while in the pathological tissues of seedlings grown in over-heated soil it has been observed by PRILLIEUX.¶ Again, it has been described by various authors in abnormal animal tissues—*e.g.*, by FARMER** in malignant growths in man, and by BALBIANI and HENNEGUY†† in the wounded tails of tadpoles (see p. 258).

MASSART goes so far as to assert that in wound-tissues nuclear division is *always* direct, but this statement has been challenged by NATHANSOHN, though it certainly seems to be true in most cases. In my own preparations there were only a very few examples of indirect division, and these were generally imperfect.

As NATHANSOHN points out, in discussing amitosis in *Spirogyra* and in higher plants, various stimuli may give rise to the same result, viz., *amitosis.*

In *Spirogyra*, NATHANSOHN obtained amitosis by placing the cells at a low temperature, and then bringing them again to a normal temperature. He also produced the same effect by treatment with a dilate solution of ether. Amitosis

* TOUMEY (27). † NATHANSOHN (28). ‡ TISCHLER (12).
§ KÜSTER (10). ‖ MASSART (26). ¶ PRILLIEUX (13).
** FARMER, etc. (29). †† BALBIANI and HENNEGUY (30).

occurred spontaneously in *Spirogyra* growing in a dish in which were numerous bacteria and Protozoa. But with higher plants the same methods were unsuccessful.

NATHANSOHN discusses the relation between mitosis and amitosis, whether the two types are to be regarded as equivalent, or as the two ends of a series, and if, in the latter case, amitosis is to be regarded as the simple and primitive type which give rise gradually to mitosis, or whether amitosis is to be considered as a result of degeneration.

That direct nuclear division is, in some cases at least, due to a degeneration of the nuclei and the cells containing them, seems to be certain. Amongst such cases are to be numbered intumescences and some callus, possibly all callus, certainly that which is rapidly formed in great quantity.

NATHANSOHN'S experiments with *Spirogyra* also seem to point to the same conclusion and to show that one of the factors amongst the causes of degeneration is the want of oxygen caused by narcotisation, by the presence of other organisms, or by too low a temperature in *Spirogyra*, and by the lack of intercellular spaces in intumescences, wound-tissues, and galls.

Again, whether nuclear division is direct or indirect seems to depend upon *nutrition*. In the cells of intumescences and callus there is little protoplasm and much watery cell sap, and therefore little food material available for the actively growing and dividing nuclei. And it is possible that this very rapidity may exert a mechanical influence on division, direct division being much more rapid than indirect.* (*Cp.* p. 38.)

In the case of karyokinetic division there are two types, one, the simpler, occurring in vegetable cells, the other, and more complicated, occurring normally in reproductive tissues, but, as FARMER has lately pointed out, to be found also in certain peculiar tissues in both plants and animals, notably in ferns, in the proliferating tissues formed during apogamy and apospory, and in malignant growths in man.

In the tissues in which amitosis occurs the food supply is small, in ordinary vegetable cells it is much increased, but it reaches a maximum in reproductive cells, in which the most complex type of mitosis occurs. The close relation between nutrition and the various types of reproduction has been proved experimentally by KLEBS,† who has shown that it is possible in certain fungi to determine the kind of reproduction which shall take place by the kind and amount of food given.

In both plants and animals it is not infrequent to find both mitosis and amitosis occurring in the same tissues. But in the cases observed by me the mitotic divisions are almost always imperfect—again pointing to degeneration. FARMER observes that in malignant growths in man "amitosis is very frequent."‡ It is important to note

* In etiolated poplar shoots grown in a damp atmosphere, in darkness, at a temperature of 35° C., the nuclear division is *exclusively direct*.

† KLEBS (31).

‡ FARMER (29), p. 503.

that both in plants and animals abnormal nuclear phenomena occur only in *rapidly growing tissues.* In plants the nuclear phenomena were most marked in rapidly-formed intumescences and wound-callus, while in animal tissues FARMER states that the phenomena observed by him only occurred in *rapidly growing tissues of a malignant character*, not in the slowly formed tumours known as benign.

Again, FARMER regards the remote cause of malignant outgrowths to be various stimuli causing continuous irritation. In plants the formation of intumescences is certainly due to stimuli acting continuously, but rapidly, on an organism in such a state of irritability that it is able to respond. Intumescences in a plant seem therefore to be comparable to malignant growths, such as cancer, in animals.

Some experiments made by BALBIANI and HENNEGUY* are particularly instructive in this connection.

They grafted together two pieces of a tadpole's tail, or the end of a tail applied to the part from which it had been cut off from a previously anæsthetised tadpole, and placed them in damp air. *In about an hour* the two pieces had grown together by the formation of new tissue produced by the rapid growth and division of cells. In some cases this tissue occurred in the form of outgrowths ("bourgeons"), comparable, as it seems to me, to the wound-callus, or intumescences of vegetable tissues. Moreover, the nuclear division was exclusively direct in the newly-formed wound-tissue, though indirect division was seen in the adjoining normal cells. As the authors point out, such a rapid formation of new tissue would be impossible by indirect nuclear division, as each mitosis takes about three hours, whereas, by the amitotic method, a considerable amount of new tissue had been formed in little more than an hour.

Later, in the new tissues, direct division was succeeded by the indirect method. The authors consider that, in such cases, amitosis cannot be regarded as degenerative (as they admit it to be in certain cases), but as *regenerative*, and as a rapid method used by the organism when there is no time for the complicated phenomena of karyokinesis to take place.

There is obviously a most striking resemblance, both in rapidity of formation, and also in the amitotic nuclear division, between the wound-tissue produced in the case of the tail of a tadpole and of the stem of a tree. And there is a similar resemblance in the formation of pathological growths on unwounded organs in both plants and animals.

If such processes in plants and animals are really comparable, as seems probable, then a study of them in plants, which, though complex enough, is yet simpler than in animals, should help to elucidate that difficult but all important problem of the causes, remote and proximate, of the formation of malignant growths in man, and will once more show the necessity for co-operation amongst workers in the various departments of biological science.

* BALBIANI and HENNEGUY (30).

I have again to thank Professor MARSHALL WARD for his kindness in allowing me to work in the University Laboratory and in the Botanic Garden, and for the suggestions he has made during the course of my investigations.

I also desire to thank Mr. LYNCH for his ready help at the Botanic Garden.

Summary.

Experiments with various species of plants show that, for the formation of intumescences, *moist air*, *heat*, *oxygen*, and in some cases *light*, are the necessary external factors, the internal factors are *irritability*, and either *active powers of assimilation* or abundance of *stored food material.* The internal causes are *extremely local*, as shown by the formation of intumescences upon small isolated fragments of leaves. The turgescence, which is the first visible stage in the formation of intumescences is, therefore, due to some *osmotic substance* formed in the cells themselves, and not conveyed from a distance. This substance appears to be *oxalic acid.*

Anatomical investigations show that there are various distinct types of intumescences on leaves which may be classified according to their structure and mode of origin. Intumescences, especially those on stems, bear a strong resemblance to *wound-callus* and to *lenticel* outgrowths.

In intumescences and wound-callus *amitotic nuclear division* is the rule, such amitosis being due to degeneration caused by the excessively rapid growth.

There are also many points of resemblance, especially as regards rapidity of formation and amitotic nuclear division, between certain wound-tissues in plants and animals, and also between certain rapidly-formed pathological growths in unwounded organs in both plants and animals, which are caused not by any parasitic organism, but simply by the influence of external stimuli acting upon a plant or animal in such a condition of irritability that it is able to respond.

Notes on Methods.

Permanent preparations were made by fixing the material in FLEMING's solution, or in picro-formal in fixing solution. Other methods were used, but these, especially the latter, gave the best results. In most cases hand sections proved more useful than those cut with a microtome, as the latter were usually too thin.

For staining the nuclei, gentian violet and eosin in oil of cloves were generally employed, but some good results were also obtained with diamant, fuchsin, and licht-grün.

The nuclei were all examined under an oil immersion lens, Zeiss $\frac{1}{12}$ apochrom., and eye-piece No. 4, and drawn with a camera lucida.

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EXPLANATION OF PLATES.

PLATE 14.

Fig. 1*a*.—Photograph of a potato plant covered with intumescences after two days under a bell-glass in a hot-house.

Fig. 1*b*.—A shoot from a potato plant similarly treated. (Reduced $\frac{2}{3}$.)

Fig. 2.—Leaf of *Solanum aculeatissimum*, with intumescences on the under side of the lamina (slightly reduced).

Fig. 3.—Fresh intumescence from leaf of *Pavonia arabica* ($\frac{1}{2}$-in. obj., oc. 2) (reduced).

Fig. 4.—Base of intumescence in *Pavonia arabica* (microtome section). *a*. intumescence, *b*. stalk cells, *c*. epidermis of leaf, *d*. mesophyll cells.

Fig. 5.—Fresh intumescence of *Vitis rotundifolia* ($\frac{1}{2}$-in. obj., oc. 2, reduced $\frac{1}{2}$), showing apical stoma.

Fig. 6.—Cells from a fresh intumescence of *Ampelopsis Veitchii*. *n.* nuclei, *s.* starch in leucoplasts, *o.* oil.

Fig. 7.—Section of intumescence on leaf of *Ipomea Woodii* (unstained, in glycerine jelly), showing oil stained black with the osmic acid of the Flemming's solution.

Fig. 8.—Section of intumescence from leaf of *Acalypha marginata* (½, 2), showing oil as in Fig. 7.

Plate 15.

Fig. 9.—Section of intumescence on leaf of *Gmelina hystrix*. (½, 2.)

Fig. 10.—Section of intumescence on leaf of *Eucalyptus botryoides* (obj. D., oc. 2).

Fig. 11.—Section of intumescence on leaf of *Eucalyptus diversicolor*.

Fig. 12.—Section of leaf of *Vitis vinifera* with intumescences. (D., 2.)

Fig. 13.—Section of outgrowths on leaf of *Hibiscus vitifolius* grown under red glass. (D., 2)

Fig. 14.—Section of leaf of *Hibiscus vitifolius* with outgrowths, grown under yellow glass. (½, 2.)

Fig. 15.—Section of outgrowths on leaf of *Hibiscus vitifolius* grown under white-washed glass. (½, 2.)

Fig. 16.—Young rapidly-formed outgrowths on leaf of *Hibiscus vitifolius*. (2-in. obj., 2 oc.)

Plate 16.

Fig. 17.—The same, more highly magnified. (D., 2.) (Reduced ½.)

Fig. 18.—Older stage of a rapidly-formed outgrowth on *Hibiscus vitifolius*. (D., 2.) (Reduced ½.)

Fig. 19.—Old outgrowth on leaf of *Hibiscus vitifolius*. (D., 2.)

Fig. 20.—Young intumescence on potato leaf. (D., 2.) (Reduced.)

Fig. 21.—Intumescence on leaf of potato. (Reduced ½.)

Fig. 22.—Older intumescence on potato leaf.

Fig. 23.—Constituent cells (fresh) of a potato intumescence which is disintegrating.

Fig. 24.—Old outgrowth on leaf of *Solanum aculeatissimum*.

Plate 17.

Fig. 25.—Nuclei in outgrowth on the stem of *Populus alba*.

a, *b*, *c*. Typical nuclei with varying degrees of reticulation of nucleoplasm.

d. Nucleus with very large nucleolus.

e. Nucleus with two nucleoli in contact.

f. Nucleus with two separated nucleoli.

g. Elongated nucleus with two nucleoli.

h. Nucleus with two nucleoli and no membrane.

i. A similar nucleus with three nucleoli.

k. Two daughter nuclei after division. A membrane is present except along the line of separation.

l. Two daughter nuclei without membranes.

m. A cell containing two nuclei each with two nuclei, one nucleus is dividing.

n. A nucleus dividing by budding.

o and *p.* Two nuclei, showing imperfect karyokinesis.

Fig. 26.—Nuclei from an outgrowth on the stem of *Populus nigra.*

a. Two adjoining cells, one with a single nucleus and the other with two nuclei. In all the nuclei the nucleolin is in many fragments.

b. Another cell with two nuclei.

c. A large nucleus with deeply stained granules round the clear zone.

d. A similar nucleus.

e. A nucleus with two nucleoli.

f. Two nuclei, apparently just after division, one with one nucleolus and the other with two. In the nucleoli are granules more deeply stained than the surrounding nucleolin.

g. A nucleus with granules of nucleolin.

h. A nucleus without membrane and with two nucleoli with fragments of nucleolin.

i. Two elongated dividing nuclei, each with one large and one small nucleolus.

k. A three-lobed nucleus with three nucleoli.

l. A nucleus like (*i*) in an earlier stage of division.

m. A nucleus made up of four separate parts, "segmented nucleus" of PRILLIEUX.

n. Two nuclei between which a new cell wall is forming.

o, p, q. Imperfect karyokinesis.

Fig. 27.—Nuclei from a stem intumescence of *Hibiscus vitifolius.*

a. Nucleus with two nucleoli and coarsely granular nucleoplasm.

b and *c.* Nuclei after division. Two in each cell.

d. Imperfect karyokinesis (*cp.* 26, *o* and *p*).

Fig. 28.—Nuclei from the callus of *Chrysanthemum Broussonetii.*

a. Dividing nucleus.

b. Nucleus dividing indirectly and showing definite chromosomes. The only well-marked case seen in any callus or intumescence.

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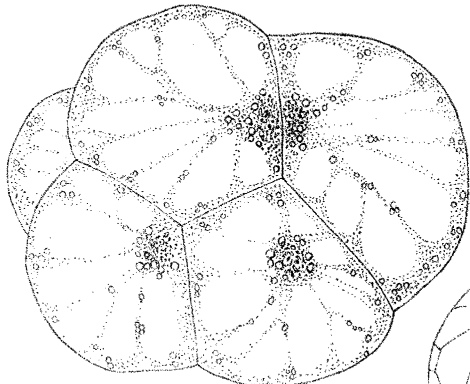

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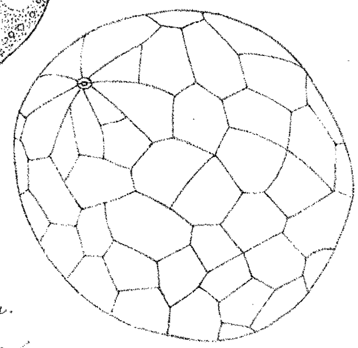

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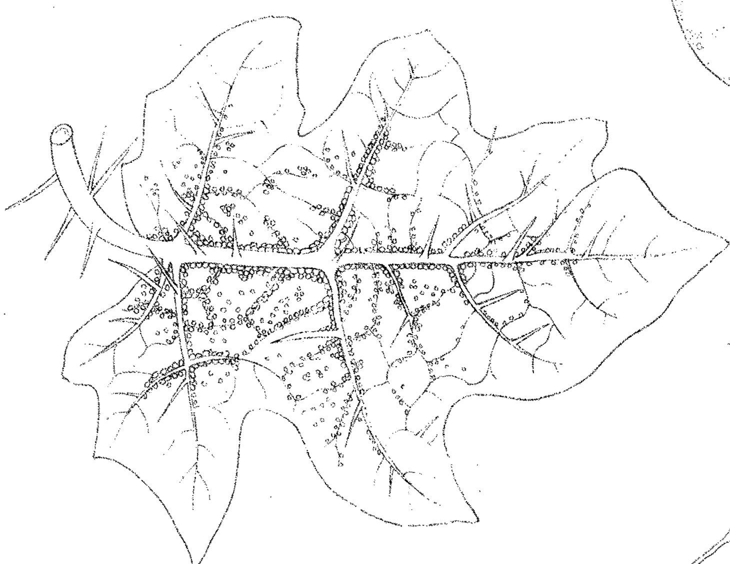

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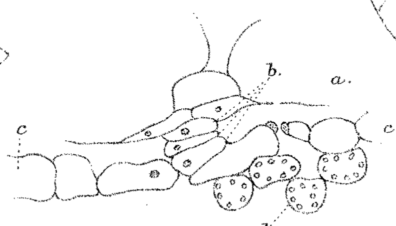

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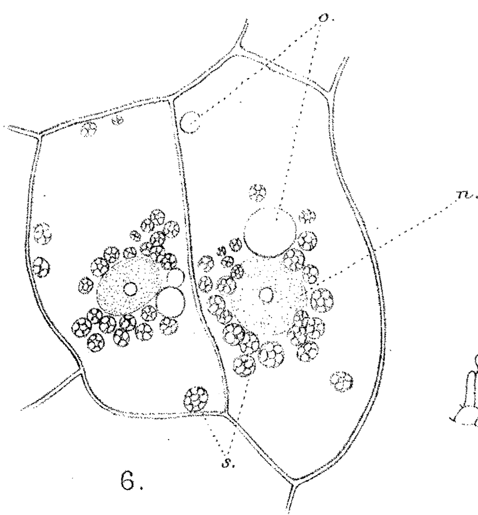

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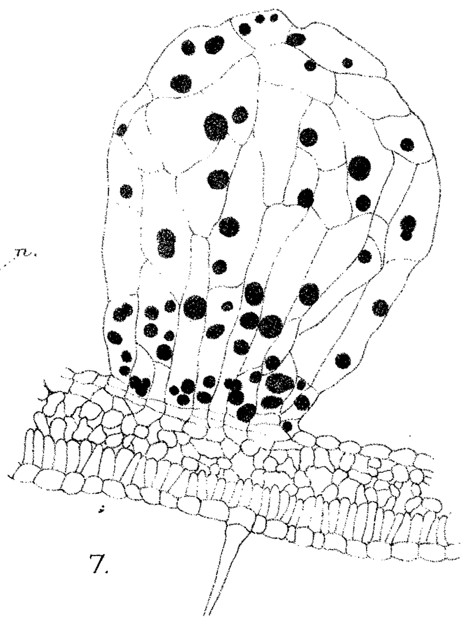

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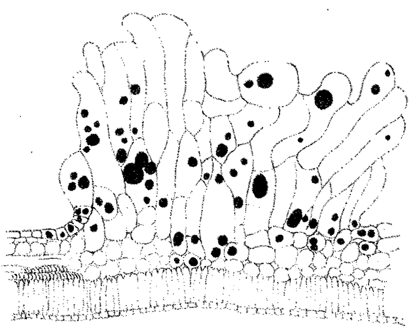

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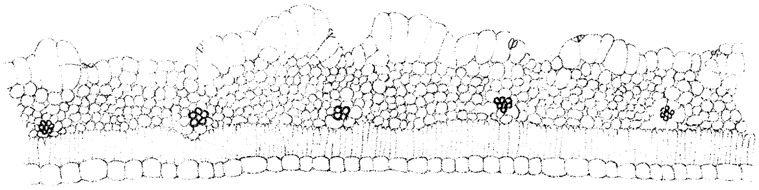

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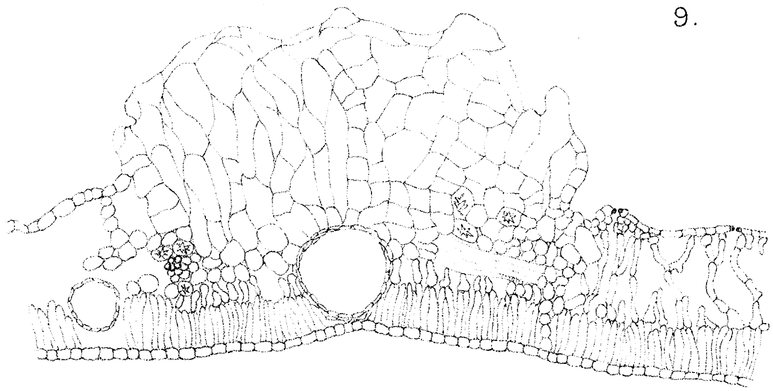

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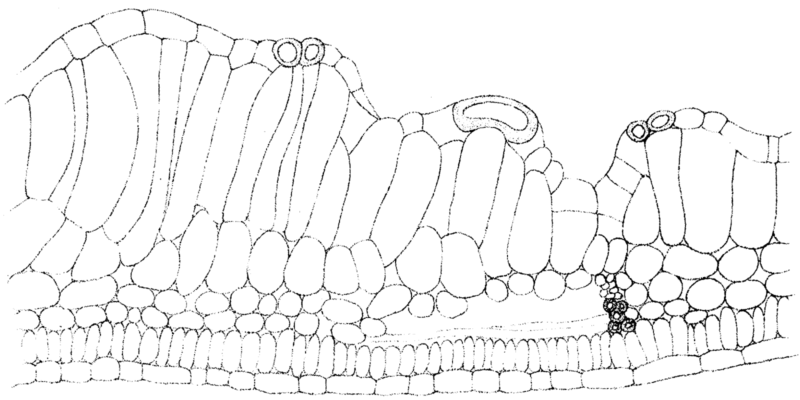

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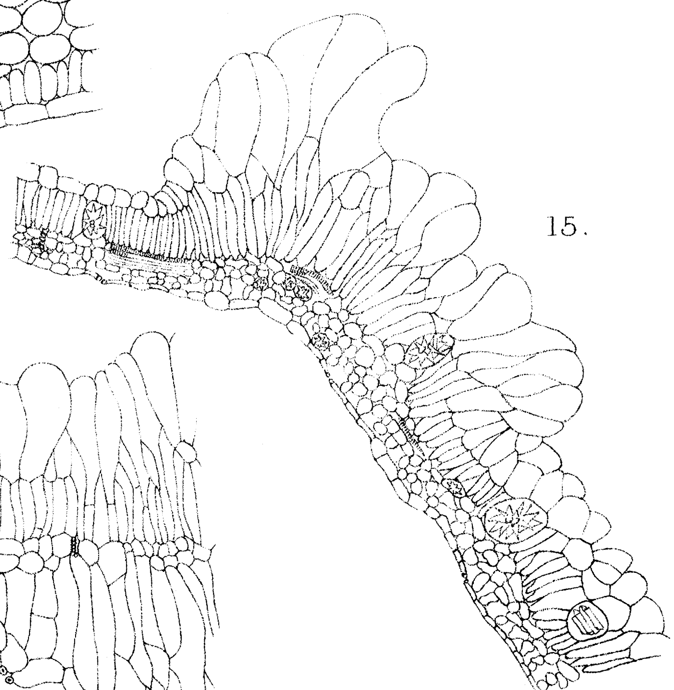

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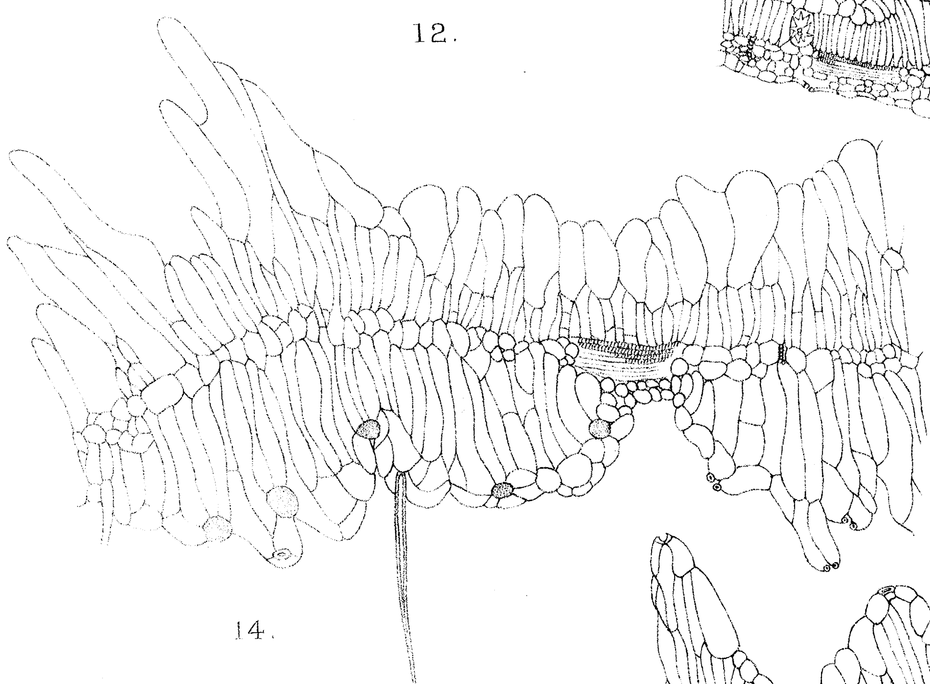

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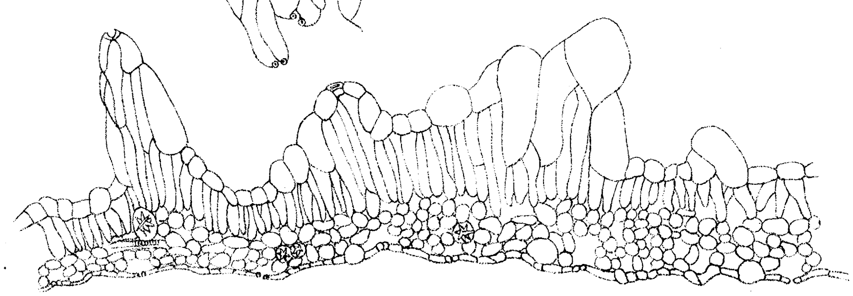

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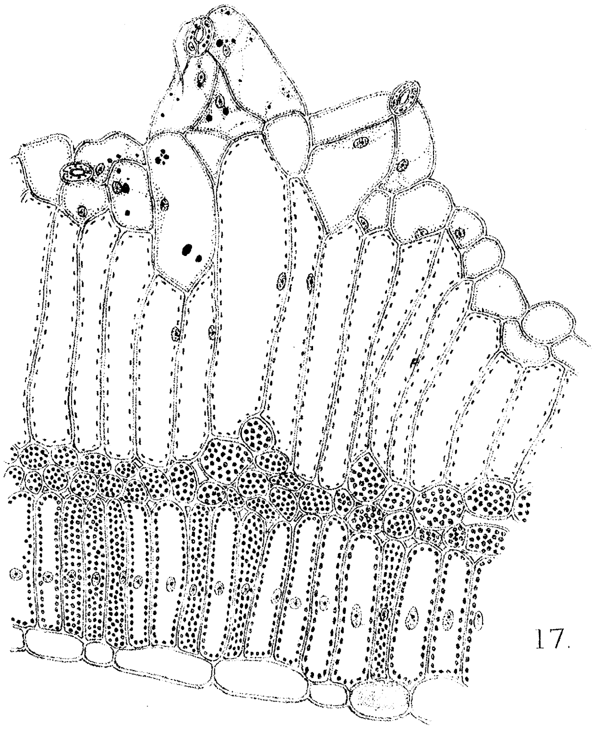

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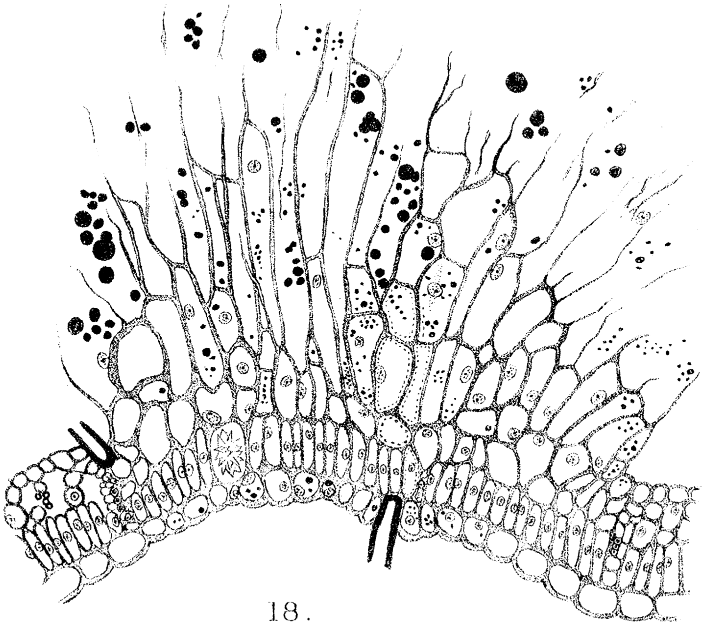

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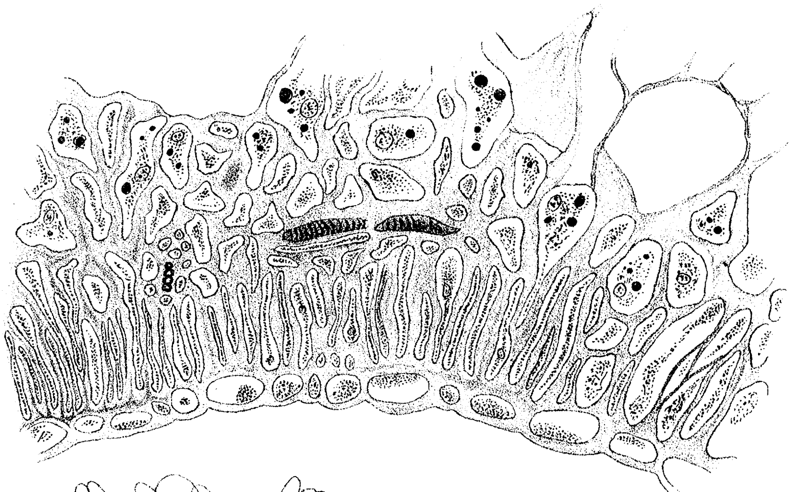

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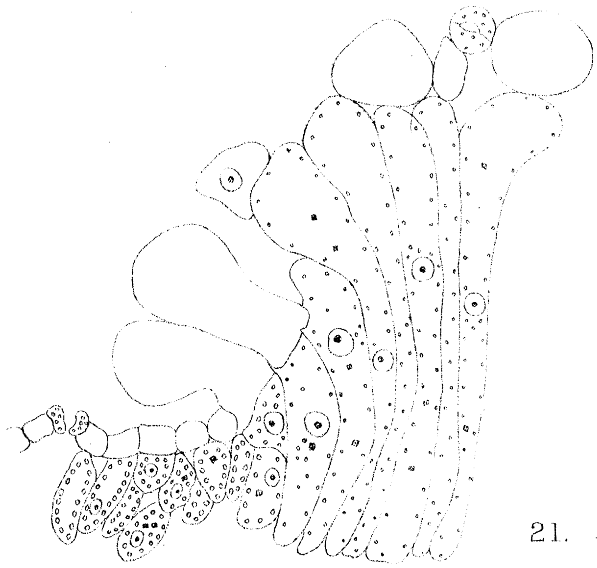

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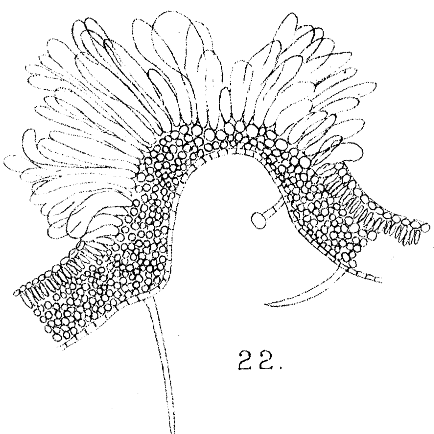

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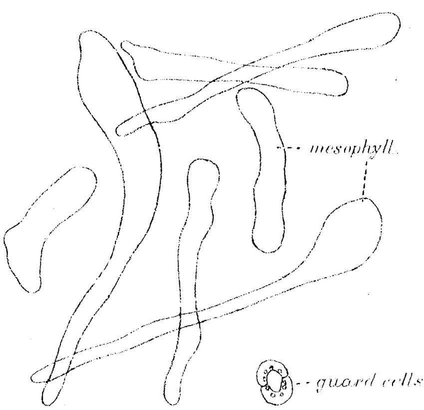

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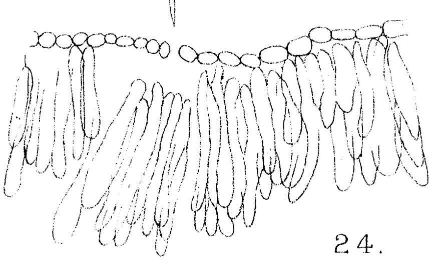

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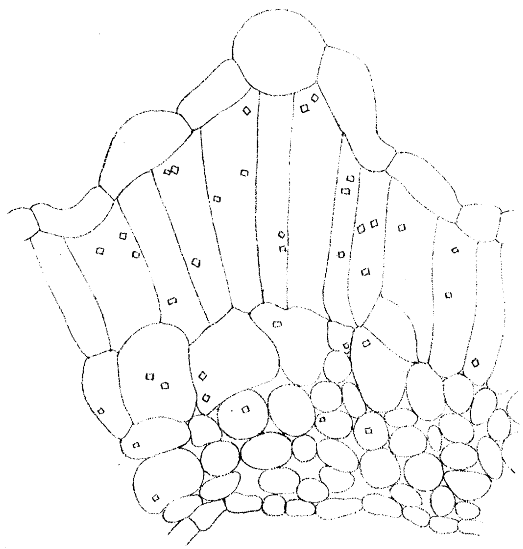

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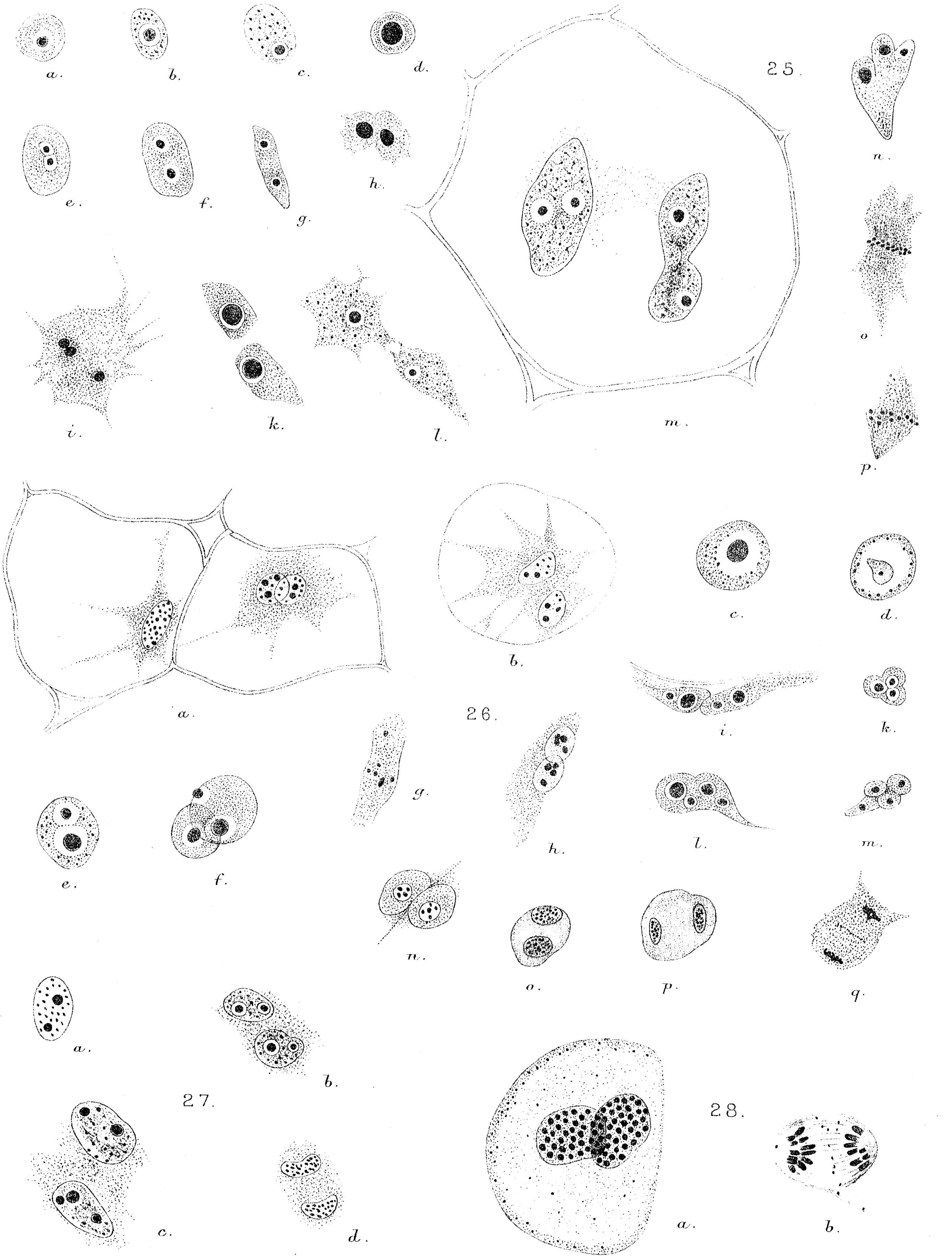

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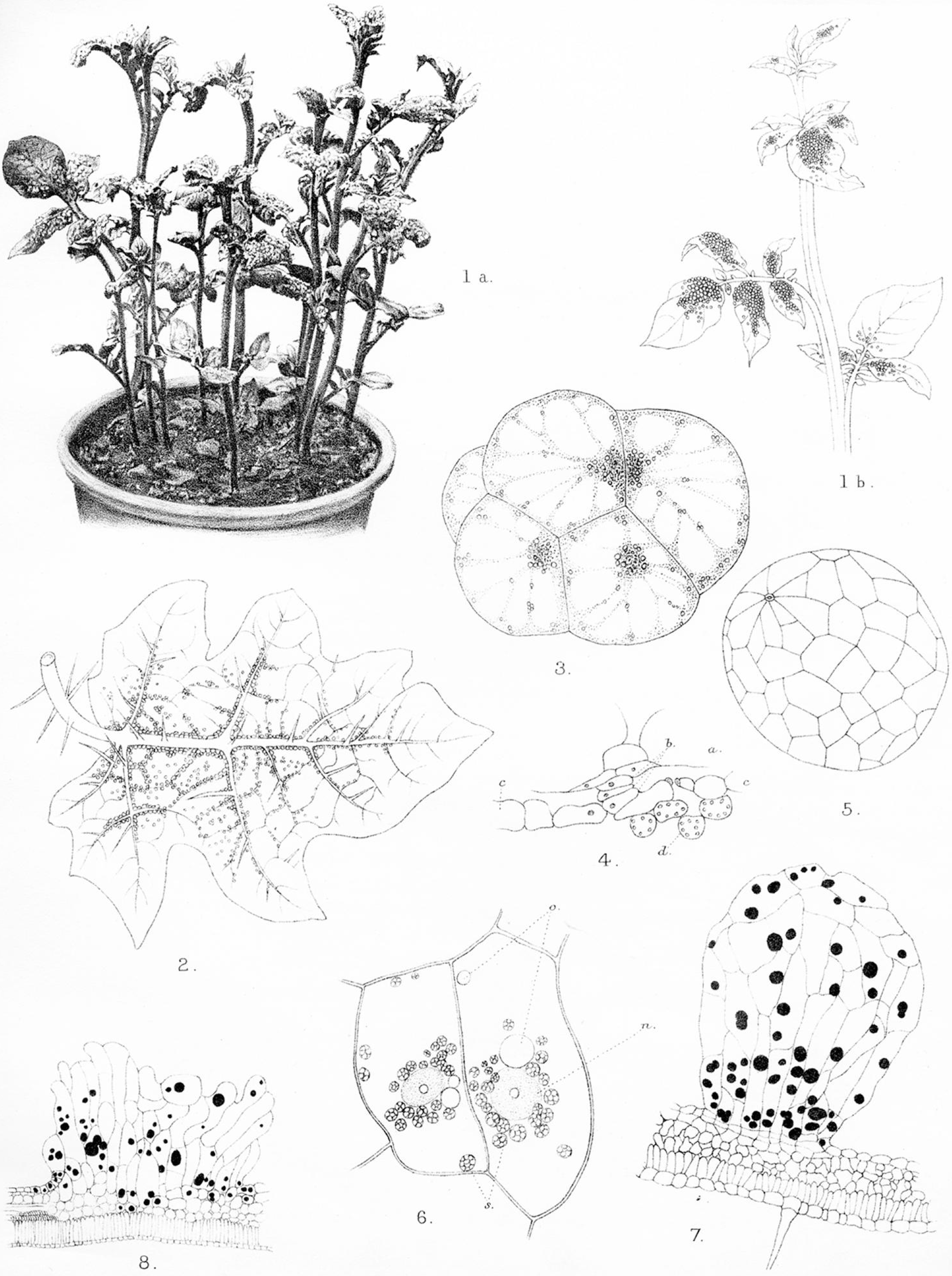


PLATE 14.

Fig. 1*a*.—Photograph of a potato plant covered with intumescences after two days under a bell-glass in a hot-house.

Fig. 1*b*.—A shoot from a potato plant similarly treated. (Reduced $\frac{2}{3}$.)

Fig. 2.—Leaf of *Solanum aculeatissimum*, with intumescences on the under side of the lamina (slightly reduced).

Fig. 3.—Fresh intumescence from leaf of *Pavonia arabica* ($\frac{1}{2}$-in. obj., oc. 2) (reduced).

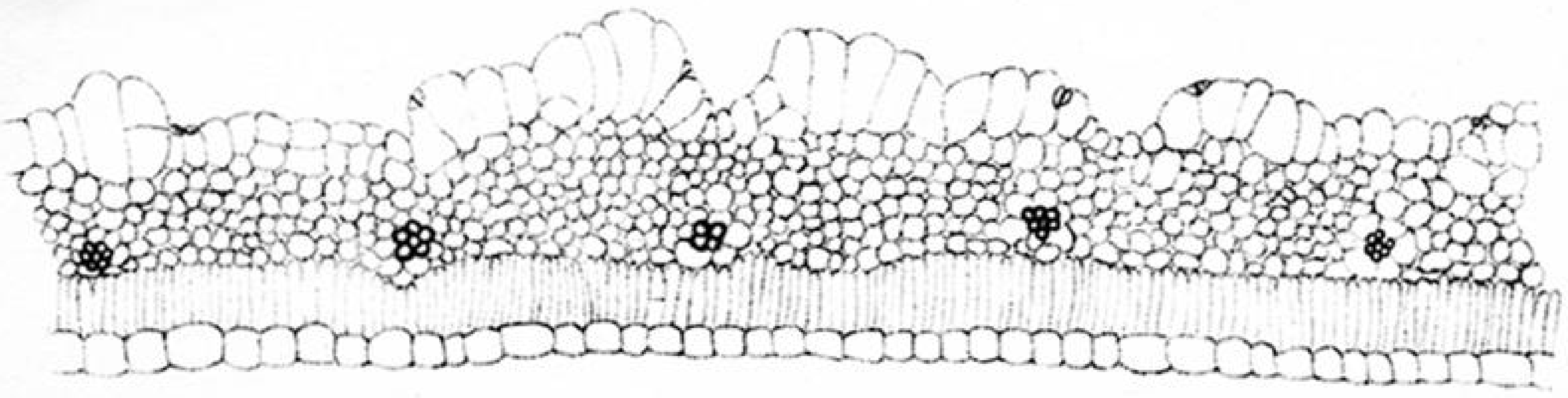
Fig. 4.—Base of intumescence in *Pavonia arabica* (microtome section). *a*. intumescence, *b*. stalk cells, *c*. epidermis of leaf, *d*. mesophyll cells.

Fig. 5.—Fresh intumescence of *Vitis rotundifolia* ($\frac{1}{2}$-in. obj., oc. 2, reduced $\frac{1}{2}$), showing apical stoma.

Fig. 6.—Cells from a fresh intumescence of *Ampelopsis Veitchii*. *n*. nuclei, *s*. starch in leucoplasts, *o*. oil.

Fig. 7.—Section of intumescence on leaf of *Ipomea Woodii* (unstained, in glycerine jelly), showing oil stained black with the osmic acid of the Flemming's solution.

Fig. 8.—Section of intumescence from leaf of *Acalypha marginata* ($\frac{1}{2}$, 2), showing oil as in Fig. 7.



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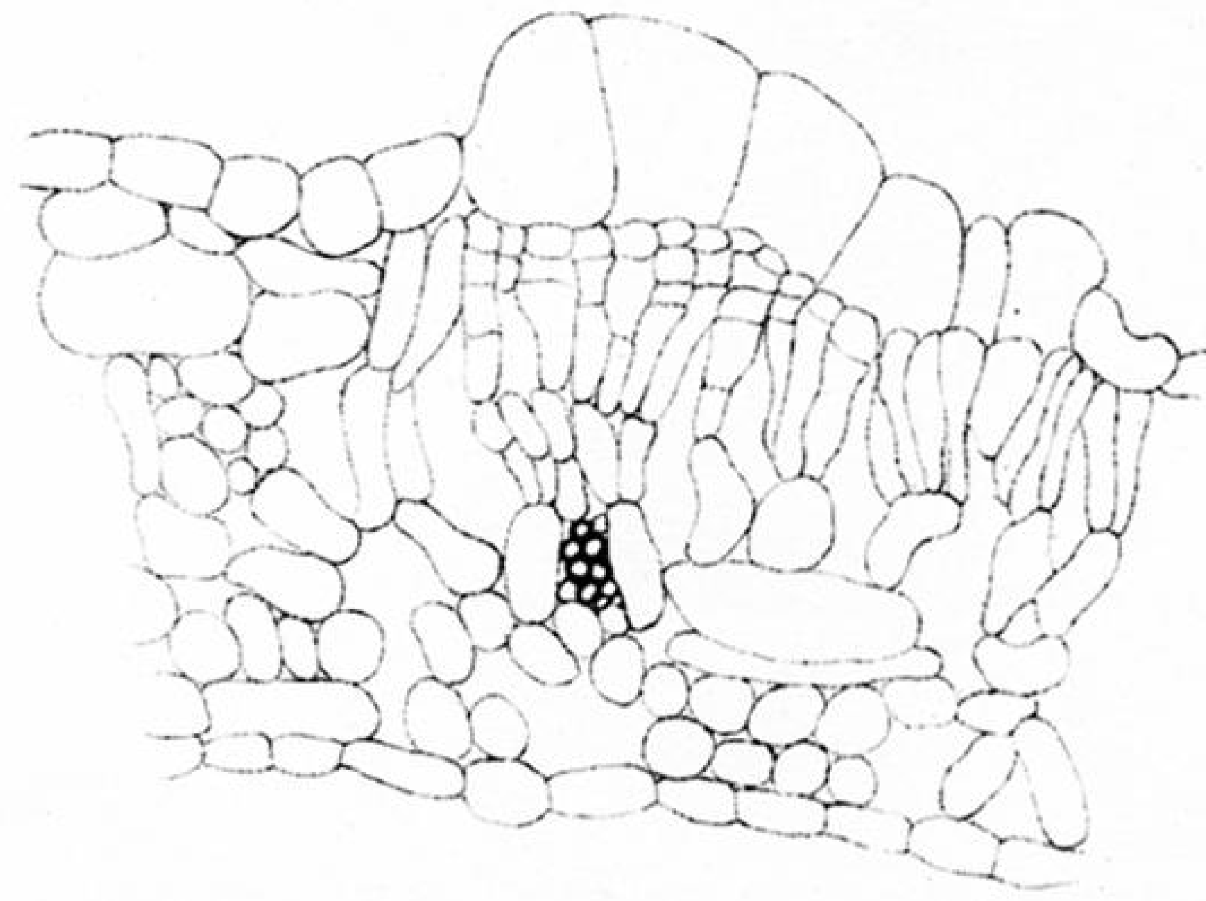

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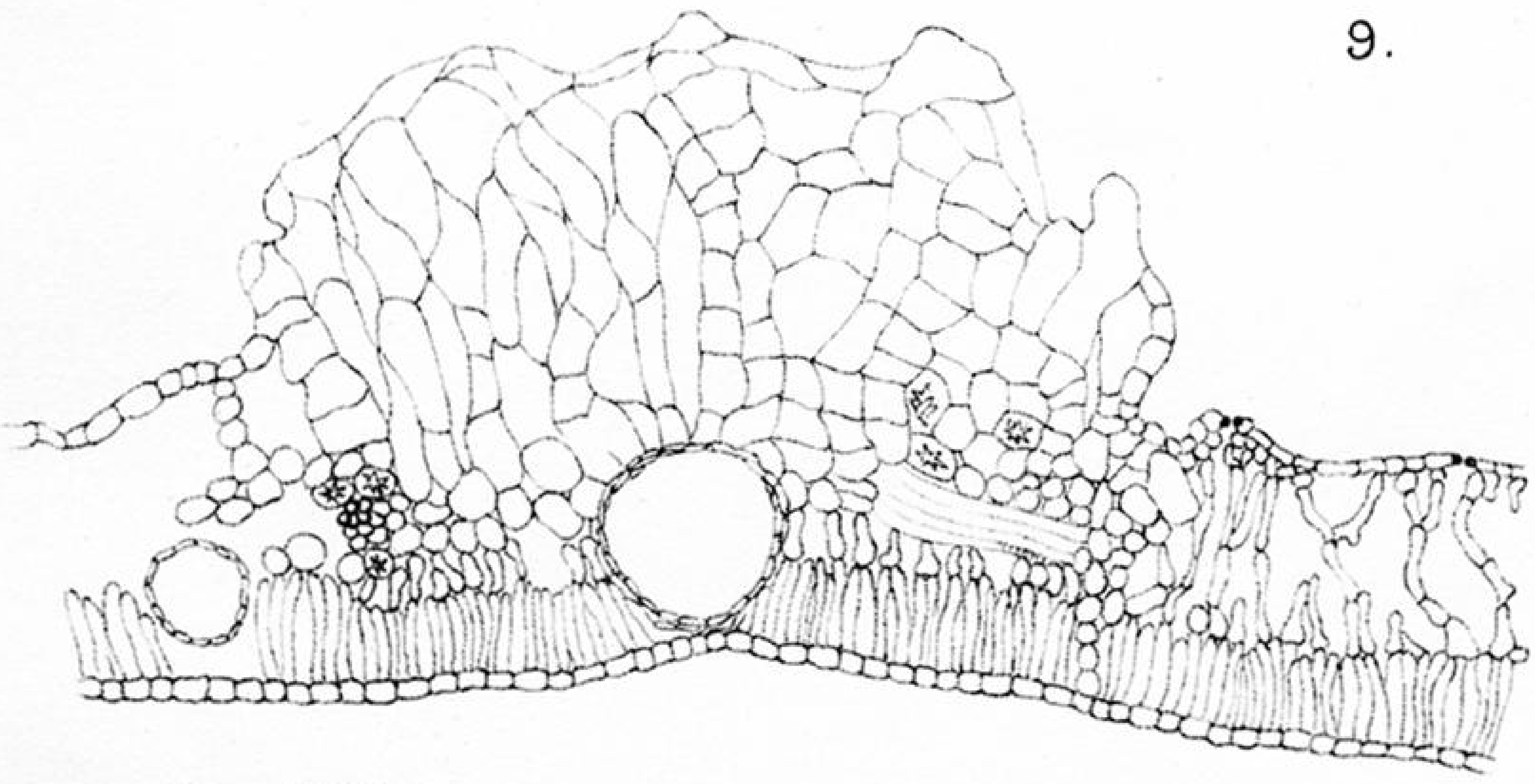

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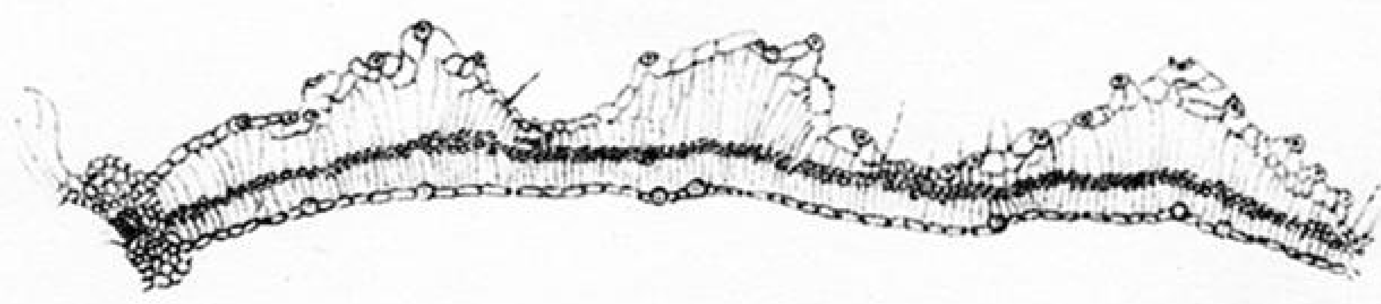

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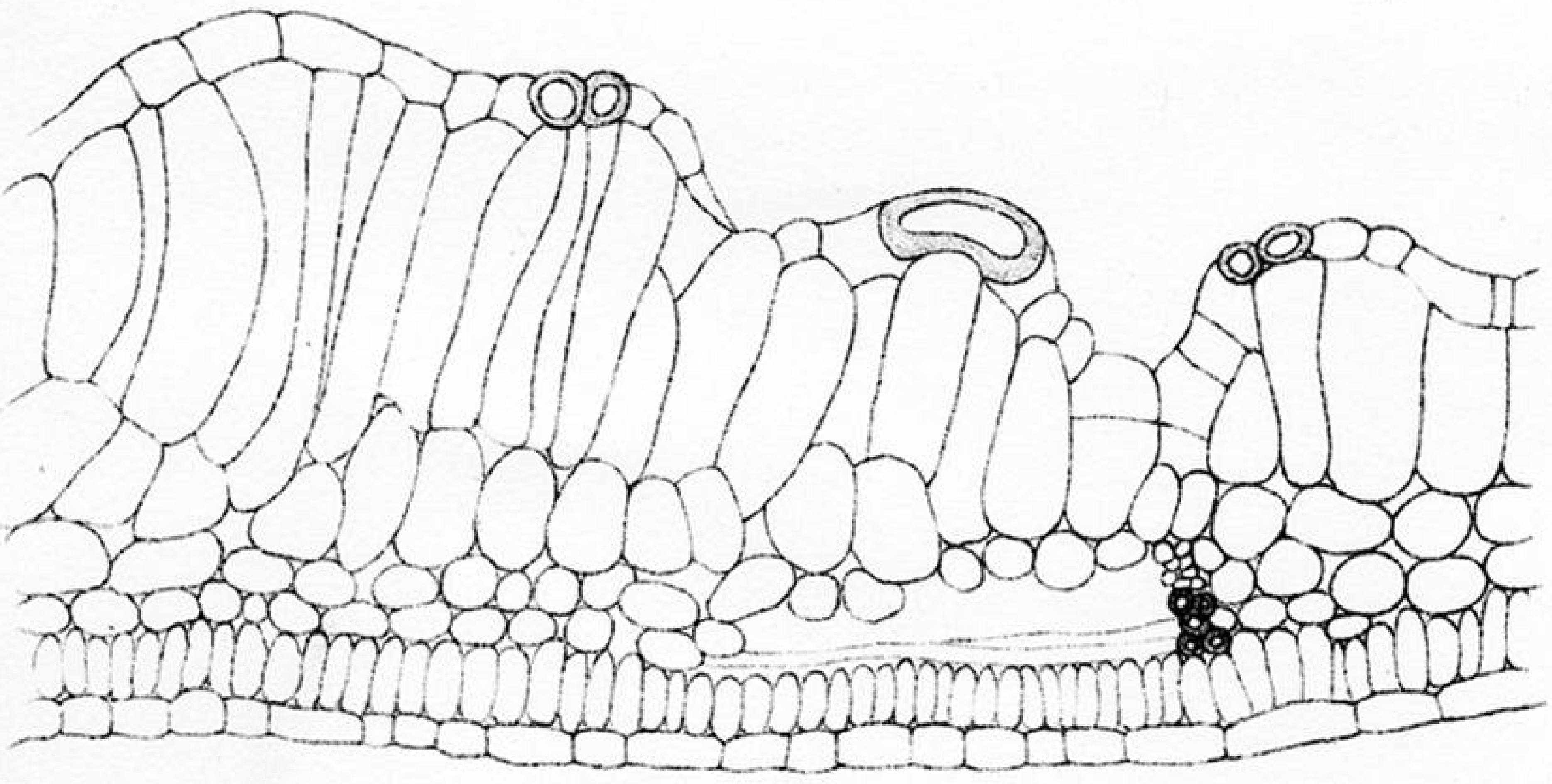

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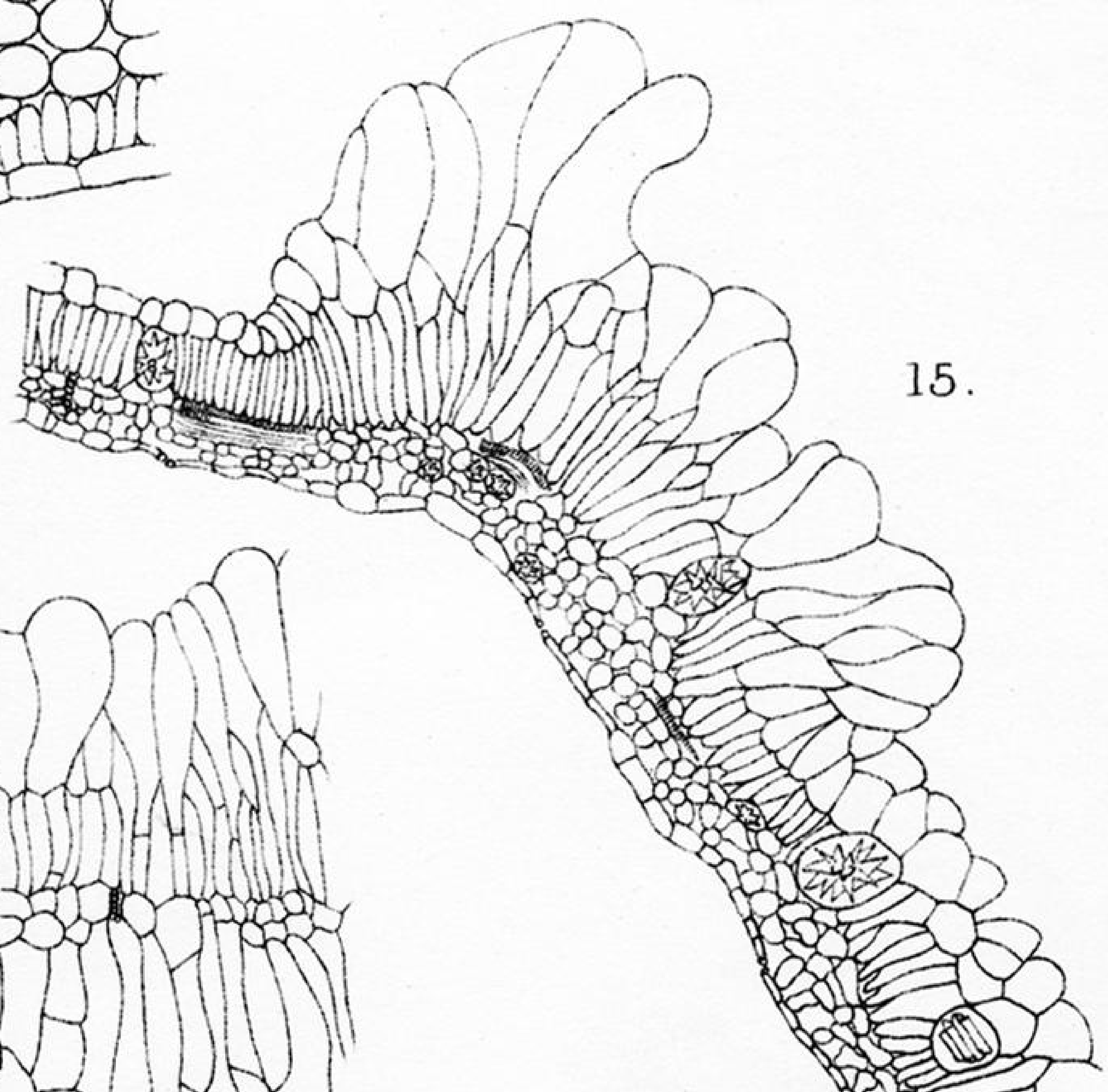

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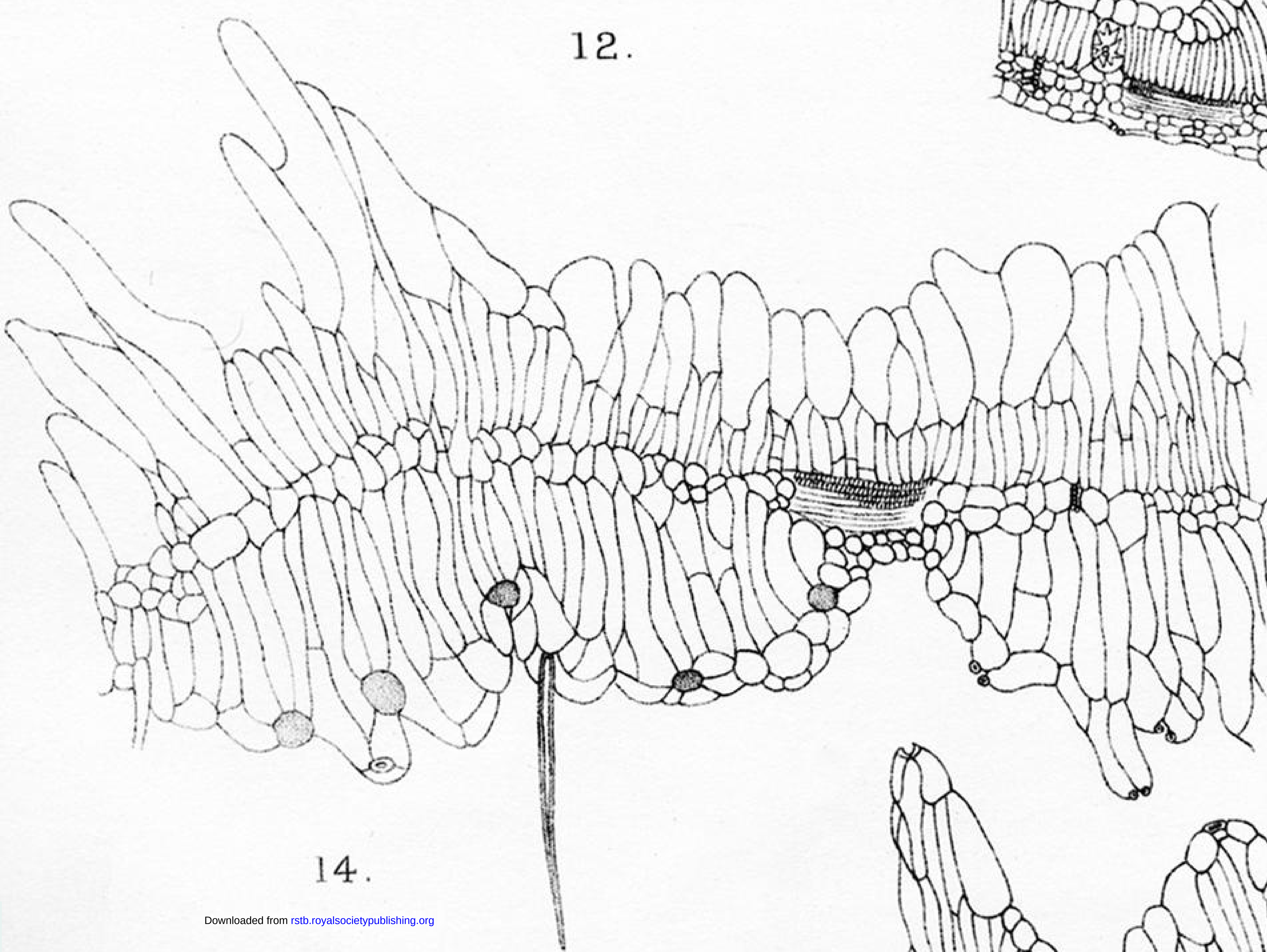

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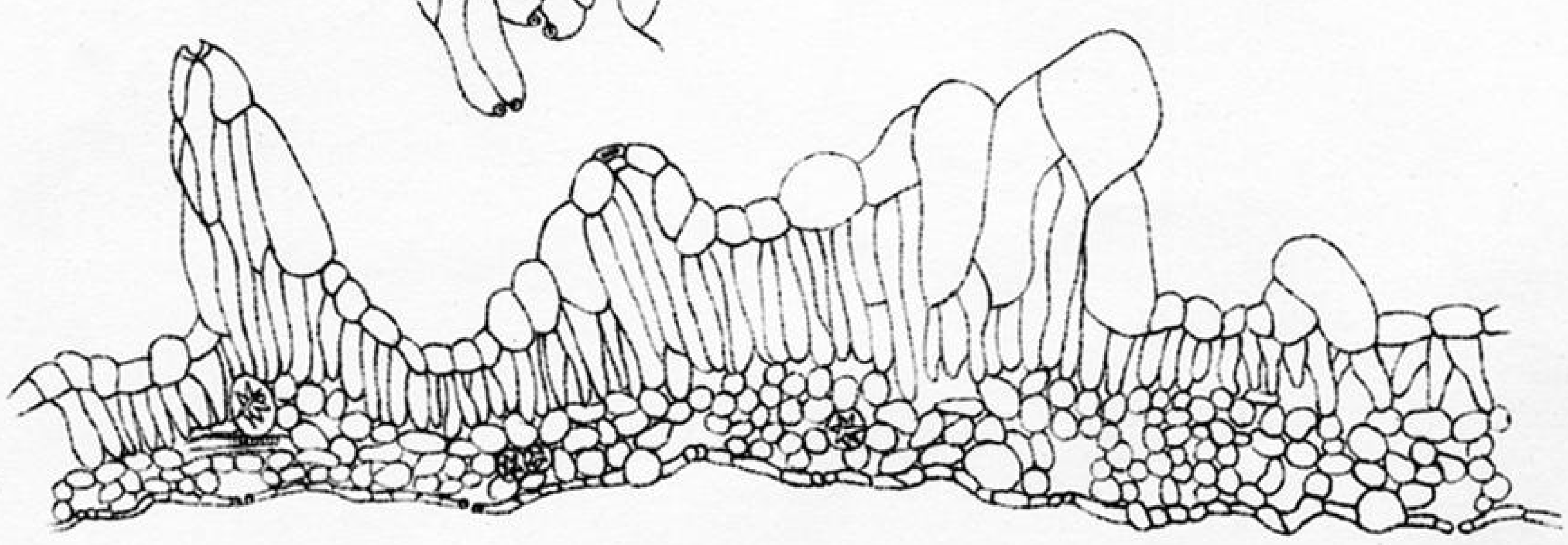

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Plate 15.

Fig. 9.—Section of intumescence on leaf of *Gmelina hystrix.* ($\frac{1}{2}$, 2.)

Fig. 10.—Section of intumescence on leaf of *Eucalyptus botryoides* (obj. D., oc. 2).

Fig. 11.—Section of intumescence on leaf of *Eucalyptus diversicolor.*

Fig. 12.—Section of leaf of *Vitis vinifera* with intumescences. (D., 2.)

Fig. 13.—Section of outgrowths on leaf of *Hibiscus vitifolius* grown under red glass. (D., 2)

Fig. 14.—Section of leaf of *Hibiscus vitifolius* with outgrowths, grown under yellow glass. ($\frac{1}{2}$, 2.)

Fig. 15.—Section of outgrowths on leaf of *Hibiscus vitifolius* grown under white-washed glass. ($\frac{1}{2}$, 2.)

Fig. 16.—Young rapidly-formed outgrowths on leaf of *Hibiscus vitifolius.* (2-in. obj., 2 oc.)

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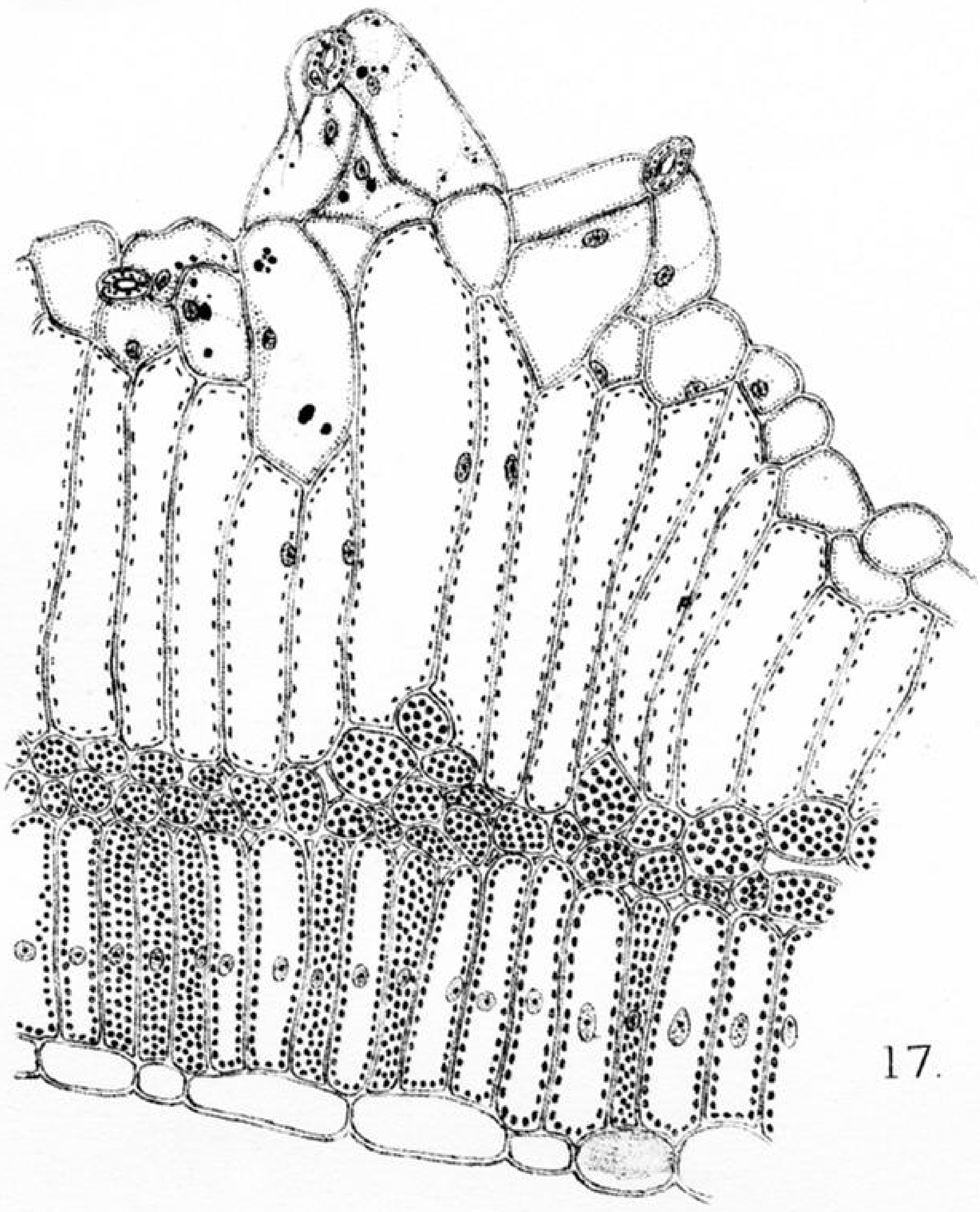

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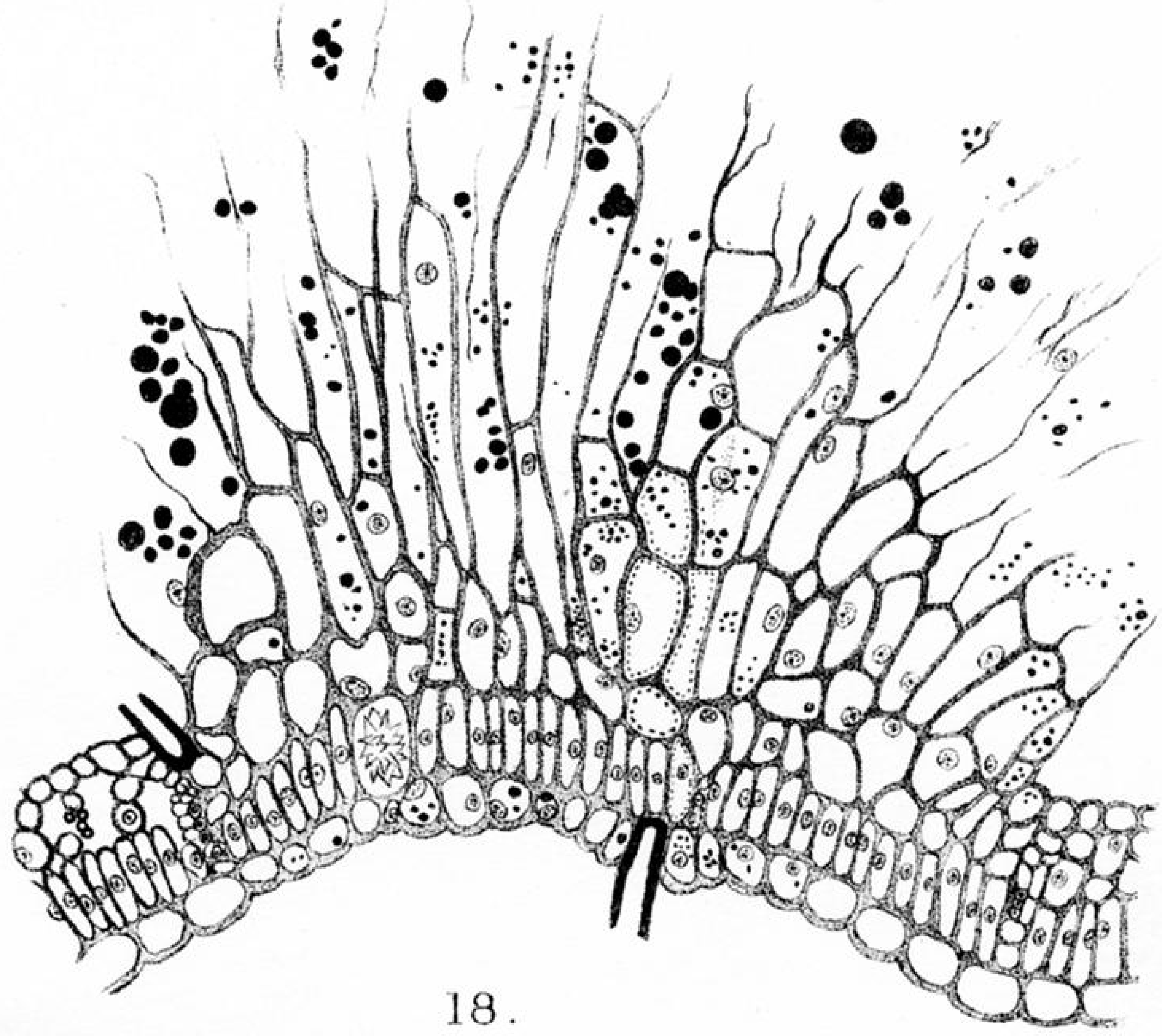

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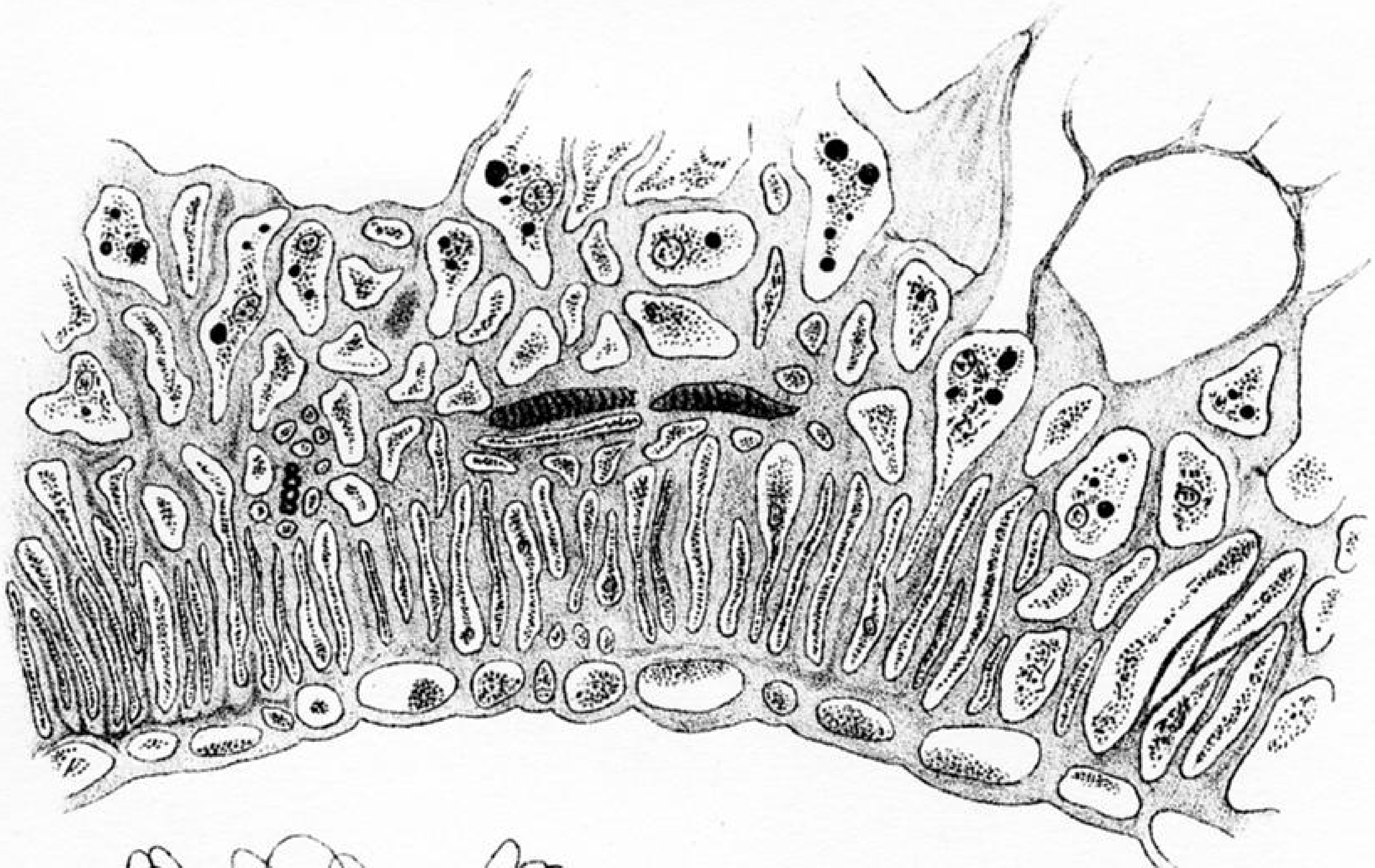

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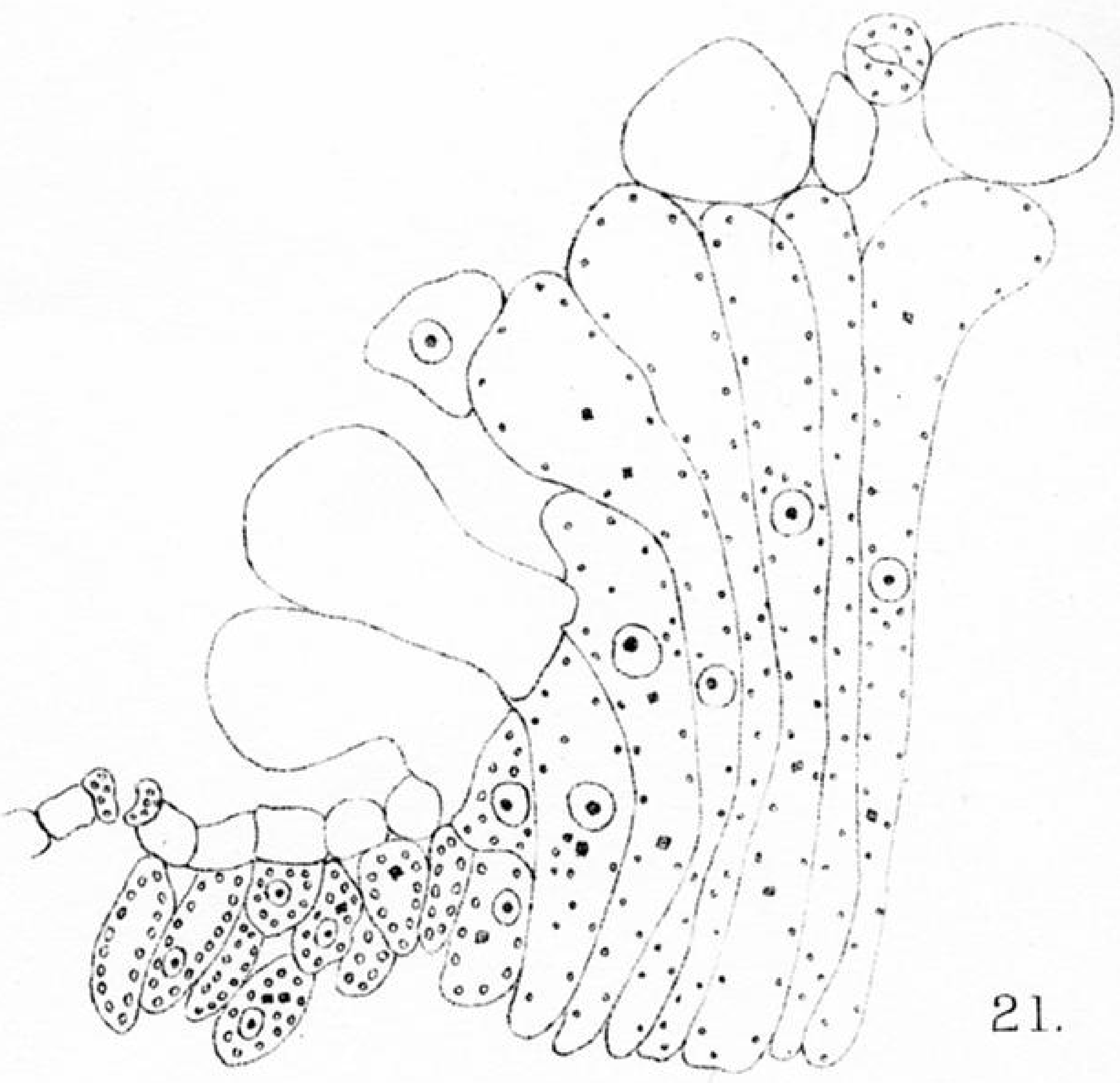

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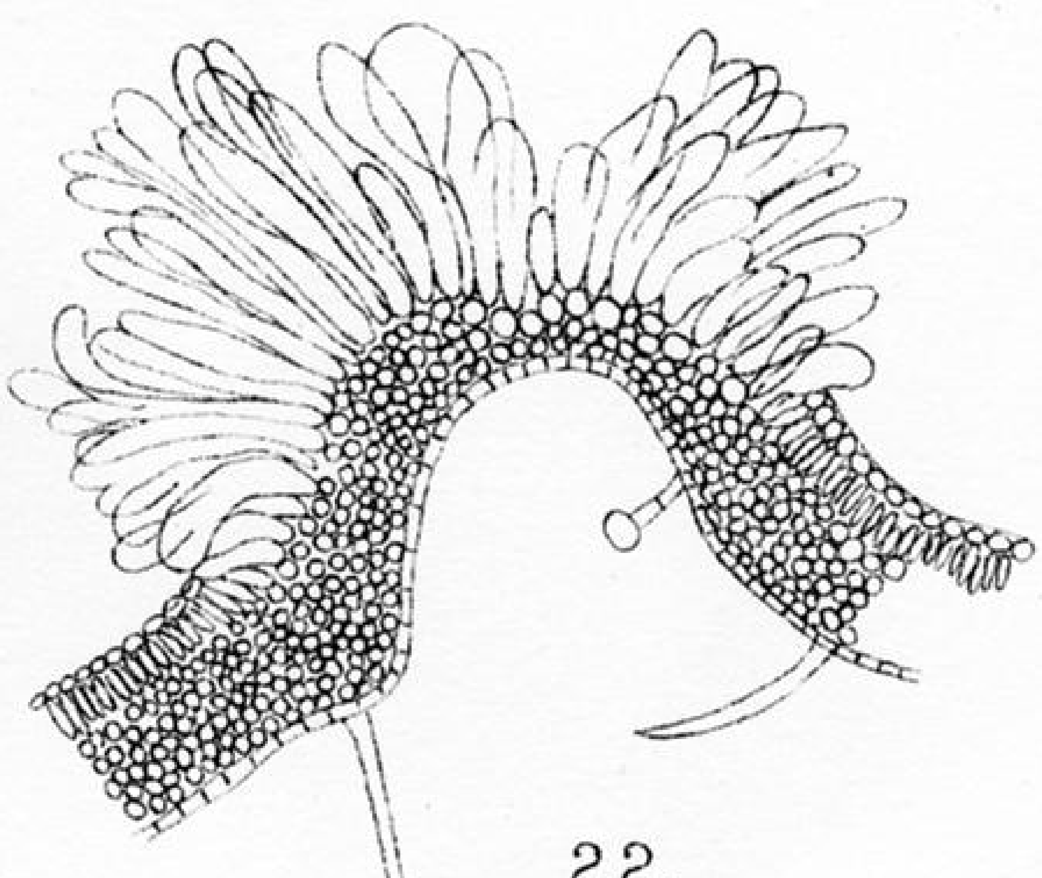

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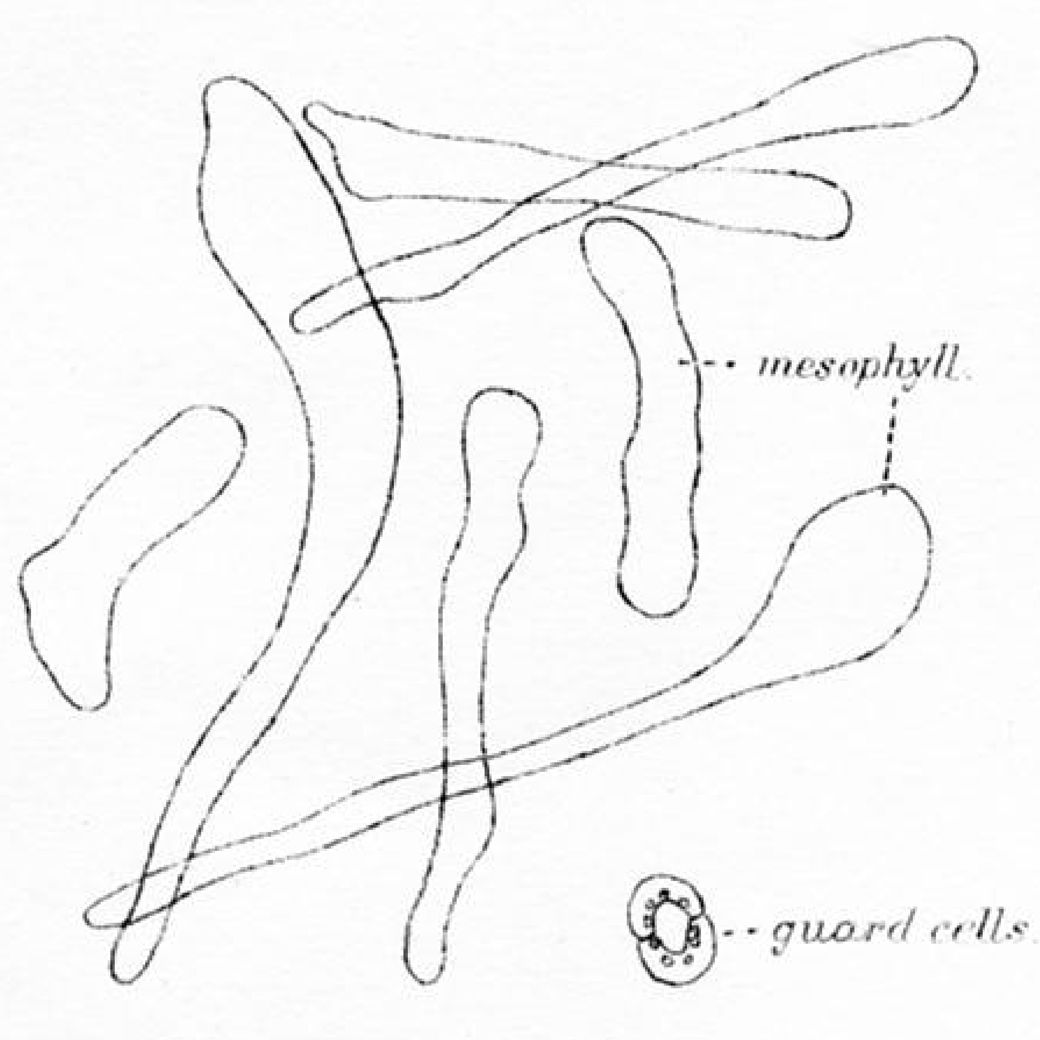

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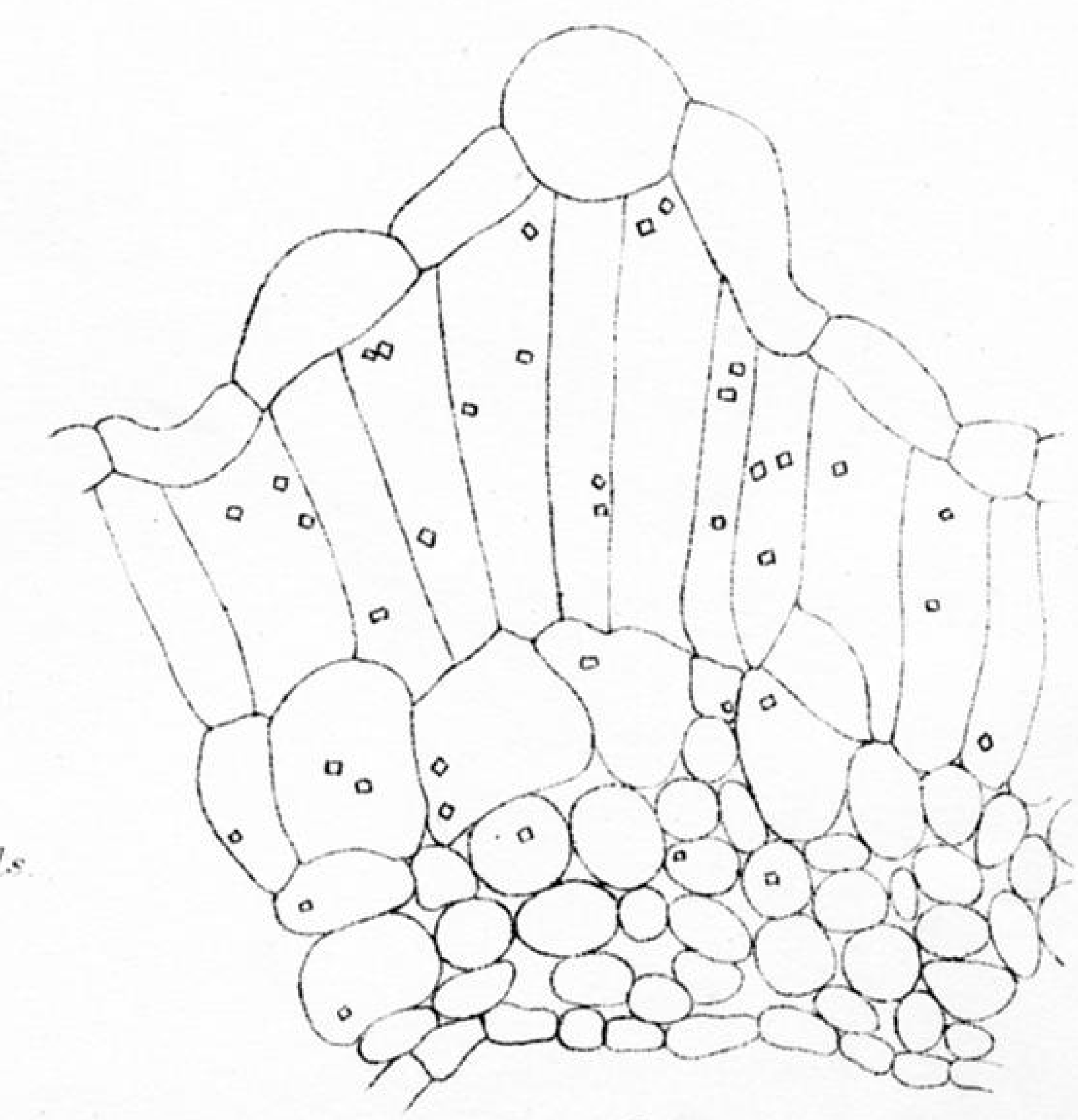

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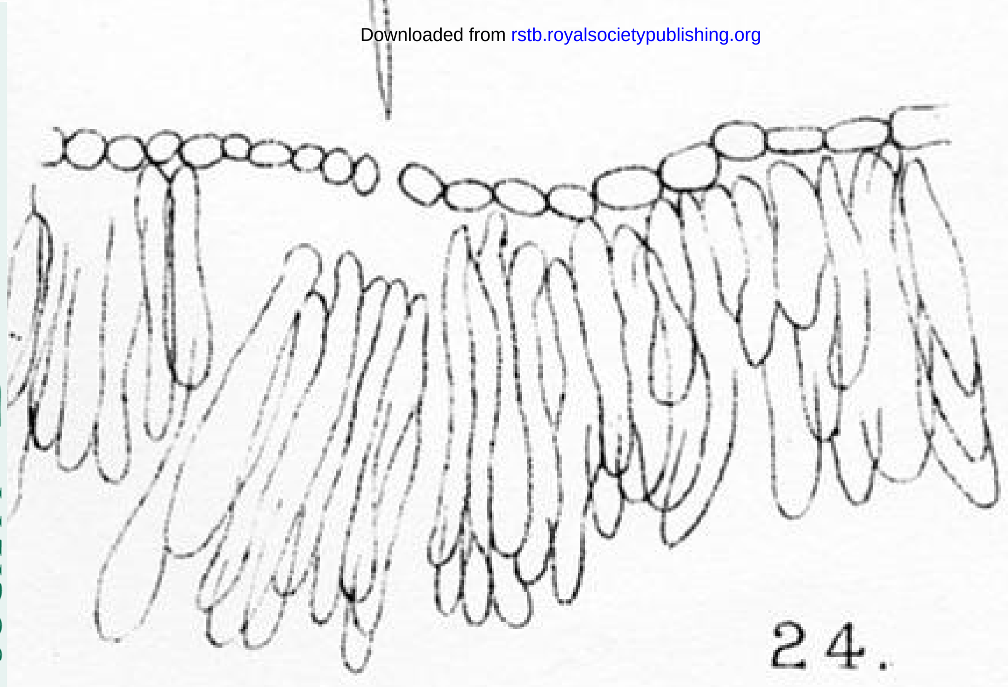

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Plate 16.

Fig. 17.—The same, more highly magnified. (D., 2.) (Reduced ½.)

Fig. 18.—Older stage of a rapidly-formed outgrowth on *Hibiscus vitifolius*. (D., 2.) (Reduced ½.)

Fig. 19.—Old outgrowth on leaf of *Hibiscus vitifolius*. (D., 2.)

Fig. 20.—Young intumescence on potato leaf. (D., 2.) (Reduced.)

Fig. 21.—Intumescence on leaf of potato. (Reduced ½.)

Fig. 22.—Older intumescence on potato leaf.

Fig. 23.—Constituent cells (fresh) of a potato intumescence which is disintegrating.

Fig. 24.—Old outgrowth on leaf of *Solanum aculeatissimum*.

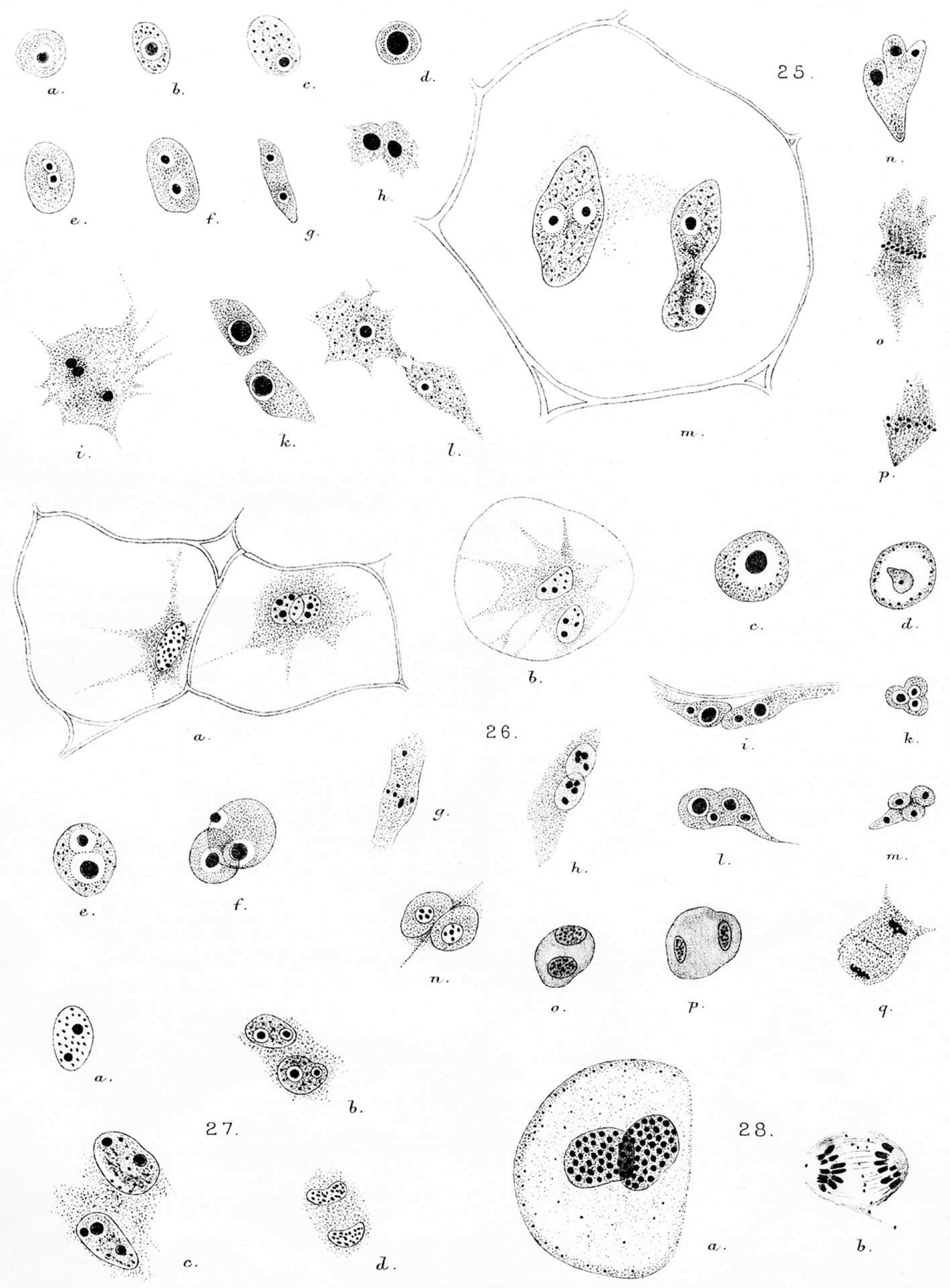

PLATE 17.

Fig. 25.—Nuclei in outgrowth on the stem of *Populus alba*.

a, *b*, *c*. Typical nuclei with varying degrees of reticulation of nucleoplasm.

d. Nucleus with very large nucleolus.

e. Nucleus with two nucleoli in contact.

f. Nucleus with two separated nucleoli.

g. Elongated nucleus with two nucleoli.

h. Nucleus with two nucleoli and no membrane.

i. A similar nucleus with three nucleoli.

k. Two daughter nuclei after division. A membrane is present except along the line of separation.

l. Two daughter nuclei without membranes.

m. A cell containing two nuclei each with two nuclei, one nucleus is dividing.

n. A nucleus dividing by budding.

o and *p*. Two nuclei, showing imperfect karyokinesis.

Fig. 26.—Nuclei from an outgrowth on the stem of *Populus nigra*.

a. Two adjoining cells, one with a single nucleus and the other with two nuclei. In all the nuclei the nucleolin is in many fragments.

b. Another cell with two nuclei.

c. A large nucleus with deeply stained granules round the clear zone.

d. A similar nucleus.

e. A nucleus with two nucleoli.

f. Two nuclei, apparently just after division, one with one nucleolus and the other with two. In the nucleoli are granules more deeply stained than the surrounding nucleolin.

g. A nucleus with granules of nucleolin.

h. A nucleus without membrane and with two nucleoli with fragments of nucleolin.

i. Two elongated dividing nuclei, each with one large and one small nucleolus.

k. A three-lobed nucleus with three nucleoli.

l. A nucleus like (*i*) in an earlier stage of division.

m. A nucleus made up of four separate parts, "segmented nucleus" of PRILLIEUX.

n. Two nuclei between which a new cell wall is forming.

o, *p*, *q*. Imperfect karyokinesis.

Fig. 27.—Nuclei from a stem intumescence of *Hibiscus vitifolius*.

a. Nucleus with two nucleoli and coarsely granular nucleoplasm.

b and *c*. Nuclei after division. Two in each cell.

d. Imperfect karyokinesis (*cp*. 26, *o* and *p*).

Fig. 28.—Nuclei from the callus of *Chrysanthemum Broussonetii*.

a. Dividing nucleus.

b. Nucleus dividing indirectly and showing definite chromosomes. The only well-marked case seen in any callus or intumescence.