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ERRATA

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P. 50, line 17 from top, for fig. 15 read fig. 14

P. 50, line 18 from top, for fig. 16 read fig. 15; also insert fig. 16 after leaf

P. 80, last line, for have connection read have no connection

P. 142, legend of fig. 4, for verticle read vertical

P. 272, line 16 from top, for fig. 15 read fig. 18

P. 277, line 2 from bottom, for 5 read 6, and insert between 4 and 6 the following: 5. The nucellus forms a beaklike prolongation, which disintegrates after the embryo is formed.

P. 279, line 14 from top, for **5** read **3**

VOLUME LXIII NUMBER 1

THE
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JANUARY 1917

OENOTHERA LAMARCKIANA MUT. VELUTINA

HUGO DEVRIES

(WITH PLATE I)

One of the rarest mutations of *Oenothera Lamarckiana* is that in which those qualities are changed which constitute the characters of the twin hybrids *laeta* and *velutina*.[1] This is the more striking because in hybrid combinations these twins appear often and, as it seems, easily. But in the same way many other mutations, which apparently might reasonably be expected, have not as yet been observed and therefore must be assumed to be at least very rare. Why some mutations are common and others rare is still an open question.

From the behavior of the twin hybrids in crosses we may deduce that the mutant *velutina* must be in the main recessive to the parent species, and that the mutant *laeta* should be dominant over the *velutina*. If there were but one character involved, this would mean that the mutant *laeta* must be externally like *O. Lamarckiana*, and the same conclusion would have to be admitted if there were more characters indissolubly bound together. This being granted, the *laeta* could, of course, never be expected to appear as a mutant.

For some years, however, my cultures tend to show that the mutations observed in the group of the Oenotheras are far more compound phenomena than I was formerly inclined to assume. This also seems to be the case with the splittings which so often

[1] DE VRIES, HUGO, On twin hybrids. BOT. GAZ. 44:401–407. 1907.

occur after hybridization, and especially with those which appear in the first hybrid generation. If we apply this view to the twin hybrids *laeta* and *velutina*, the possibility is at once revealed that the components of this group of characters might not always be so indissolubly connected, and that some deviating combination of these qualities might still produce a mutant *laeta*, different from the type of *Lamarckiana*. As a matter of fact, a pure and complete mutant *velutina* has appeared in my cultures, but a *laeta* has never been seen as a mutant. In crossing this *velutina* with the parent species, however, twin hybrids arose, one of which may be designated as *laeta*, as will be shown later.

The question whether the characters of mutants and of hybrids among the Oenotheras are single or built up of a less or greater number of theoretically independent units now seems to be one of principal interest to me. Until now, however, the analysis of the qualities of the twin hybrids *laeta* and *velutina* has been difficult and unreliable on account of the presence of the hereditary qualities of their other parent. We can make inferences from these by comparing the twins issued from the crosses of different species with *O. Lamarckiana*, but we can hardly expect to get a complete analysis in this way. The mutant *velutina* is free from these specific admixtures, and therefore may afford a better material for experiments in this direction. I intend to study it from this point of view by means of a number of crosses, most of which I made during the summer of 1915, and shall give here only a description of the mutant itself, and of those hybridizations which give proof of its right to the name of *velutina*.

In order to avoid the confusion which might easily arise from the similarity of the names *O. Lamarckiana* hyb. *velutina* and *O. Lamarckiana* mut. *velutina*, I will give a synonym to the latter and call it *O. Lamarckiana* mut. *blandina*, or briefly *O. blandina*. In descriptions the use of this latter term will be obviously the easier. *O. blandina* has throughout its life and in all its organs a paler tinge than *O. Lamarckiana*.

O. blandina is, in all respects and at every stage of its evolution, strikingly different from *O. Lamarckiana* and easily recognizable. Its marks become visible with its very first leaves, when still in the

seed pans, about 4–6 weeks after sowing. At the time when the seedlings must be planted in larger boxes, their marks are fully reliable, even in hybrid mixtures. Their leaves are narrow and a pale yellowish green, whereas those of *O. Lamarckiana* are broad and a deeper green. As the rosettes increase the number and the size of their leaves, the differences between the two types are seen to increase also, and after some weeks more, when the young plants are ripe for transplanting into the garden, *O. blandina* is clearly a type of its own and can easily be counted off in the mixtures. In May the rosettes of *O. Lamarckiana* gradually become very stout, but those of *O. blandina* remain slender. The leaves are so narrow as not to touch each other regularly nor to cover the ground between them. They resemble those of *O. rubrinervis*, but lack the brittleness, the typical bending of the petiole and blade, and the specific color of this form. Moreover, they show a high degree of fluctuating variability in their color. This is always gray, on account of the hairiness of the surface, but it varies between a normal green and a more or less pale and yellowish tinge. The paler they are, the poorer they are in chlorophyll, and therefore the palest individuals soon begin to show a slower growth. They stay behind the others the more, the paler they are. These differences increase if the culture is densely planted, and even at the time of flowering the paler individuals may be seen to be much weaker and shorter than the others. In ordinary cultures, however, when the plants are at a distance of 20 cm. or more from their next neighbors, and grown in a well manured soil, these differences gradually disappear and are no longer visible when the first flowers open. In order to fertilize pale individuals beside the green ones, I had to mark them in early youth. From the self-fertilized seeds of the green ones the seedlings are on the average less pale than those of the paler parents, but the difference, although obvious and unmistakable on the beds in springtime, soon disappears as the summer begins. From time to time there is even a partial variability, such as, for instance, a green branch on a stem with pale leaves. This shows that there is no racial difference between the green individuals and the pale ones, even as in the cases of *Oenothera* (*Lamarckiana*×*atrovirens*) *gracilis* and of *O.* (*Lamarckiana*×

Hookeri) *velutina*.[2] Since making these experiments (1912–1913) I have cultivated *O. blandina* so as to reduce the paleness of its leaves to the first youth of the rosettes, and to have no diminution of the individual strength of my seed-bearing specimens on account of it.

At the time of flowering the plants are much more slender than those of *O. Lamarckiana*, and are in their main features very much like the *velutina* hybrids of *O. biennis*×*Lamarckiana* and of *O. Lamarckiana*×*O. biennis Chicago*, and especially like the latter, with which in some instances they can easily be confounded. The leaves on the stem are narrow, reaching about two-thirds the breadth of those of *Lamarckiana* if compared by equal length. In the beginning they are folded along their midvein, but later they become flattened, and this curious character may then be seen only in the bracts of the inflorescence. The bubbles, which are so characteristic of the leaf blades of *O. Lamarckiana*, are absent in the mutant. I shall designate this lack of bubbles by the term "smooth."

The flowers of *O. blandina* are cup-shaped, whereas those of the parent species are more or less quadrangular. The size is the same, the color is as bright, and the stamens show no marked difference; the supply of pollen is very large in both cases. The stigma is widely spread out above the anthers, the distance being even somewhat larger in the mutant (1 cm. against 0.5 cm.). The flower buds are almost twice as thick in the mutant, more regularly and more deeply colored with red brown lines and spots, and much more hairy. This color and this hairiness extend over the tube of the flower and the ovary, and in a less degree over the top of the stem and the young bracts. The small free tips at the top of the flower bud are thick in *O. blandina* but thin in *O. Lamarckiana*. The differences of the fruits are small, except for the hairiness. The most striking character of *O. blandina*, however, is seen at the end of the flowering period, when the spikes are long and the lower fruits begin to ripen. At that period the spikes are very slender, with few fruits and long internodes, whereas on the spike of *O. Lamarckiana* the fruits are densely crowded. I counted the fruits

[2] Gruppenweise Artbildung. Berlin, 1913, p. 164, where *O. atrovirens* still bears the name of *O. cruciata* (*fig. 73*); and p. 116, *fig. 46*, for the twin hybrids of *O. Hookeri*.

on a length of half a meter in the middle part of the spike, at the end of September, and found 30 of them on *O. blandina*, but 75 on *O. Lamarckiana*, both on very vigorous annual specimens. From this the internodes of the spike are 1.7 against 0.7 cm., or more than twice as long as those of the parent species. The average numbers of flowers which open on a spike during an evening are inversely proportional to these figures. For many crosses I have castrated five successive flower buds of *O. Lamarckiana* on one day and pollinated them the next day, whereas the crossing of 5 flowers on a spike of *O. blandina* usually lasts 4 or more days, which makes quite a difference in the technical work.

Of course, there are a number of distinguishing points of less value, but their description would remain vague unless strict averages could be given; and since all characters are more or less dependent upon the conditions of soil and culture, it is doubtful whether even averages would be reliable. I shall return to this point in the description of the hybrids.

In comparing this description with that given in my book on *Gruppenweise Artbildung* for *O.* (*Lamarckiana*×*O. biennis Chicago*) *velutina*, it will easily be seen that the two belong to the same type. In the garden, when groups of 10–30 plants are compared, this similarity is of course far more striking. It is at once clear that *O. blandina* must be a true and pure *velutina*.

If we now try to resume this description in such terms as to distinguish a number of probable units, the combination of which might constitute the type of *velutina*, I might propose the following: (1) slender stature; (2) long internodes of the flower spike; (3) leaves narrow, folded longitudinally and smooth, that is, without bubbles; (4) flowers cup-shaped; (5) hairiness of all organs; (6) abundance of red color in the younger parts. It is obvious, however, that some of these points may go together and depend upon one unit, but on the other hand it must be conceded that this list may be far from complete. Most of these assumed units are recessive to the corresponding qualities of *O. Lamarckiana*, but the smoothness is dominant over the bubbles, which are evidently due to a lack of growth parallel to the surface of the blade of the leaf. This enables us to separate smoothness from the larger part

of the other characters by means of crosses, as we shall see when dealing with the hybrids.

I shall now describe the origin of *O. blandina* and the pedigree of the race derived from it. As a matter of fact, this mutant did not arise directly from *O. Lamarckiana*, but through another mutant race as an intermediate. This was the fertile race of *O. lata* issued from an original fecundation of my normal *O. lata* by means of the pollen of *O. semilata*. This race is described in my book *Gruppenweise Artbildung* on pp. 256, 257. In the fourth generation of this race, issued from the seeds of 1904 sown in 1905, 1907, and 1908, three specimens of *O. blandina* were observed, two in 1907 and one in 1908. Besides these, the cultures consisted of specimens of *O. lata* and *O. Lamarckiana* in the usual proportions, and of other mutants such as *O. nanella*, *O. oblonga*, and *O. scintillans*. The size of the cultures, however, was too small to calculate percentage figures.

The full name of the new type, therefore, would be *O. Lamarckiana* (mut. 1888 *lata*×mut. 1895 *semilata*) mut. 1907–8 *blandina*, leaving out the fecundation of the *lata* specimens of the pedigree by the pollen of *O. Lamarckiana*.

The second generation was derived separately from one mutant of 1907 and from that of 1908, both after guarded and artificial pollination. The seeds of the first mutant were sown in 1913 and gave a culture of 25 flowering individuals and 45 others which were pulled up shortly before flowering. Both groups were uniform, apart from the variation in the green color already mentioned. They were fully like the simultaneous culture of the third generation of the other strain, and for this reason this first line has not been continued.

The seeds of the mutant of 1908 were sown in 1912 and gave a culture of 67 flowering plants, all of which repeated the type of their parent. Besides these, I sowed part of the seed somewhat later in the season (June), and obtained a large group of rosettes of radical leaves, of which, however, only one survived our long, wet winter. This specimen flowered in 1913 on the main spike and on a number of branches, was very vigorous, but not strikingly stouter than the annuals of that summer.

In 1912 I saved the seeds of 4 self-fertilized plants separately. Two of them had been green from their first youth and the two others had been of a pale color in the beginning. The result was, in 1913, a small but distinct advantage of the two first sowings over the two latter sowings. I have chosen the first ones for the crosses to be described later on, and repeated this third generation in 1914 in order to give the plants more space and a better manured soil, and to compare such vigorous individuals with others growing in a dry and poor soil. The results of this comparison have been described elsewhere; they showed that the seeds produced by the two groups were different. Almost all the seeds of the strong plants contained a healthy germ, but among the seeds of the weaker individuals there were about 25 per cent of empty ones. The germination showed even a larger difference, giving about 80–90 per cent of seedlings for the normal seeds and only about 50 per cent for those of the weaker plants.[3] I shall return to this phenomenon in the second part of this article.

The fifth generation was derived, in 1915, from seeds of 4 self-fertilized individuals of 1914, chosen as the best ones among the stronger of the two groups. Sixty plants from one parent were planted in my experimental garden on good soil and with plenty of space, in order to be used for crosses. The remainder embraced about 3000 plants (between 600 and 800 from each parent) and were set out in another garden in order that the degree of mutability of this race might be studied. It was found to be rather small, although almost all of these plants have flowered and have been carefully tried at different stages of their evolution, from their germination to the time when their last flowers faded away in August and their first fruits ripened.

The result of these repeated inspections has been that 4 mutants, belonging to one type, were discovered, but that no other deviation could be observed. This gives a percentage figure of 0.1. If we compare this with the table given on p. 337 of my *Gruppenweise Artbildung*, we see that *O. blandina* falls into the group of those mutants (*O. nanella* and *O. rubrinervis*), the mutability of which has

[3] Über künstliche Beschleunigung der Wasseraufnahme in Samen durch Druck. Biol. Centralbl. **35**:175. 1915. In this article *O. blandina* has been provisionally designated as *O. Lamarckiana* mut. nov. B.

become much smaller than that of the parent species, which latter is given there as 2.2 per cent. The transition of one or more unit characters into the inactive condition was considered there as the probable cause of this change, and the same conception may obviously be applied to our present case.

Although the mutability of *O. blandina* is thus seen to be very small, it does not follow that it is wholly absent for other mutations than the one mentioned. Casual mutations parallel to those of *O. Lamarckiana* may be expected to appear from time to time, either in the pure strains or after hybridizations with other, still less mutable, species. This has occurred once in my garden. Among the offspring of a cross between *O. blandina* and *O. Cockerelli*, a species from Colorado, an individual arose in 1915 which showed the marks of *O. lata* combined with those of *O. Cockerelli*, and agreed with the description given in my book (p. 254). It proves that the mutability of *O. blandina* into *lata* is not wholly absent.

The mutation from *O. blandina*, 4 specimens of which occurred in my culture of 1915, was a strikingly new type, quite different from all the mutations produced by *O. Lamarckiana* and its derivatives until now. It was distinguished at once by its linear leaves, which could be seen in the boxes before the young plants were planted out on the beds. The 4 mutants were brought into the glass-covered part of my garden, where 2 of them have flowered. The 2 others remained small, produced stems, but died in the fall before making any flower buds. Of the flowering specimens one was also small and therefore was not used as a seed-bearer, but the other reached about 1 m. in height, was very richly branched, and bore, from July to October, many hundreds of flowers and fruits. All these flowers had the same type, consisting of narrow petals instead of the large cordate ones of the parental form. The petals did not belong to the type called *cruciata*, inasmuch as they had not the least sign of the sepalody characteristic of *O. cruciata* and its allies. Their color was uniformly yellow, not differing from that of *O. Lamarckiana*. The breadth varied from 0.5 to 1.5 cm. for a length of 3 cm. The form was ovate, with some small indentations along the margin, and the tip was narrowed and more or less spirally twisted. This latter mark, which was best visible during

the time of the most abundant flowering, has induced me to choose for this mutation the name of *O. spiralis*.

As to the other marks, they were probably all evolved under the influence of the very narrow leaves, which could not produce food enough for very stout individuals or organs. The leaves measured 5–6 mm. in breadth by a length of 8–10 cm.; they were smooth (without bubbles) as in the parent, not folded longitudinally, only a little hairy, and dark green. The internodes were long, reaching 2 cm. or more, and the foliage was therefore thin and the whole habit slender. The flower buds were less hairy than in *O. blandina*, but more so than in *O. Lamarckiana*, and broader than would be expected from the narrow petals. The stigma was above the anthers, which contained a good supply of pollen, making artificial self-pollination and crossing quite easy. The fruits were rather thin, and somewhat smaller than those of *O. blandina*, a little more conical, and less hairy.

It should be pointed out that the origination in 4 specimens, one from one parent and the 3 others together from another parent, is analogous to the production of *O. blandina* itself, which arose in 3 specimens from one lot of seeds. It points to an internal condition of heritable mutability and suggests the expectation that under a better climate and with more suitable conditions of cultivation the number of simultaneous mutations in the same direction might increase sensibly.

CROSSES OF O. BLANDINA WITH OTHER SPECIES.—In order to give proof that *O. blandina* is really the mutant *velutina*, I made some crosses with such species as are known to split *O. Lamarckiana* and some of its other derivatives into the twin hybrids *laeta* and *velutina*. It is obvious that with the loss of the active qualities of *laeta* this capacity of undergoing a splitting must disappear. The mutant *velutina* corresponds to the latent or inactive condition of these qualities, and if combined with splitting species it must therefore give rise only to one of the twins, the hybrid *velutina*. In other words, the crosses with these species must be expected to produce no *laeta* but only *velutina* hybrids, and these must be exactly the same as the *velutina* issued from the corresponding crosses of *O. Lamarckiana*.

Under normal conditions the splitting of this latter species occurs in nearly equal groups of both the twins, but as a matter of fact the external circumstances are, with us, often such as to diminish the percentage of *laeta* quite sensibly. These deviations, however, have been amply studied in my work on *Gruppenweise Artbildung;* they show what size the cultures must be in order to prove the absence of *laeta*. I cultivated 60–80 offspring for each culture, and repeated the same cultures during 2 years, making on the average 140 specimens for each cross. The first year I compared them with the hybrids *laeta* and *velutina* derived from the corresponding crosses of my new dimorphic mutant *O. cana*,[4] which happened to be at hand in a complete set; but the second year I have sown the twin hybrids issued from crosses of *O. Lamarckiana* itself with the same species as were used for the crosses of *O. blandina*. The material is externally the same in both cases, and quite as good for the comparison, but in the latter instance the proof is a more direct one. I grew the hybrids of *O. blandina* and the control cultures side by side, and compared them from the beginning of germination in February until the ripening of the first fruits in September. The differences between the two twins of a cross are large and striking,[5] from their very first leaves, and it was therefore impossible that a *laeta* among the hybrids of *O. blandina* could have escaped observation. As a matter of fact, no single *laeta* appeared, although altogether over 500 hybrids were cultivated.

For these crosses I chose 2 heterogamic and 2 isogamic species, and made the combinations in both groups in the opposite directions, so as to use *O. blandina* twice as a pistil and twice as a pollen parent. I made these pollinations in 1913, in the third generation of my race, choosing the most vigorous individuals which had had a normal green color from their very youth. The crosses with the heterogamic species were *O. syrticola* Bartlett[6]×*O. blandina* and *O. blandina*×*O. biennis Chicago*. Those with the isogamic forms

[4] De Vries, Hugo, New dimorphic mutants of the Oenotheras. Bot. Gaz. **62**:249–280. *figs. 5.* 1916.

[5] For descriptions and photographs as well as for colored plates of the twins, see *Gruppenweise Artbildung*.

[6] This is the *O. muricata* L. of my *Gruppenweise Artbildung*.

were *O. Hookeri*×*O. blandina* and *O. blandina*×*O. Cockerelli*.[7] These combinations would have given the twins *laeta* and *velutina* if *O. Lamarckiana* had been used instead of *O. blandina*. Now, however, they gave only the hybrid *velutina*, showing that the splitting capacity of the parent species is absent in the mutant.

From these facts it follows that in *O. blandina* the *laeta* qualities of *O. Lamarckiana* are not only externally, but also internally, in the *velutina* condition. The former, therefore, is to be considered as a pure mutant *velutina*.[8]

I shall now describe the single crosses as briefly as possible.

O. syrticola×*O. blandina* was made on 2 biennial plants of the first named species in July 1913, the pollen of a green *blandina* being used in the one case, and that of a pale green one in the other case. The seeds of the first one were sown in 1914, those of the other in 1915. Both of them gave cultures of 70 healthy offspring, making a total of 140, of which 25 and 10 specimens were allowed to flower and to ripen their fruits, after the others had been pulled up, in June, before flowering. They were compared in every month of 1914 with the twins of *O. syrticola*×*O. cana*, and in 1915 with those from a cross of *O. syrticola*×*O. Lamarckiana*, made in 1913 on a specimen of the same group of biennial plants as used for the cross with *O. blandina*. All of the 140 hybrids were evidently *velutina* and exactly like those of the control cultures.

O. blandina×*O. biennis Chicago*.—This cross was made on two specimens of 1913, the pollen of the same parent plant being used in both of them. They gave uniform cultures of 60 and 80 plants, of which 25 and 10 were allowed to make long spikes of flowers and fruits. The others were annual plants also, but were thrown away in June, as soon as they reached a height of 30–40 cm., and showed their marks so as not to leave the least doubt concerning their *velutina* qualities. In 1914 I compared them with the hybrids of *O. cana*×*O. biennis Chicago*, and in 1915 with those of *O. Lamarckiana*×*O. biennis Chicago*, which I cultivated in the first generation

[7] For description and figures of these species see *Gruppenweise Artbildung*.

[8] Of course, other combinations, or combinations of the loss of the splitting capacity with other external marks must be admitted to be possible. As a matter of fact, such combinations occur from time to time, as, for example, in *O. oblonga;* see *Gruppenweise Artbildung*, p. 266.

as well as in the second, from another cross. The flowering individuals of all these crosses reached, in September, a height of more than 2 m., and all the *blandina* hybrids were, during all the stages of their evolution, like the *velutina* of the controlling cross.

O. blandina×*O. Cockerelli*.—Seeds of only one cross of 1913 were tried, both parents being annuals. One part was sown in 1914, another in 1915; size of the cultures, 60+80=140 specimens, of which 25 and 21 flowered. One of the latter was the *lata* mutant previously mentioned. In June 1914, there was not the least doubt concerning the identity of all the 60 specimens with *O.* (*cana*× *Cockerelli*) *velutina*, but in 1915 those young plants which had not been planted out in order to flower were a little too crowded. I therefore pulled out those which were indubitably *velutina*, and planted all the dubious ones on a separate bed, giving them just as much space as in ordinary cultures. About one-half of these (11 plants) flowered in September, but all of them displayed, at that time, the characters of *O.* (*Lamarckiana*×*Cockerelli*) *velutina*, so as to leave no room for doubt. No *laeta* has appeared among the offspring of this cross.

O. Hookeri×*O. blandina*.—Only one *Hookeri* was crossed in 1913 with one specimen of *blandina* of my race. The seeds gave 60 offspring in 1914 and 85 in 1915, of which 25 and 10 flowered. In the crosses of *O. Lamarckiana* and its derivatives with this Californian species, the *velutina* have almost the features of *O. Hookeri* itself, showing only a small influence of the other parent. I compared my hybrids in both years with first generation hybrids, using for the comparison both the reciprocal crosses of *O. cana* of 1913 in one year and the hybrids of a cross *O. Hookeri*×*Lamarckiana* of 1909 in the next year. Although the specimens, which were not allowed to flower, made stems of only a few centimeters, or stayed in the conditions of rosettes of radical leaves, the type of *O. Hookeri* was strongly pronounced in them. All of them had the long narrow leaves of *velutina*, and no *laeta* occurred in the whole culture of 145 plants.

Resuming these details, we see that 565 hybrids of *O. blandina* with splitting species have been studied, and that all of them bore the unquestionable features of the *velutina* of the corresponding crosses of *O. Lamarckiana* and of *O. cana*.

CROSSES OF O. BLANDINA WITH O. LAMARCKIANA.—If the *laeta* qualities have become latent and inactive in *O. blandina*, we should expect that this mutant would have acquired the property of splitting itself these qualities in *O. Lamarckiana*. The confirmation of this expectation must obviously strengthen the conclusion from which it started. And since *O. nanella* and other mutants may give rise to the same hybrid twins as the parent species, we may expect *O. blandina* to split them also. I made both the reciprocal crosses with the parent species and one with the dwarf, and all 3 cases have corroborated my conception. I made the crosses in 1913 in the third generation of my race, and cultivated the first generation in 1914, repeating it in 1915. The splitting occurred in all 3 cases as expected, giving nearly equal groups of the two types. One of these types exactly corresponded to *O. blandina* itself; in comparing it from its first youth up to the time of flowering and fruiting, I could not discover any difference. This one should be considered as the *velutina*, therefore, and will be called *O.* (*Lamarckiana*×*blandina*) *velutina* a.s.o. The other type was evidently a *laeta*. During some stages of its evolution it was almost wholly like *O. Lamarckiana*, but later it changed its appearance and displayed some of the characters which usually distinguish the different forms of the hybrid *laeta* from their parents. For this reason I shall call it *O.* (*Lamarckiana*×*blandina*) *laeta*, or briefly *O. blandina laeta*, implying by this name only the presence of one or more characteristics of the *laeta* type, but not necessarily all of them.

According to the species which determine the splitting in *O. Lamarckiana*, the types of hybrid *laeta* may be divided into two groups. One of them is small-flowered and ordinarily tall, corresponding to the *rigida* type described and figured in my book (pp. 73, 80, 81). To this type appertain the *laeta* produced by *O. biennis*, *O. muricata*, *O. Cockerelli*, and *O. biennis Chicago*. The other type has large flowers; it embraces only the *O.* (*Hookeri*× *Lamarckiana*) *laeta* and its reciprocal. The flowers have the same size in both parents, and therefore this size is not changed in the hybrids. In the cross of *O. Lamarckiana* and *O. blandina* the same rule prevails, the flowers of the hybrid being not rarely even somewhat stouter than those of the parents.

O. blandina laeta shows the greatest affinity to *O. Hookeri laeta*, not only in the flowers, but also in other respects, as, for example, in the stature at the time of flowering, which in both hybrids comes much nearer to that of *O. Lamarckiana* than any of the small-flowered hybrid *laeta*. Far more interesting, however, is the similarity in its behavior in the second generation, after *self-fertilization*. The *Hookeri laeta* are the only *laeta* as yet known to split; all *laeta* of other extraction and all the *velutina* as yet studied give a uniform progeny. But the *Hookeri laeta* split in every generation into *laeta* and *velutina* which are exactly like the original twins.[9] The same phenomenon is seen in *O. blandina laeta*, although as yet I have only cultivated one second generation from one cross. This was *O. blandina*×*O. Lamarckiana*, made in 1913. The first generation in 1914 gave 59 per cent *velutina* and 41 per cent *laeta*, and the progeny of the latter split into the same two types in 1915, giving 67 per cent *velutina* and 33 per cent *laeta*. Why the large-flowered *laeta* should split, but the small-flowered type remain constant, is a question which will have to be studied later.

O. blandina laeta has been, throughout its whole evolution, exactly the same type in the 3 crosses already mentioned, and whose progeny I cultivated in both years side by side. In the seed pans and the transplanting boxes the young plants are almost exactly like *O. Lamarckiana*, resembling this form far more than any of the hybrid *laeta* do. This condition prevails until the beginning of flowering, during which period the leaves of the stem are somewhat broader and less covered with bubbles than in the parent species. This difference is then seen to increase gradually and becomes evident in the lower bracts of the inflorescence, which are broad, especially at their base, smooth, and wholly or almost without bubbles. As the spike develops, the difference from the parental type becomes greater. The fruits are less crowded and somewhat stouter, and the plants gradually reach a greater height than specimens of *O. Lamarckiana* planted at the same time and under the same conditions. Although the differences are still small, apart from the smoothness of the leaves, the plants of *O. blandina laeta* cannot be mistaken for *Lamarckiana* during all the time of flowering, which may last more than 2 months.

[9] See the pedigrees in *Gruppenweise Artbildung*, p. 131.

It seems probable that the increased breadth and the diminished bubbles of the higher leaves of the stem and of the bracts of the inflorescence are expressions of a single change, which must consist in a thorough stretching of the blade parallel to its surface. If this be so, we may conclude that the bubbles, which are so characteristic of *O. Lamarckiana*, are due to some deficiency in this stretching and thereby constitute a recessive character. If this conclusion be granted, the smoothness of the leaves of *O. blandina* must be dominant in its crosses with *O. Lamarckiana*, and in this way be transferred to both of its twins, causing the one to be a *laeta* instead of a pure *Lamarckiana*. We are thereby provided with a beginning of an experimental analysis of the marks of mut. *velutina*, as already discussed.

Here I might insert some considerations concerning the mutative origin of *O. blandina*. We have seen that *O. Lamarckiana* and *O. nanella*, when crossed with this new form, repeat its characters in part of the offspring. In the same way a mutant *velutina* may be produced by the conjugation of a mutated sexual cell with a normal one. Thus it is not necessary to assume the accidental meeting of two mutated gametes, which would obviously make the chance of the mutation occurring very much smaller still. It is sufficient to suppose that only the female elements of the original *O. laeta* have mutated in this way, although we cannot know whether this change might not have taken place in the male cells. And since *O. blandina* behaves as an isogamous species, both hypotheses seem to be equally probable. In both cases mutants of the *laeta* type should be expected to appear also, but as they would be very rare and not discernible in the beginning from the *Lamarckiana* specimens which always develop out of a part of the seeds of *O. lata*, they would surely have been overlooked in the years 1907–1908, when the mutation into *blandina* occurred.

I shall now give a more detailed description of my experiments. The crosses were made in 1913 and the first generation was cultivated twice for every cross, once in 1914 and once in 1915.

O. blandina×*O. Lamarckiana*.—A biennial specimen of the latter form was chosen and its pollen placed on the stigma of two individuals of the thoroughly green type of *O. blandina*. The seeds of one cross were sown in 1914, and those of the other in 1915.

The first culture consisted of 23 *laeta* and 34 *velutina*, making a total of 58, with 41 per cent *laeta*. In 1915 the figures were 46 *laeta* and 39 *velutina*, or 54 per cent *laeta* in 85 specimens. Although the size of the cultures was small, they evidently point to a division in nearly equal groups. The two types were clearly different from the beginning and could easily be counted out in June before the production of the stems. In 1915 I separated them in March, at the time of planting into the boxes, in order to control my estimate later on, and in April planted the *laeta* in one group and the *velutina* in another half of the bed. In 1914 I had 25 and the following year 10 flowering plants, half of which belonged in each case to the *laeta* type and the other half to the *velutina* type. The *laeta* have already been described; the *velutina* were in no respect and at no time different from ordinary *O. blandina*.

The second generation from seed of one of the *velutina* plants embraced 30 flowering and 40 younger specimens, all of which exactly repeated the marks of their parent. From the seeds of one self-fertilized *laeta*, however, I got the splitting group already described. Its two types were the same as in the previous generation. I recognized the splitting in the seed pan, but counted them only in June after planting out 15 *laeta* and 15 *velutina*. All in all I had 80 plants, of which 26 were *laeta* and 54 *velutina*, or 33 per cent *laeta*, which is somewhat less than in the first generation. All the 30 specimens of the bed richly flowered and ripened their first fruits before being thrown away.

O. Lamarckiana×*O. blandina*.—A biennial plant of the species was crossed in 1913 with a green individual of the mutant. The seeds were sown partly in 1914 and partly in 1915. They gave the same two types as in the reciprocal cross. During the whole lifetime there were no visible differences. In the first year I had 60 plants with 22 per cent *laeta*, and in the second year 108 specimens with 25 per cent *laeta;* the remainder were *velutina*. Of these, 25 and 10 flowered, in about equal groups for both types, having been recognized and sorted out at the time of planting. The other plants were cultivated till the end of June.

O. blandina×*O. nanella*.—Two green individuals were fertilized in 1913 by the pollen of my race of *O. Lamarckiana* mut.

nanella; the seeds of the one were sown in 1914 and of the other in 1915. There were no dwarfs in this first generation, but only *laeta* and *velutina*, which were just like those of the crosses already described. I had 90 and 72 plants, with 74 and 67 per cent *laeta*. There were 25 and 10 flowering plants belonging equally to the two groups; the others were large rosettes in June.

If we compare the percentages of *laeta* given with one another we find for *O. blandina*×*O. Lamarckiana* 41 and 54 per cent, for the reciprocal cross 22 and 25 per cent, and for the experiment with the dwarfs 74 and 67 per cent; finally, for the second generation of the first cross 33 per cent. The average of all these figures is 45 per cent *laeta*, which comes as near to equality of the two groups as may be expected. The deviations from this mean are probably due mainly to the choice of the parents and to their cultural conditions.

O. rubrinervis×*O. blandina*.—Besides the 3 crosses already mentioned and discussed, I have also made the two reciprocal crosses with my race of *O. rubrinervis*. In the first generation they split in the same way, the only difference being that instead of the *laeta* another type arises. This is the *subrobusta*, which appears in the hybrid splittings of *O. rubrinervis* with other derivatives of *O. Lamarckiana*, as described in my *Gruppenweise Artbildung*. No differences were observed, although the comparison lasted from germination till the ripening of the fruits. The other type was the same as in the crosses already dealt with, and exactly like the parental type of *O. blandina*.

The cross was made in 1913 between an individual of my pure race of *O. rubrinervis* and a specimen of the third generation of *O. blandina*. One part of the seeds was sown in 1914 and another in 1915. In the first year I had 60 plants with 32 per cent *blandina*, and cultivated 18 *laeta* and 7 *blandina* until the ripening of their fruits. In the last named year I had 77 specimens, of which 61 per cent were *blandina* and of which 5 *laeta* and 5 *velutina* were left to flower. All in all, the cultures embraced 137 plants, with 45 per cent *blandina*. The others were all *subrobusta* and not different from the *subrobusta* cultures of those years resulting from other crosses.

O. blandina×*O. rubrinervis*.—For this cross I used two specimens of *O. blandina* of the third generation in 1913, the one being a pale green and the other a normal color. In 1914 each of the cultures embraced 60 plants, of which 25 flowered. The percentages for *blandina* were 48 for the green, but only 20 for the pale parent. For this reason I repeated the latter culture in 1915 and obtained from 70 plants 47 per cent *blandina*. The types of *subrobusta* and *velutina* in these cultures were exactly the same as those from the reciprocal cross.

The percentages given are obviously of the same type as those for the splitting into *laeta* and *velutina* and come as near to equality for the two types as may be expected under ordinary conditions of cultivation. I propose to grow the second generation next summer.

THE VIABILITY OF THE SEEDS OF O. LAMARCKIANA MUT. VELUTINA.—Besides the external differences between our new mutant and the parent species, there is another mark which lends a high interest to the new form. This is found in the seeds. The seeds of *O. Lamarckiana* differ from those of almost all other species (with the exception of *O. suaveolens*) in containing a large proportion of empty grains, even under the most favorable conditions of life. More than one-half of the seeds have no germ at all, although externally they are, as a rule, not distinguished from the normal ones. RENNER[10] has studied the development of these empty seeds and found that their germ is fecundated and undergoes one or two cell divisions, but then stops and dies off. He considers this phenomenon as a hereditary character of the species. It runs parallel, in this respect, to the rudimentary ovules described by GEERTS, which are characteristic of the whole group of the Oenotheras. Besides this type of empty seeds a less or larger number usually occur which stop their development at a much later stage. The proportion of these can be diminished by a better culture, and therefore they may be considered as a result of the crowding of the seeds in the capsules, combined with the limited amount of nourishment available for them.

Our new mutant *velutina* produces hardly any abortive seeds, at least under normal conditions of culture. I tried the seeds from

[10] RENNER, O., Befruchtung und Embryobildung bei *Oenothera Lamarckiana*. Flora 7:115–150. 1914.

purely pollinated capsules of 4 specimens of my culture of 1915, which was the fourth generation of my race. I took them carefully out of the fruits, mixed those of 5 successive capsules of the same spike, and counted 200 grains from each lot. I soaked them in water, pushed this into their seed coats by means of a pressure of 8 atmospheres for about 24 hours, and afterward laid them out to germinate in small glass tubes in a stove at 30° C.[11] Within 6 days the larger part of the seeds germinated, giving percentages of 85, 84, 73, and 70 for the 4 lots. I then opened the remaining grains and found fully developed germs in almost all of them. The percentage of germs, being the sum of the two trials, came to 99, 96, 96, and 93, and there is no doubt that if they had been left in the stove for a longer time, almost all of the resting germs would have shown signs of germination.

The proportion of rapidly germinating grains and that of empty ones depend in a high measure upon the external conditions of life. During the summer of 1914 I cultivated 2 lots of individuals from seeds of the same parent plant, giving to one of them the ordinary favorable conditions of my garden, and keeping the others in a dry soil without manure. The seeds of the 2 most vigorous specimens of both lots, taken from self-fertilized capsules, were tried. They contained 99 and 99 per cent of germs for one group, but only 72.5 and 73.5 per cent for the other group. The same effect was shown in the amount of grains which germinated within the first 6 days. The figures were 80–88 per cent in one case and 53 per cent in the other case.[12]

The loss of the hereditary property of producing about 50 per cent of empty grains constitutes a latent mutation accompanying the visible changes in the external structure. The same loss accompanies the mutation into *gigas*, *rubrinervis*, and some newer mutants, but does not occur in such cases as those of *O. lata*, *O. scintillans*, *O. cana*, and others (see footnote 4).

[11] This method has been followed in all the experiments to be described in the text. It is described in the following papers: The coefficient of mutation in *Oenothera biennis* L. BOT. GAZ. **59**: 190–194. 1915; and in Über künstliche Beschleunigung der Wasseraufnahme in Samen durch Druck. Biol. Centralbl. **35**:161–176. 1915.

[12] Biol. Centralbl. **35**:174–175, where *O. blandina* was provisionally indicated as mut. nov. *B*.

I made a series of crosses in order to study the nature of this latent mutation of *O. blandina*. They led to the discovery of some points which seem to deserve a more thorough study than I could give them until now.

In the first place, it is to be expected that in crosses with those species which do not produce such empty seeds the high figures of both parents will simply be repeated. I tried these cases, determining the amount of normally developed germs in lots of 200 seeds each, after the method already described. I made the crosses in 1914 and 1915, and in most cases in both the reciprocal directions. The seeds were taken from 5 successive fruits, and carefully prepared so as not to lose any small grains. The results are given in table I.

TABLE I

Cross	Percentage of germs*		
	Cross	Cross	Reciprocal
O. biennis ×blandina.....	99	—	91
O. biennis Chicago× "	95	89	94
O. Cockerelli × "	99	95	87
O. Hookeri × "	94	91	—
O. syrticola × "	87	83	—

* The dash (—) means that the cross has not been tried.

In some instances I cultivated the first generation of the hybrids in 1915 and tried their seeds after self-fertilization. I got almost the same figures: *O. blandina*×*Chicago* 86 per cent, *O. Hookeri*× *blandina* 97 per cent, and *O. syrticola*×*blandina* 93 per cent.

In trials with other species the hereditary property of *O. Lamarckiana* of making germs in only one-half of its seeds is recessive to the normal condition of producing almost only normal grains. It behaves in the same way in crosses with *O. blandina*. The results are given in table II.

Of course it is to be expected that those mutants which themselves give high percentages after self-fertilization will do the same in their crosses with the *velutina* mutant (table III).

The crosses were made in 1915. *O. rubrinervis* is the same strain as used in my *Gruppenweise Artbildung*. *O. erythrina* is a new mutant from *Lamarckiana*, of the type of the hybrid form *subrobusta* of that book; and *O. deserens* is a mutant from *O. rubri-*

nervis, originated through the loss of the typical red color, but without change in the brittleness of the stem. These new mutants will have to be dealt with in another article.

TABLE II

Cross	Culture	Percentage of germs	
		Cross	Reciprocal
O. blandina ×Lamarckiana....	1914	94	—
" × "	1915	97	95
" ×nanella.....	1915	99	—
O. Lamarckiana ×blandina.....	1914	90	—

The most interesting question in this situation, however, is that concerning the seeds of the first generation of the crosses between *O. Lamarckiana* and *O. blandina*. I made the crosses in

TABLE III

Cross	Percentage of germs	
	Cross	Reciprocal
O. blandina ×rubrinervis.....	91	97
" ×erythrina.....	99	—
" ×deserens.....	99	100

1914, cultivated the hybrids in 1915, and tried their self-fertilized seeds during the winter. From each of the 3 crosses I had 5 vigorous specimens of the *laeta* type, and an equal number of the *velutina* type. I shall deal with the *laeta* first (table IV).

TABLE IV

SELF-FERTILIZED SEEDS OF *laeta* SPECIMENS OF THE FIRST GENERATIONS

Cross	Percentage of germs				
O. Lamarckiana ×blandina.....	98	98	97	96	95
O. blandina ×Lamarckiana.....	96	96	95	92	36
" ×nanella.....	96	96	43	41	39

The 3 crosses may be taken as instances of one type and combined on this ground. We see that a splitting occurs, 11 individuals having the high percentages of the one parent, and 4 others

having the low figures of *O. Lamarckiana*. This splitting may be considered, therefore, as amphiclinous,[13] 73 per cent of the hybrids belonging to the one and 27 per cent belonging to the other parental type. The splitting is analogous to that of the cross *O. Lamarckiana*×*nanella*, which gives, under ordinary circumstances, in the first generation about 78 per cent tall and 22 per cent dwarfish specimens. It must be pointed out, however, that here the germs, which belong to the next generation, are dealt with as a mark of the first generation. In this respect they may be compared with the rudimentary ovules of GEERTS, which are not fertilized.

I tried also the *blandina* plants of the same crosses, but had fertilized only two specimens of each, which is too few to study this phenomenon. I found 77 and 69 per cent, 67 and 60 per cent, and 71 and 70 per cent of good grains among their seeds. It is remarkable that all of these plants had about the same figure, which is far less than that of the specimens of the pure race of *O. blandina* (about 95 per cent), but a splitting did not show itself.

The same difference as between the *laeta* and extracted *velutina* was shown by the *subrobusta* and the *velutina* from the crosses with *O. rubrinervis*. I found the figures as given in table V.

TABLE V

Cross	Hybrid	Percentage of germs		
O. rubrinervis×blandina	subrobusta	97	96	—
O. blandina ×rubrinervis	"	96	96	96
O. rubrinervis×blandina	blandina	70	68	—
O. blandina ×rubrinervis	"	80	75	—

It is possible, however, that this difference is only an effect of the higher exigencies of the plants of the type of *blandina*, since we have already seen that their amount of good grains is easily diminished by an unfavorable culture. Moreover, this character is not a constant one, for in trying the *blandina* plants of the second generation I found their contents of normal embryos complete, no single empty grain being found in lots of 200 seeds of two such plants.

[13] Über amphikline Bastarde. Ber Deutsch. Bot. Gesells. 33:461–468. 1915.

It is necessary, of course, to study the progeny of the *laeta* plants with a high and of those with a low percentage figure. Until now, however, I have had only an opportunity to study the offspring of one *laeta* of the first type, since I happened to have fertilized only that one in 1914. Its seeds contained 96 per cent of germs. I cultivated 15 specimens of *laeta* from these seeds, and found, for self-fertilized seeds of each of them, percentages between 91–97, with a mean of 95. In this instance, therefore, the high percentage seems to be constant.

The main result of this inquiry is that *O. Lamarckiana* mut. *velutina* has lost the property of the parent species of producing about one-half of empty grains, that this property is recessive to the normal production of almost only good grains, and that a splitting is observed in the first generation, which seems to follow the type of amphiclinous hybrids, as in the case of *O. Lamarckiana*×*O. nanella*. A further study is required to elucidate these points, especially the behavior of the seeds as a mark of the generation which produces them.

Summary

1. *O. Lamarckiana* mut. *velutina*=*O. blandina* arose from my family of *O. Lamarckiana* mut. *lata*×*semilata* among seeds of the third generation saved in 1904, in 3 specimens. Of one of these I cultivated a second generation and of one of the others 4 successive generations, embracing together over 3000 plants.

2. All these plants were exactly alike with the exception of 4 mutants which constituted a new type, *O. spiralis*. The mutation coefficient was 0.1 per cent, or about the same as for *O. rubrinervis* and *O. nanella*, and much smaller than that for *O. Lamarckiana*.

3. For the appearance of the original mutation only one sexual cell needs to be mutated, since in combining with a normal gamete it may give rise to *O. blandina*, as is shown by the splitting of both the reciprocal crosses of this form with *O. Lamarckiana*. The splitting goes into nearly equal groups of specimens like *O. blandina* and of *laeta*.

4. *O. Lamarckiana* mut. *velutina* resembles the hybrids of the type of *velutina* so much as to be considered one of them. Among

them it is the most like *O.* (*Lamarckiana*×*O. biennis Chicago*) *velutina*, without the marks of the second parental species, however. It is slender, with long internodes in the spike, and with flowers as large as those of *O. Lamarckiana*.

5. *O. Lamarckiana* mut. *velutina* is distinguished from its parent species in a very striking character. It has lost the property of producing about one-half of empty grains; almost all of its seeds contain healthy and well developed germs and germinate easily. This new quality is dominant over that of the parent. It is the same as in almost all the older species of the genus.

6. Moreover, *O.* mut. *velutina* is distinguished from *O. Lamarckiana* at least in one other dominant character, the smoothness of its leaves at the time of flowering. Secondly, it is distinguished in quite a number of characters, which seem to be more or less independent of one another, namely, slender stature, long internodes of the flower spike, narrow and longitudinally folded leaves and bracts, and cup-shaped flowers. Besides these, the richness in red color and the hairiness of all organs, especially in their youth, are very striking marks.

7. In crosses with those species which split *O. Lamarckiana* and some of its other derivatives into the twin hybrids *laeta* and *velutina*, the *O.* mut. *velutina* produces only hybrids of the *velutina* type.

8. In crosses with *O. Lamarckiana* and *O. nanella*, these forms are seen to be split by *O.* mut. *velutina* into twin hybrids, which correspond to the twins produced by other species with them, but which, of course, lack the characters of those other parents. The twins of *O. blandina* may be considered as pure *laeta* and pure *velutina*, therefore, the former having smooth leaves and bracts in the summer, the latter being identical with *O. blandina* itself.

9. The study of our new mutant reveals the existence of at least two recessive characters in *O. Lamarckiana*, namely, the bubbles of the leaf blade and the presence of typical empty seeds.

BOTANIC GARDEN
AMSTERDAM

EXPLANATION OF PLATE I

At the right, *Oenothera Lamarckiana* mut. *velutina* (*O. blandina*); at the left, *O. blandina* mut. *spiralis*.

DEVRIES on OENOTHERA

INFLUENCE OF THE LEAF UPON ROOT FORMATION AND GEOTROPIC CURVATURE IN THE STEM OF BRYOPHYLLUM CALYCINUM AND THE POSSIBILITY OF A HORMONE THEORY OF THESE PROCESSES

JACQUES LOEB

(WITH THIRTY FIGURES)

In two former publications[1] it was shown that while the stem of *Bryophyllum calycinum* prevents or retards the development of roots and shoots in the notches of a leaf, conversely the leaf accelerates the development of roots and shoots in a stem; since in a stem deprived of all leaves the roots and shoots develop later and grow more slowly than if a leaf is left on the stem. The two phenomena found a common explanation in the assumption that the leaf furnishes substances to the stem which accelerate the organ formation in the latter, while if these substances are not "sucked away" from the leaf by the stem they will accelerate the growth of roots and shoots in the notches of the leaf. These substances may be water or solutes.

In these experiments it was noticed that the leaf has also an accelerating effect upon the geotropic curvature of the stem. When stems of *Bryophyllum* are suspended horizontally by 2 threads in a jar saturated with water vapor, they will bend, becoming convex on the lower, and concave on the upper side (fig. 1), and this bending continues until finally the stems assume the shape of a U. This geotropic bending is a slow process when the stem contains no leaf, but is considerably accelerated if a leaf is left on the stem (fig. 1). The position of the leaf has a great influence, not only on the velocity of the geotropic bending and the region of the stem in which it occurs, but also upon the formation of organs in the stem. The description of this influence and of the apparently close connection between the two groups of phenomena will form the subject of this paper.

[1] LOEB, J., BOT. GAZ. **60**:249–276. 1915; **62**:293–302. 1916.

Straight stems, at least 10 cm. long, were selected for these experiments. The growing region and the 2 apical nodes were removed, the pieces chosen for experimentation containing about 4–7 or 8 nodes. Each stem was suspended horizontally by 2 threads (fig. 1), one at each end, in a jar the bottom of which was filled with water. The jar was loosely covered with a glass plate so that the stems were surrounded by an atmosphere almost completely saturated with water vapor.

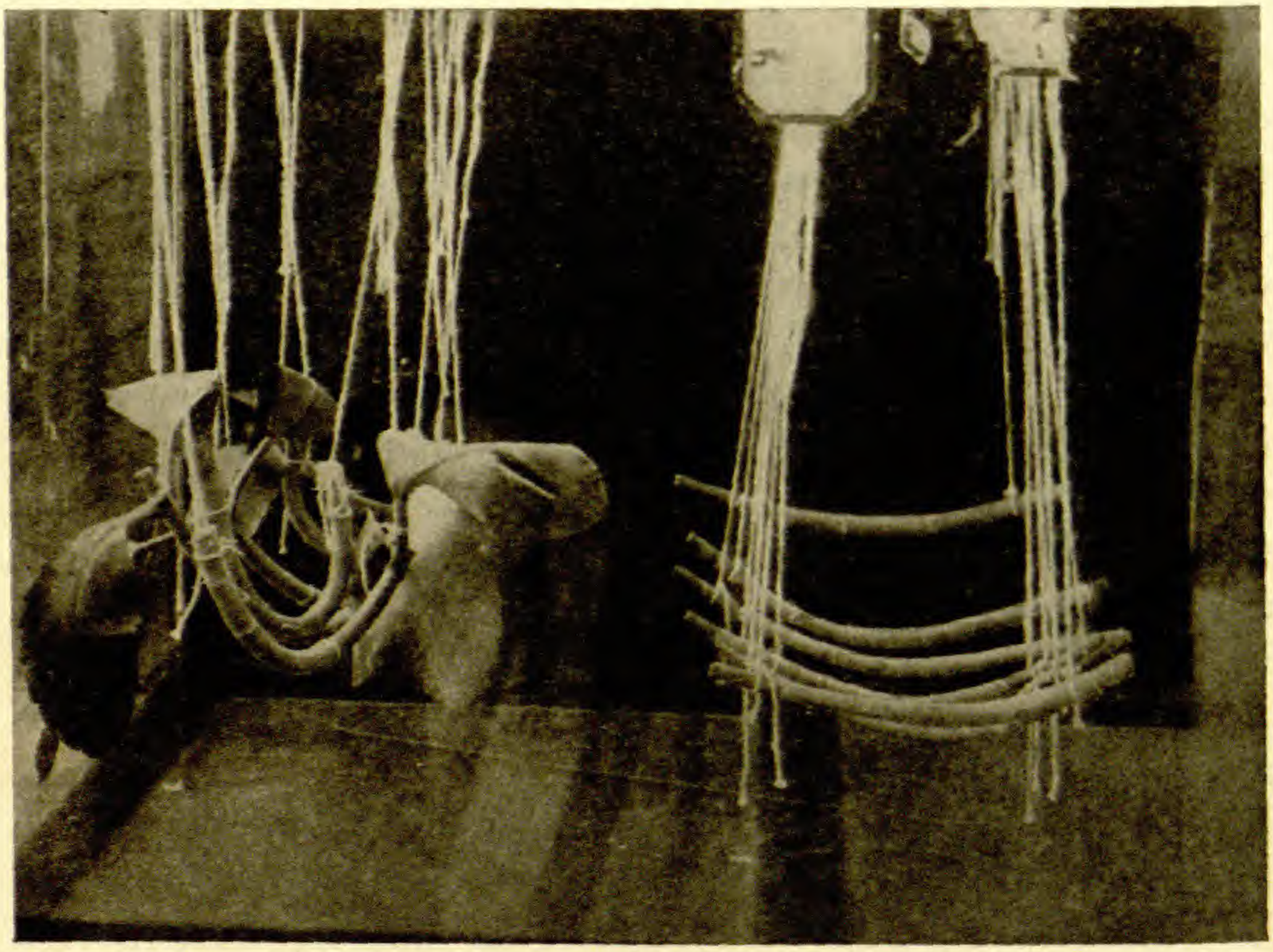

FIG. 1

The writer was rather surprised to find that stems deprived of their growing region should show geotropic curvatures. These curvatures were not confined to the nodes as is the case in grasses, but whole regions of the stem, both nodes and internodes, bent (fig. 1). We shall see later that the bending was accomplished by an increase in the length of the cortex of the under side of the horizontally placed stem, while the upper side of the stem was bent passively as a consequence of this growth.

I. Influence of the presence and absence of leaves

Fig. 1 illustrates the influence of the presence and absence of leaves upon the rapidity of geotropic curvature in the stems. The 6 stems to the right had no leaves and bent very slowly; the 6 stems to the left had each 2 leaves at the most apical node (while the other leaves were removed) and bent much more rapidly. The photograph was taken on the eleventh day after the experiment was begun. It is noticeable also that the stems containing leaves

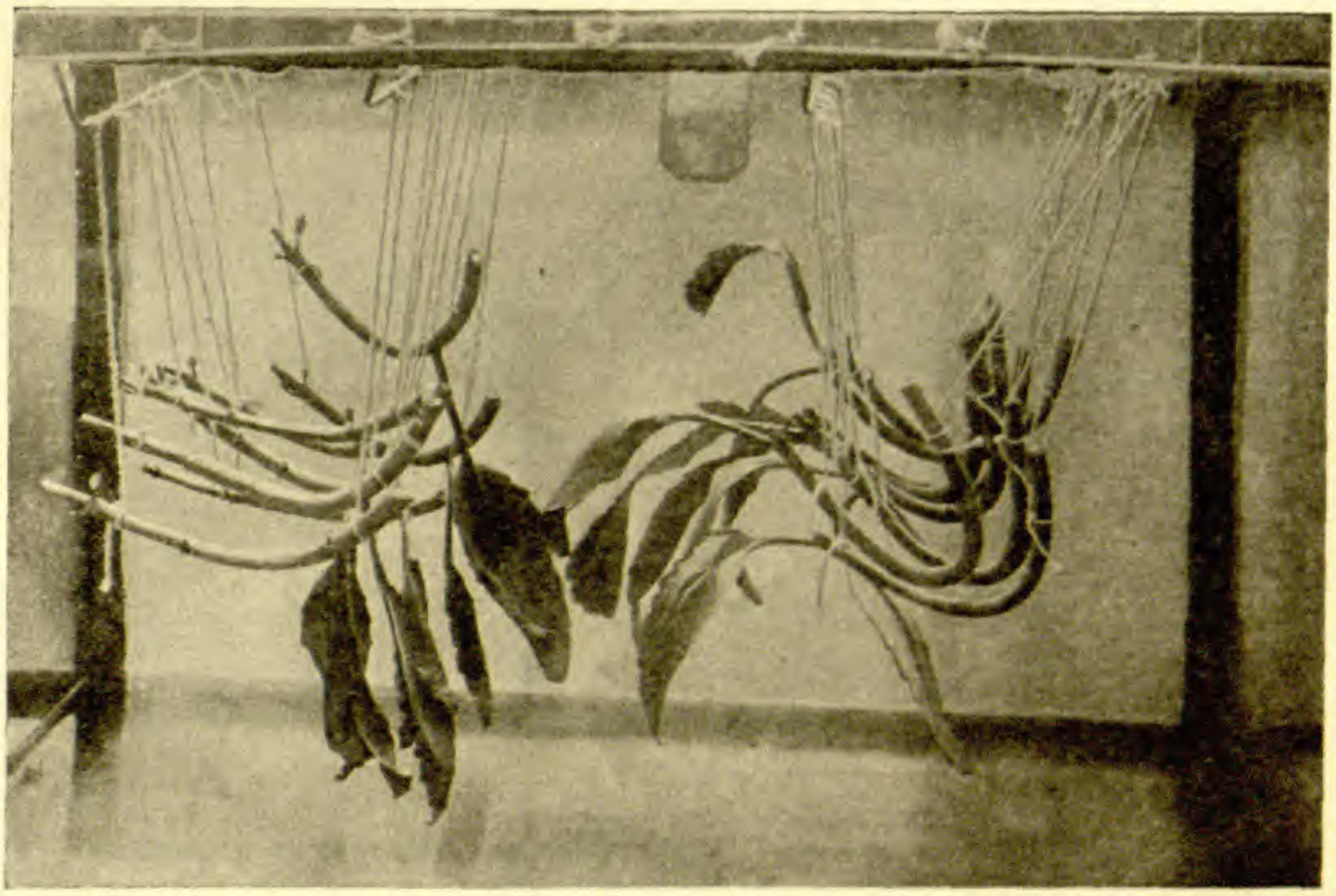

Fig. 2

formed roots (in their basal nodes) much more rapidly than the stems deprived of all leaves. This experiment, representing the accelerating influence of the apical leaves upon both root formation and geotropic curvature, never fails; and the same may be said of most of the experiments described in the following pages.

II. Influence of the position of the leaf on the stem upon geotropic curvature and organ formation

In the following experiment only one leaf was left on the stem. It was found that it made a great difference whether this leaf was at the apex or at the base. This is illustrated by fig. 2. On the

right hand in the photograph are 6 stems having one leaf left at the apex. These stems bent so rapidly and strongly that they soon reached a U-shape. On the left are 6 stems having a leaf left at the base. In this case the curvature is generally much less than

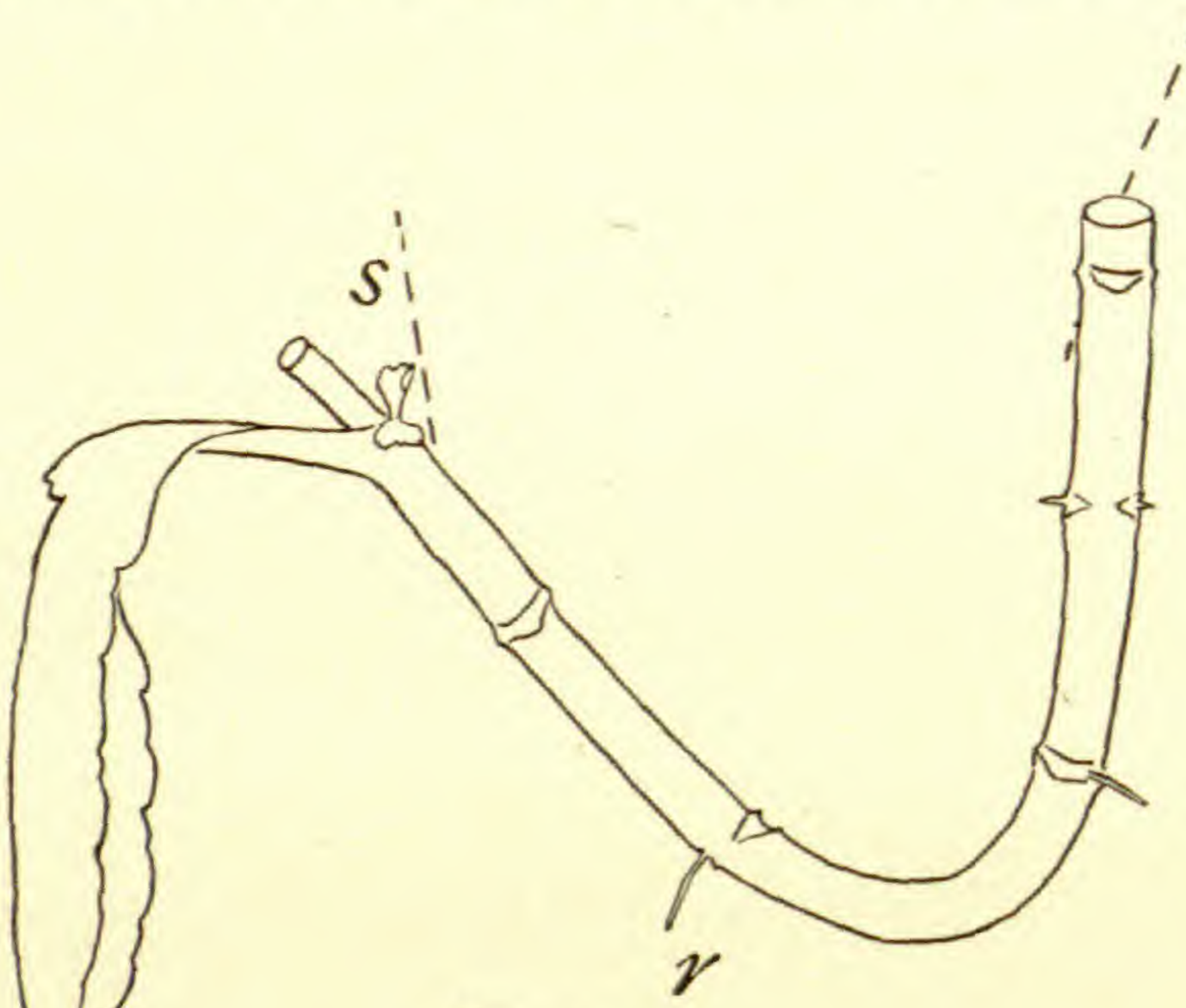

FIG. 3

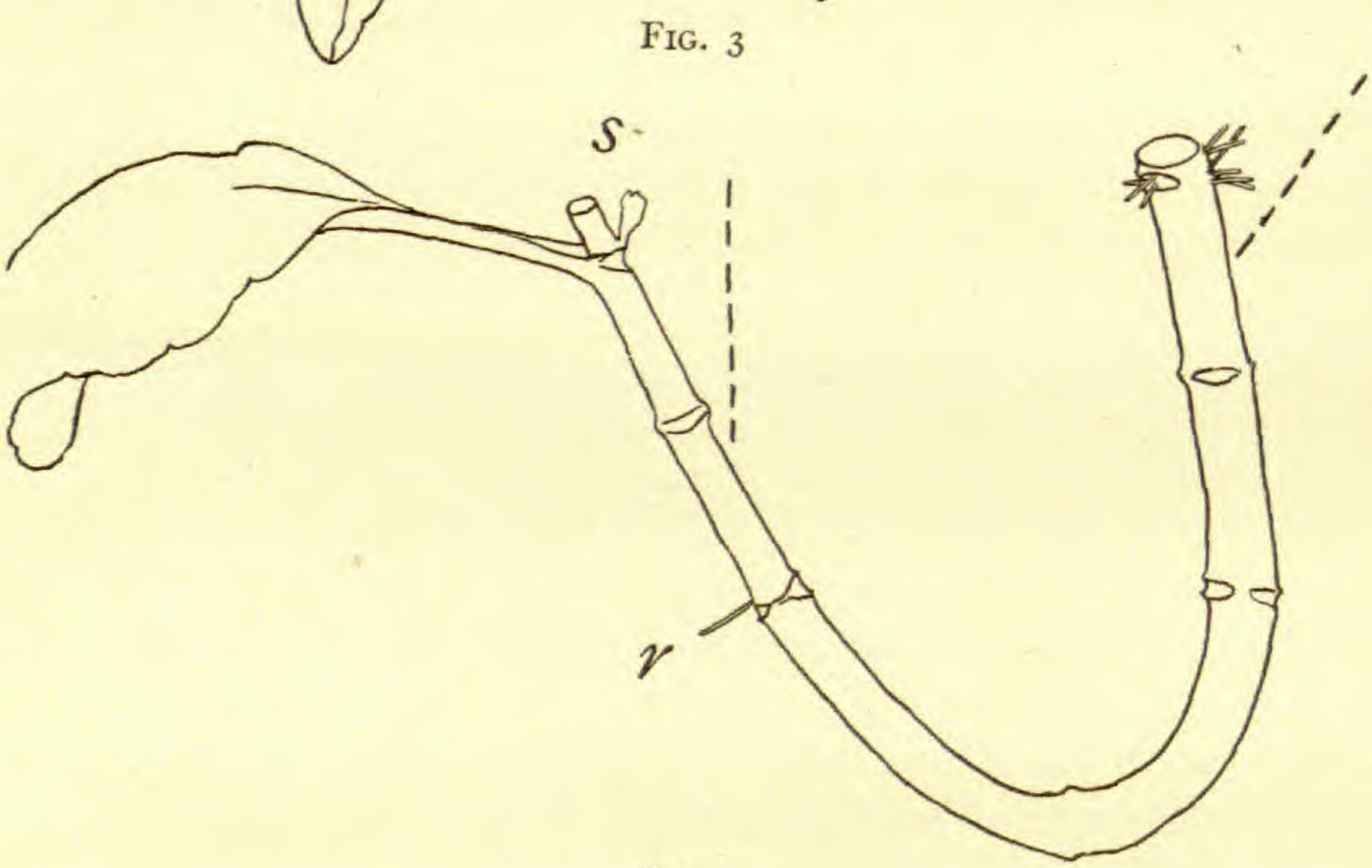

FIG. 4

when the leaf is at the apex. Both groups of stems had been suspended horizontally and both had been put into the jar at the same time. The photograph was taken on the eleventh day. This experiment also is always successful.

It is noticeable, incidentally, that while the leaf at the base accelerates the shoot formation, the one at the apex accelerates root formation. The more rapid geotropic curvature occurs in those stems in which the root formation is favored.

Aside from the influence of the position of the leaf upon the velocity and extent of the curvature, an equally striking influence exists between the position of the leaf and the localization of the curvature in the stem. When the leaf is at the apex, the curvature appears near the second node behind (basally from) the leaf (figs. 3, 4), and is confined chiefly to this region and possibly to the next node located more basally. The drawing was made 10 days after the experiment began.

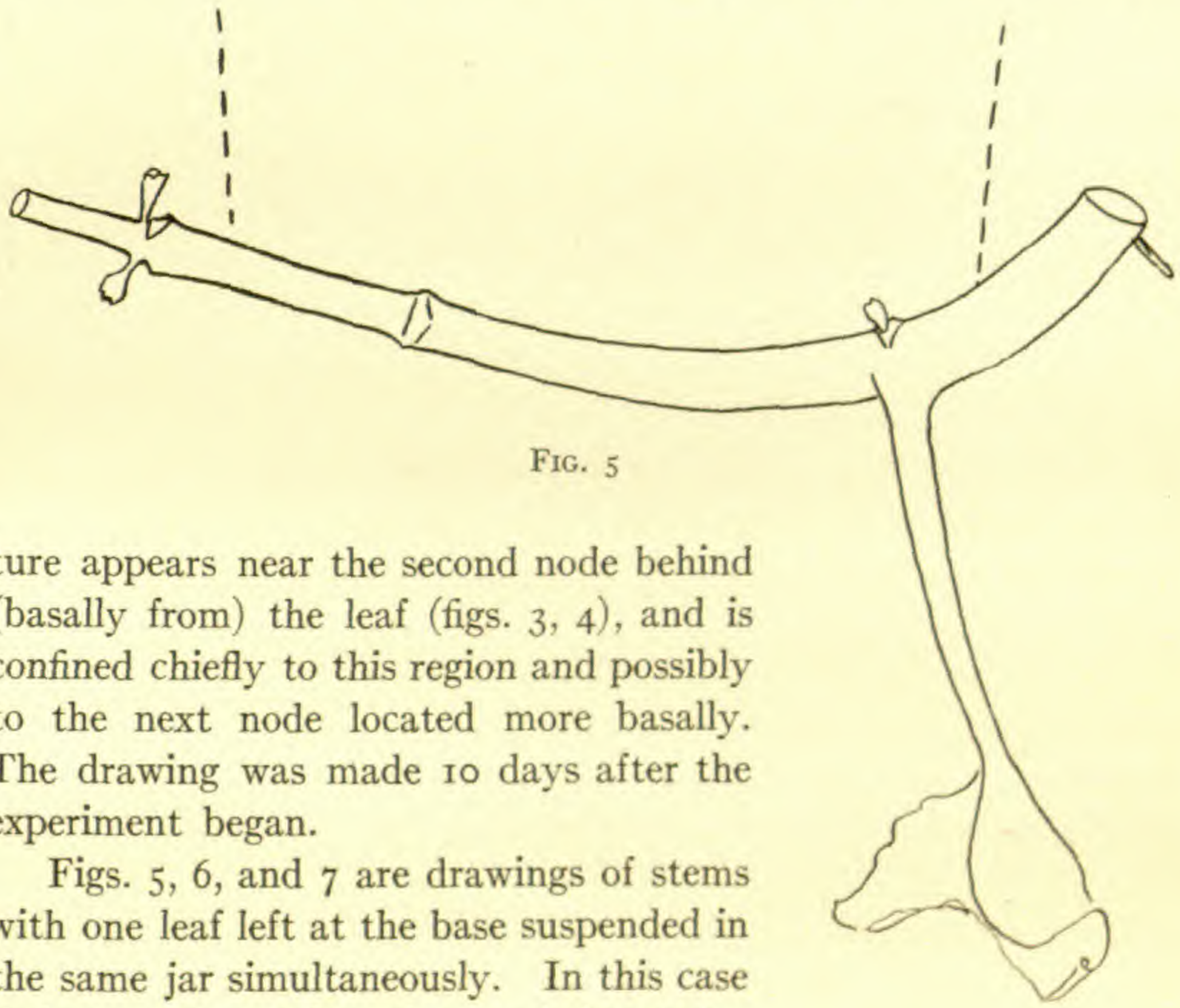

FIG. 5

Figs. 5, 6, and 7 are drawings of stems with one leaf left at the base suspended in the same jar simultaneously. In this case little curvature takes place and the curvature which occurs is near the region where the leaf is located. Fig. 6 is an extreme case. It seems that the amount of curvature increases with the length of the piece of internode left behind the basal leaf. The photograph in fig. 2 shows also the difference in the localization of curvature according to whether the leaf left is at the apex or at the base.

In the experiments thus far mentioned the leaf was on the lower side of the stem. When the leaf is left on the upper side of a

horizontally suspended stem, the geotropic bending is slower than when it is on the lower side, but the bending will also be much more rapid and more intense when the leaf is left at the apex (figs. 8, 9, 10) than when it is left at the base (figs. 11, 12, 13). In the latter case the bending is again slight, and what little curvature occurs is confined to the immediate neighborhood of the basal leaf. Fig. 13

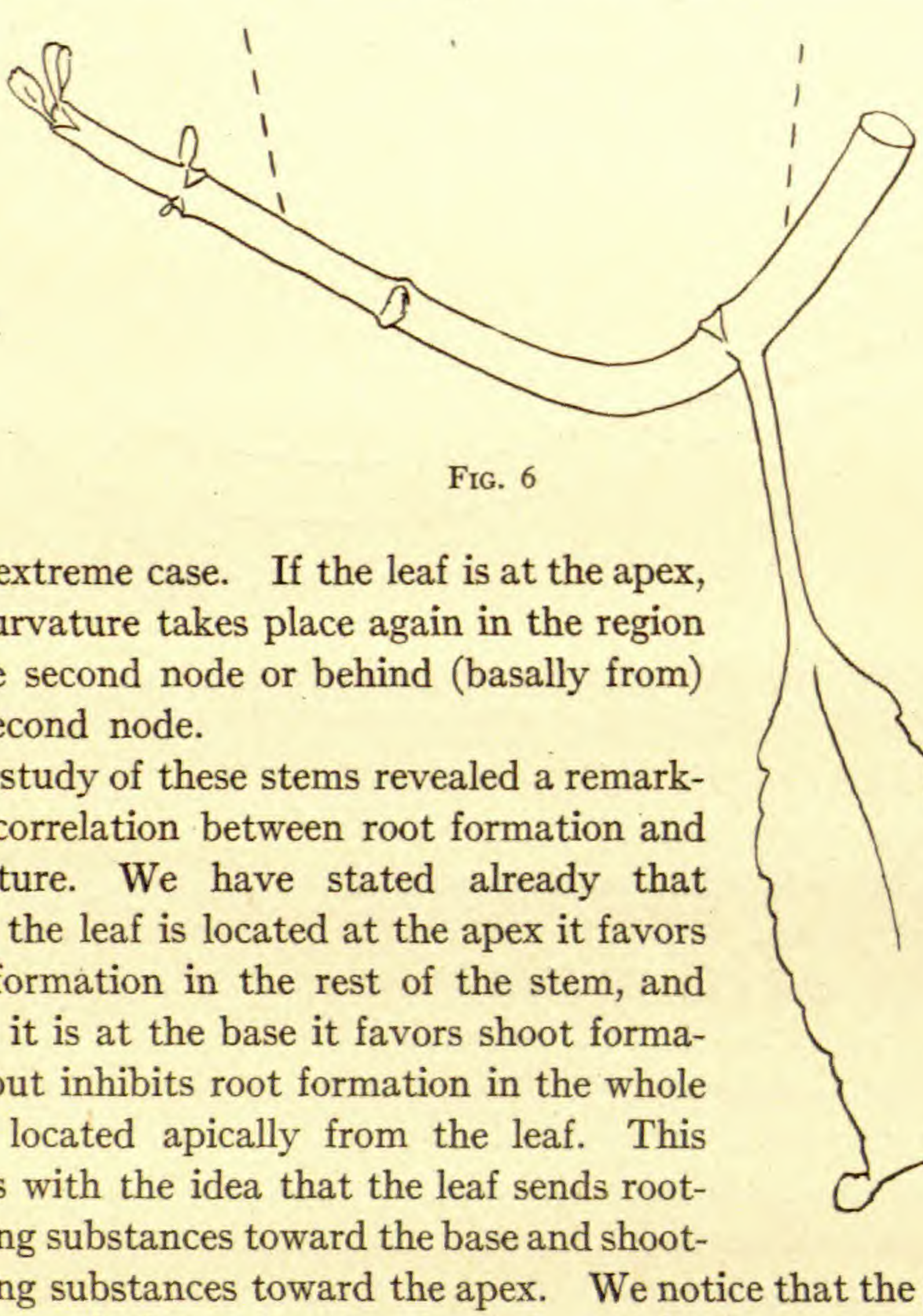

FIG. 6

is an extreme case. If the leaf is at the apex, the curvature takes place again in the region of the second node or behind (basally from) the second node.

A study of these stems revealed a remarkable correlation between root formation and curvature. We have stated already that when the leaf is located at the apex it favors root formation in the rest of the stem, and when it is at the base it favors shoot formation but inhibits root formation in the whole stem located apically from the leaf. This agrees with the idea that the leaf sends root-forming substances toward the base and shoot-forming substances toward the apex. We notice that the geotropic curvature is favored or accelerated most in those stems in which an apically located leaf is left, and in such stems root formation is favored also. The correlation between root formation and geotropic curvature is still more striking, however, if we consider the location of the roots formed. When the leaf left is at the apex,

roots usually appear in the second (and often the fourth) node behind the leaf, where the geotropic curvature also begins. This is obvious in *r*, figs. 3, 4, 8, 9, and 10. When the leaf left is at the base, the root formation is considerably less (as is also the geotropic curvature), and what there is of root formation occurs again in the region where the main geotropic curvature also occurs; namely, in the lower basal node (*r*, figs. 11, 12, 13). When the leaf is at the base, root formation is more favored when the leafless basal node

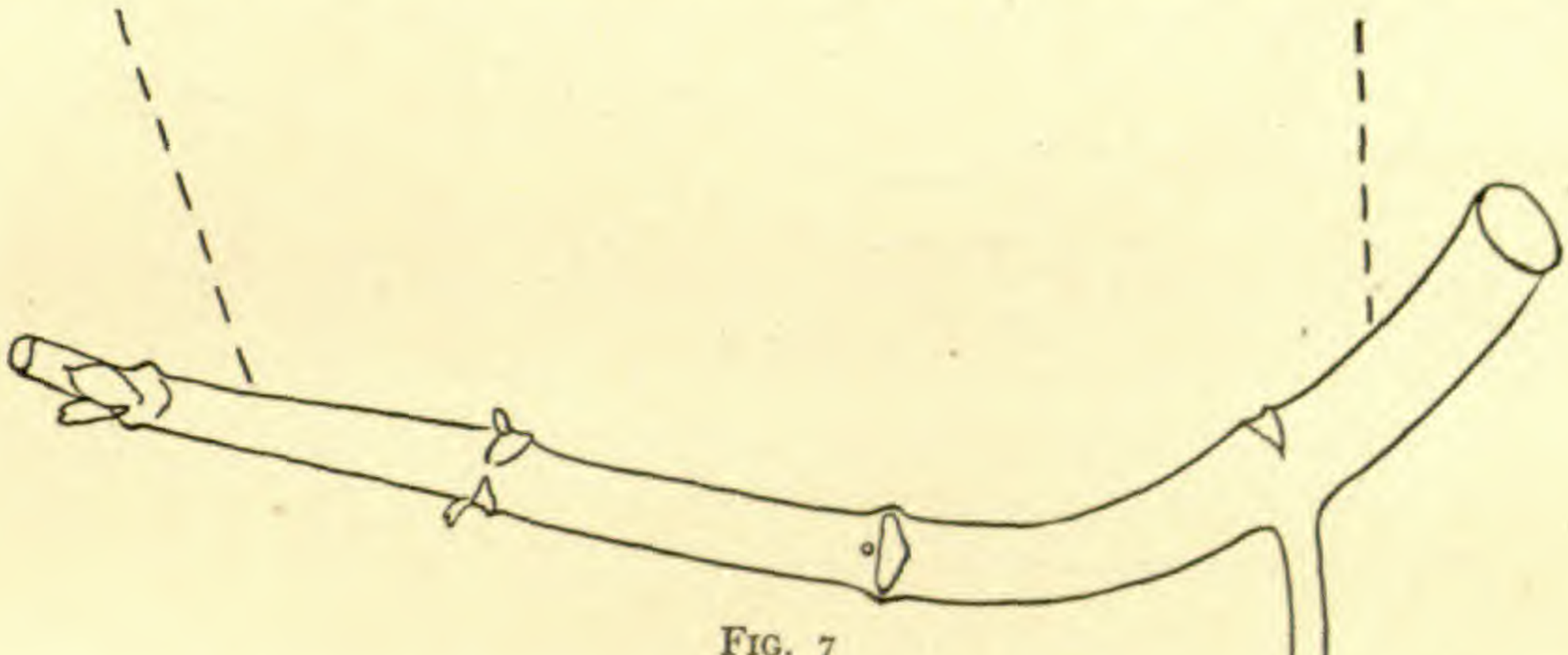

FIG. 7

is below, as in figs. 11, 12, and 13, than when it is above, as in figs. 5, 6, and 7, where no roots have appeared as yet in the leafless basal node on the upper side, although both series of experiments were started simultaneously.

This tendency of the roots to form more easily in the nodes of the under side of a horizontal stem is an important link in the chain of circumstances connecting root formation and geotropic curvature, since the growth causing this curvature is confined to the cortex of the under side of the stem.

III. EXPERIMENTS ON STEMS SPLIT LONGITUDINALLY

In order to find out the mechanism of geotropic curvature, stems were split lengthwise and suspended horizontally in jars saturated with water vapor. Each half stem had one leaf left either at the apex or at the base. Figs. 14–20 give the typical results of a series of such experiments on the seventeenth day.

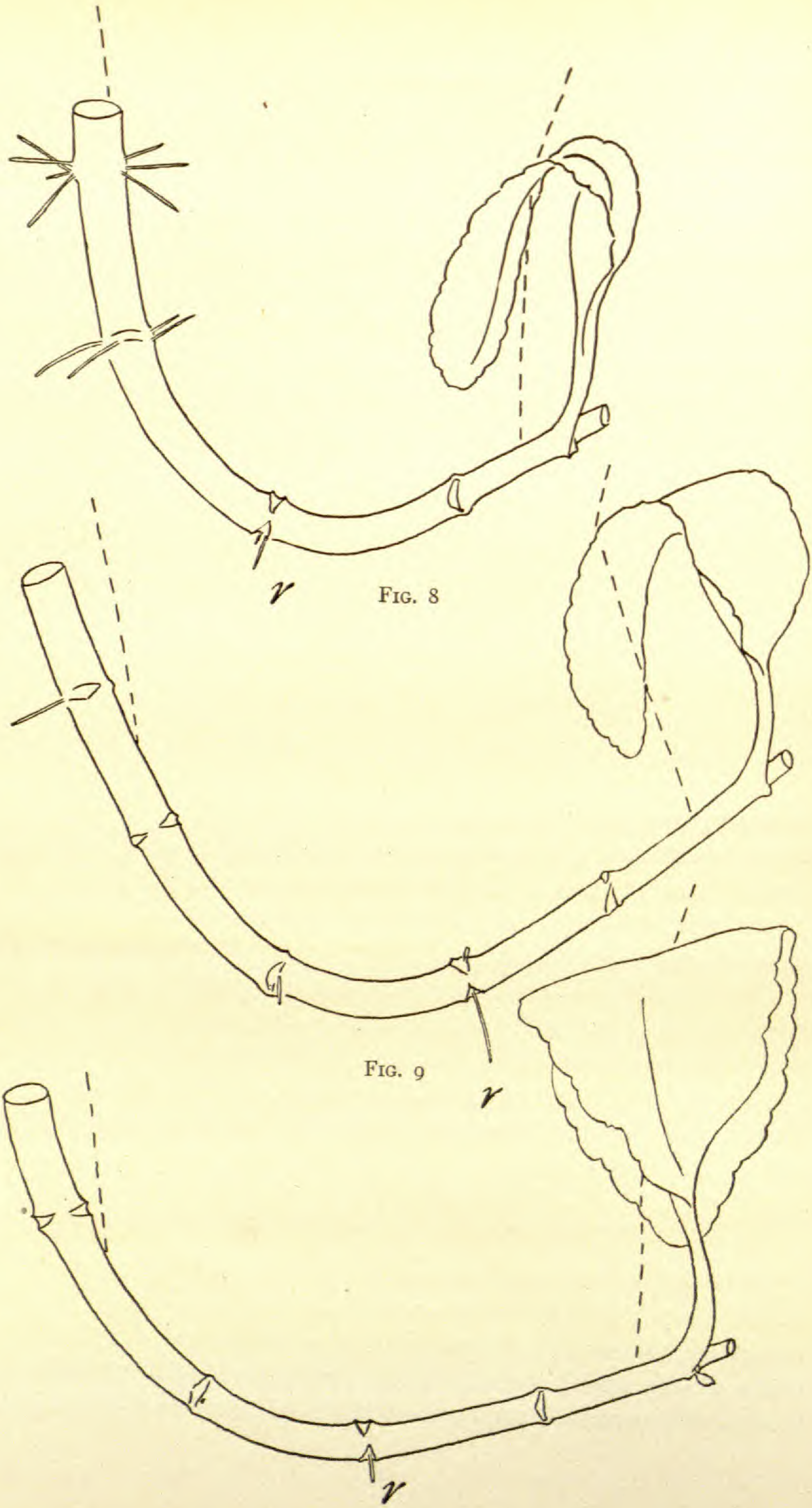

FIG. 8

FIG. 9

FIG. 10

Figs. 14, 15, 16, and 17 give the appearance of the lower halves of the stems (that is, stems suspended horizontally in such a way that

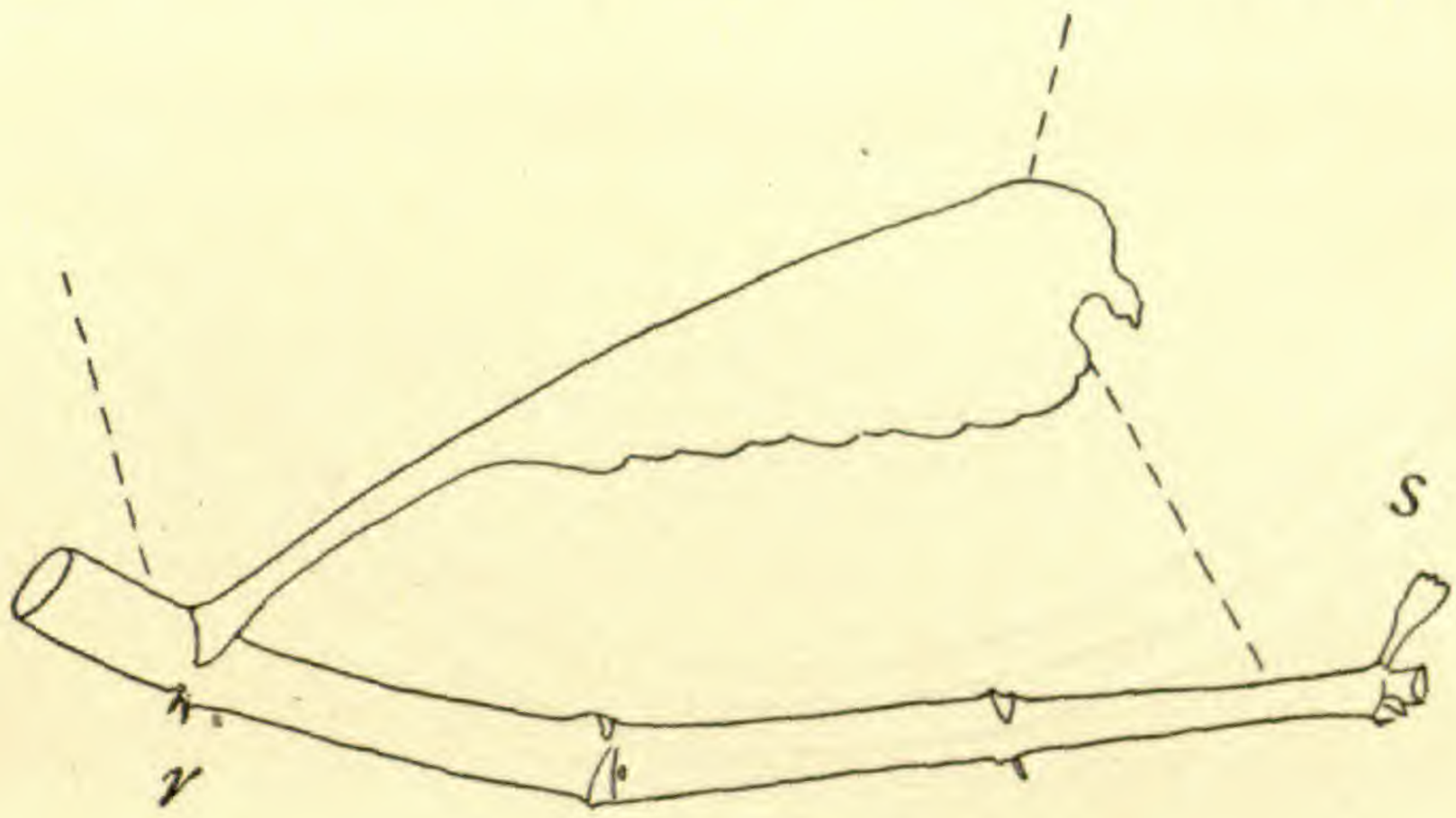

FIG. 11

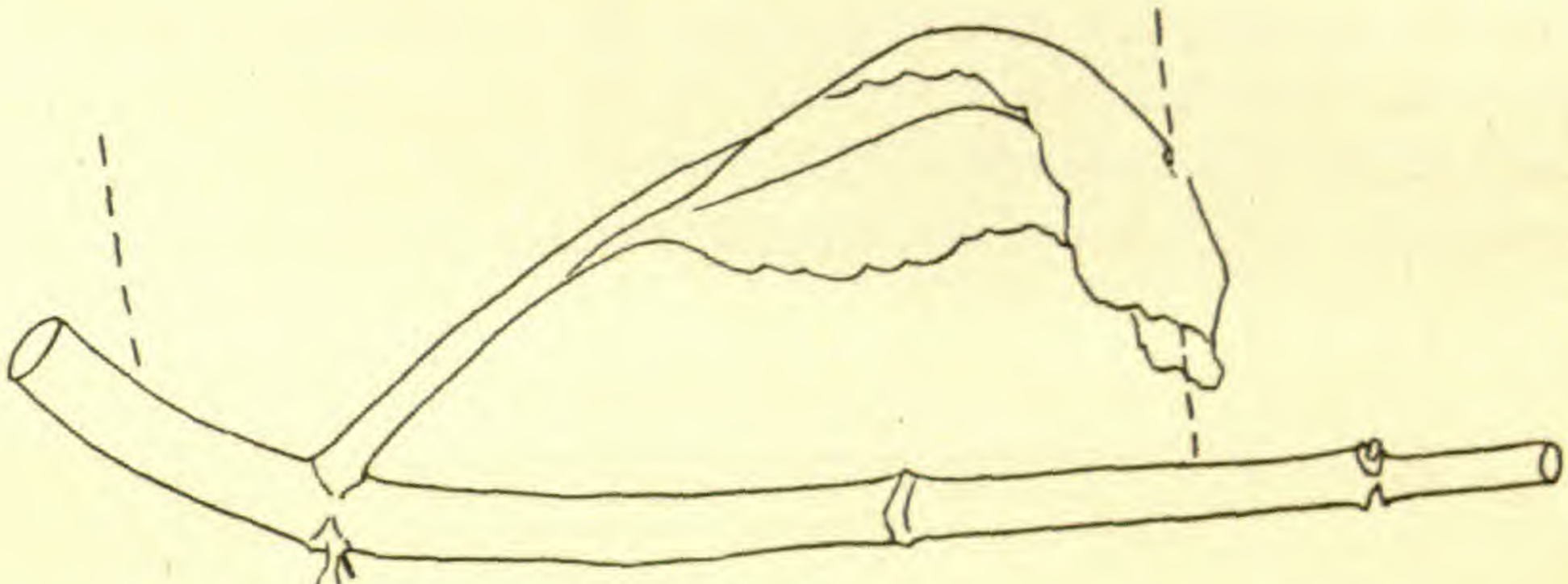

FIG. 12

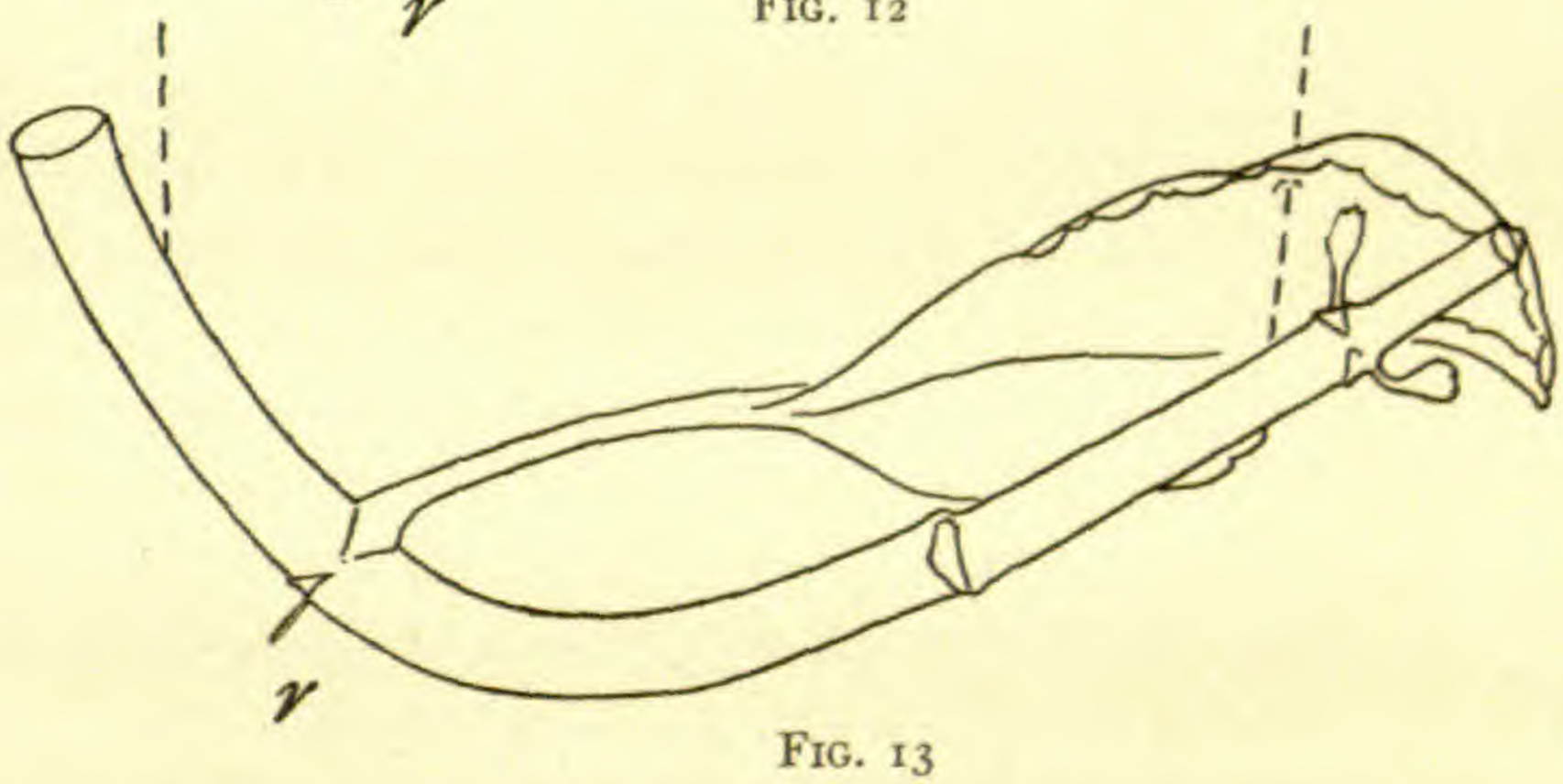

FIG. 13

the cortex was below, and the cut surface above) on the seventeenth day. When the apical leaf is preserved, the bending is rapid and extensive, as in fig. 17. When the basal leaf is preserved (figs. 15,

16), either no bending occurs, as in fig. 15, or it is confined to the region of the leaf or of the first node in front of the basal leaf (fig. 16). When no leaf is left, as in fig. 14, a slow bending takes

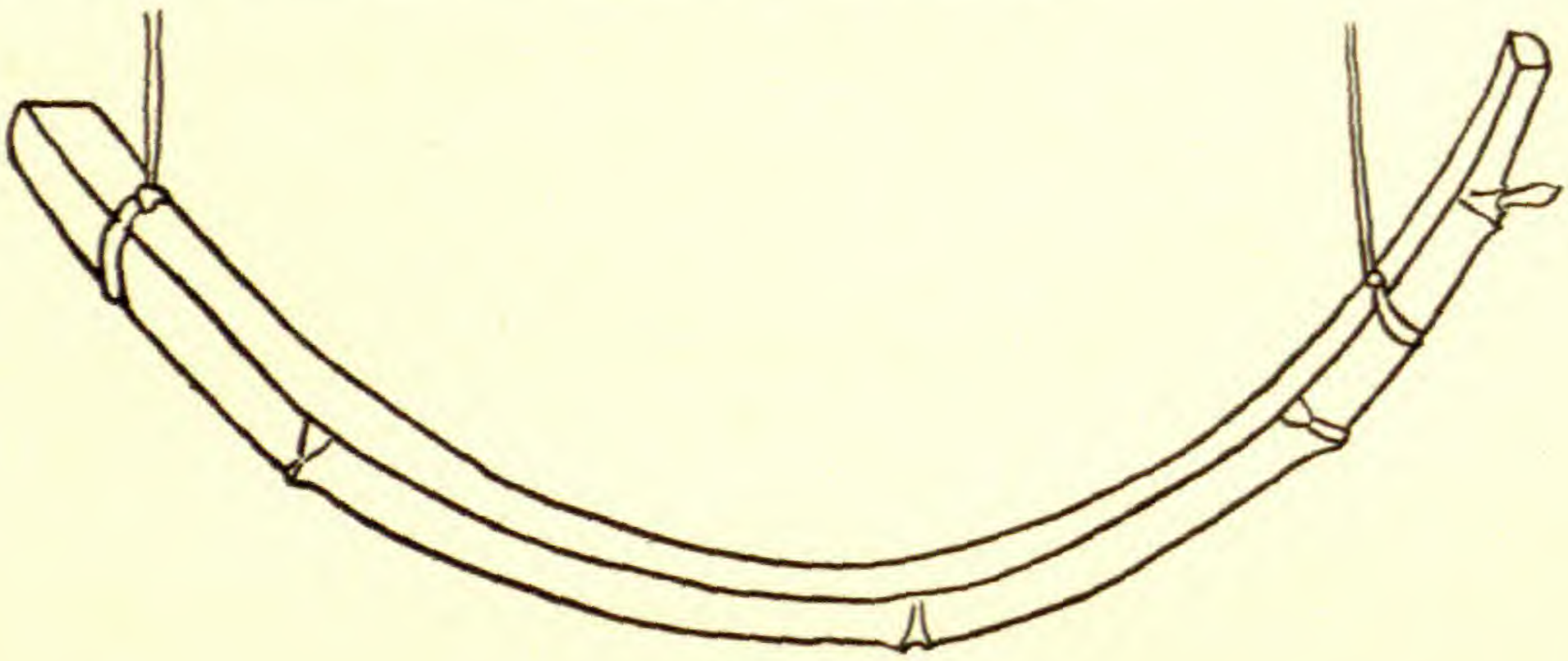

FIG. 14

place, more rapid and extensive than in fig. 15, but less rapid than when the apical leaf is left, as in fig. 17. In the other halves of the stem which were suspended with the cortex above (figs. 18, 19, 20), practically no geotropic bending takes place, for the reason that

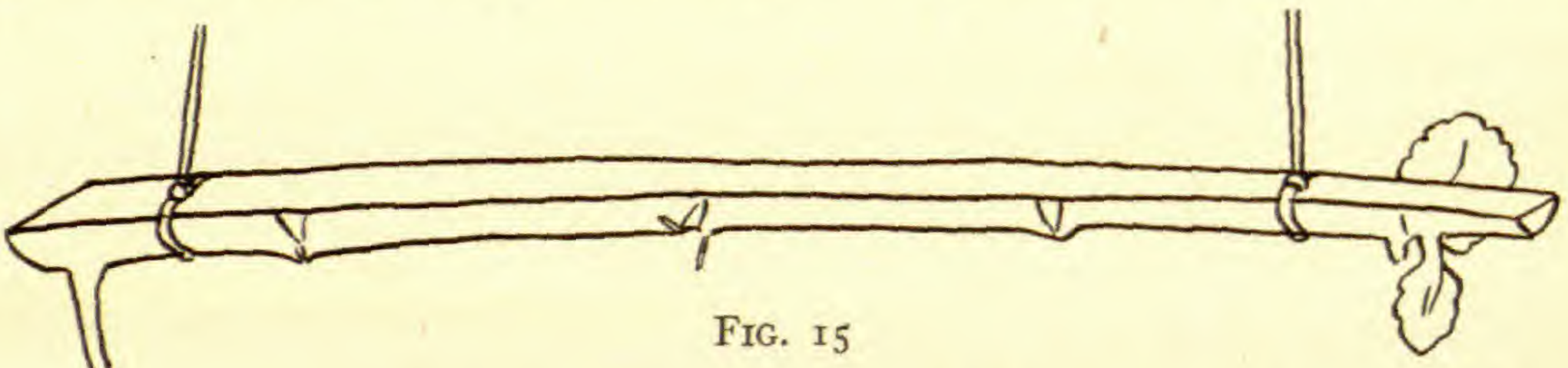

FIG. 15

the geotropic bending of the stem of *Bryophyllum* is due, as we shall see, to the active growth of the cortex on the lower side, which is lacking in those halves in which the cut surface forms the lower side, as in figs. 18–20. All these drawings were made on the seventeenth day of the experiment. Fig. 21 gives an indication of how regularly the results described in figs. 14–17 occur.

It was of interest to study the reaction of split stems in which the leaf was above and the cortex below. For this purpose it was necessary to split the stem only to the apical or basal node in which the leaf was preserved (figs. 22, 23), but not in its entire length.

When the leaf is at the apex and above (fig. 22), geotropic curvature of the stem occurs, but not so rapidly as when the leaf is below;

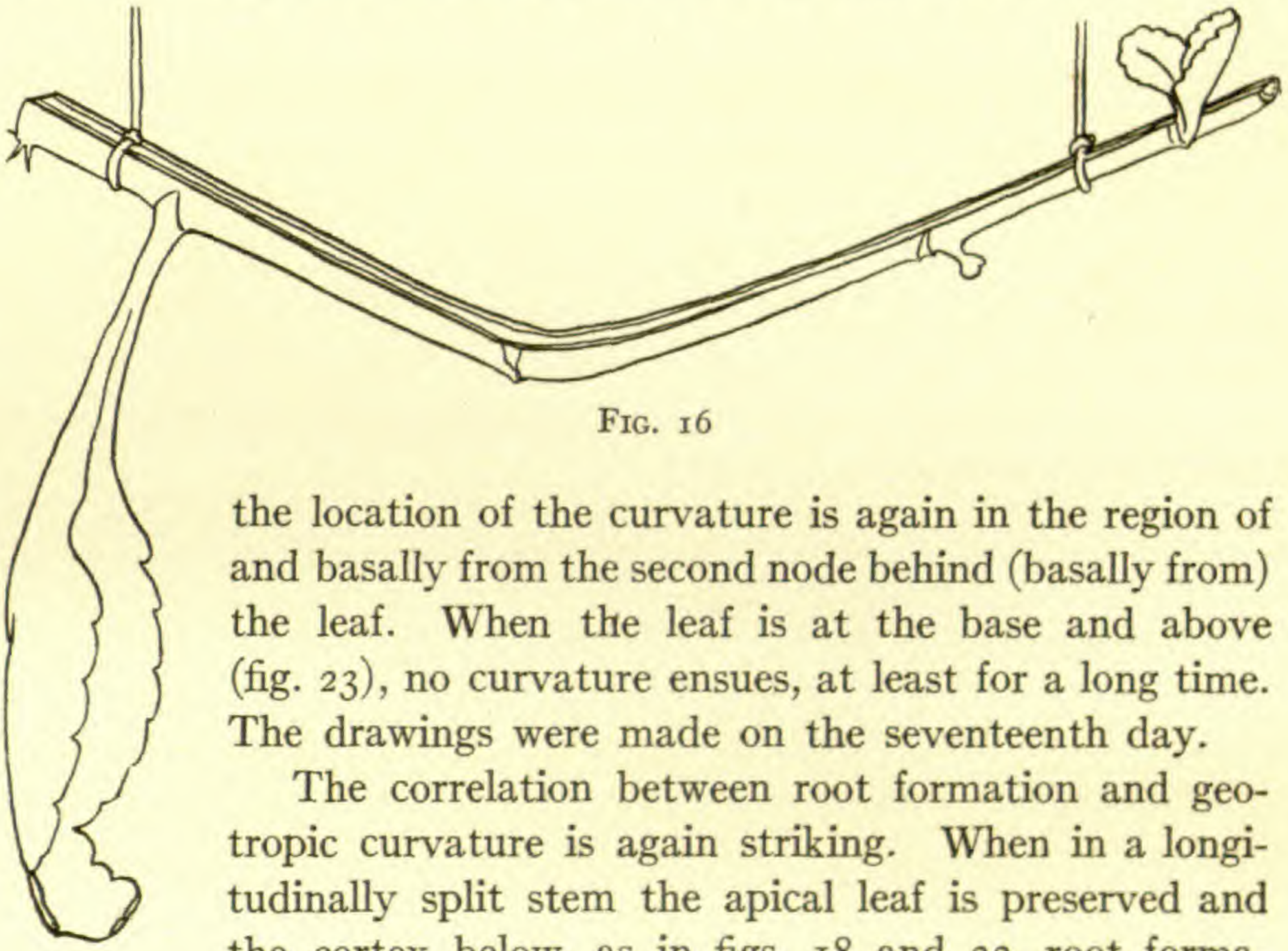

FIG. 16

the location of the curvature is again in the region of and basally from the second node behind (basally from) the leaf. When the leaf is at the base and above (fig. 23), no curvature ensues, at least for a long time. The drawings were made on the seventeenth day.

The correlation between root formation and geotropic curvature is again striking. When in a longitudinally split stem the apical leaf is preserved and the cortex below, as in figs. 18 and 22, root formation occurs in the second node behind the leaf, in the region

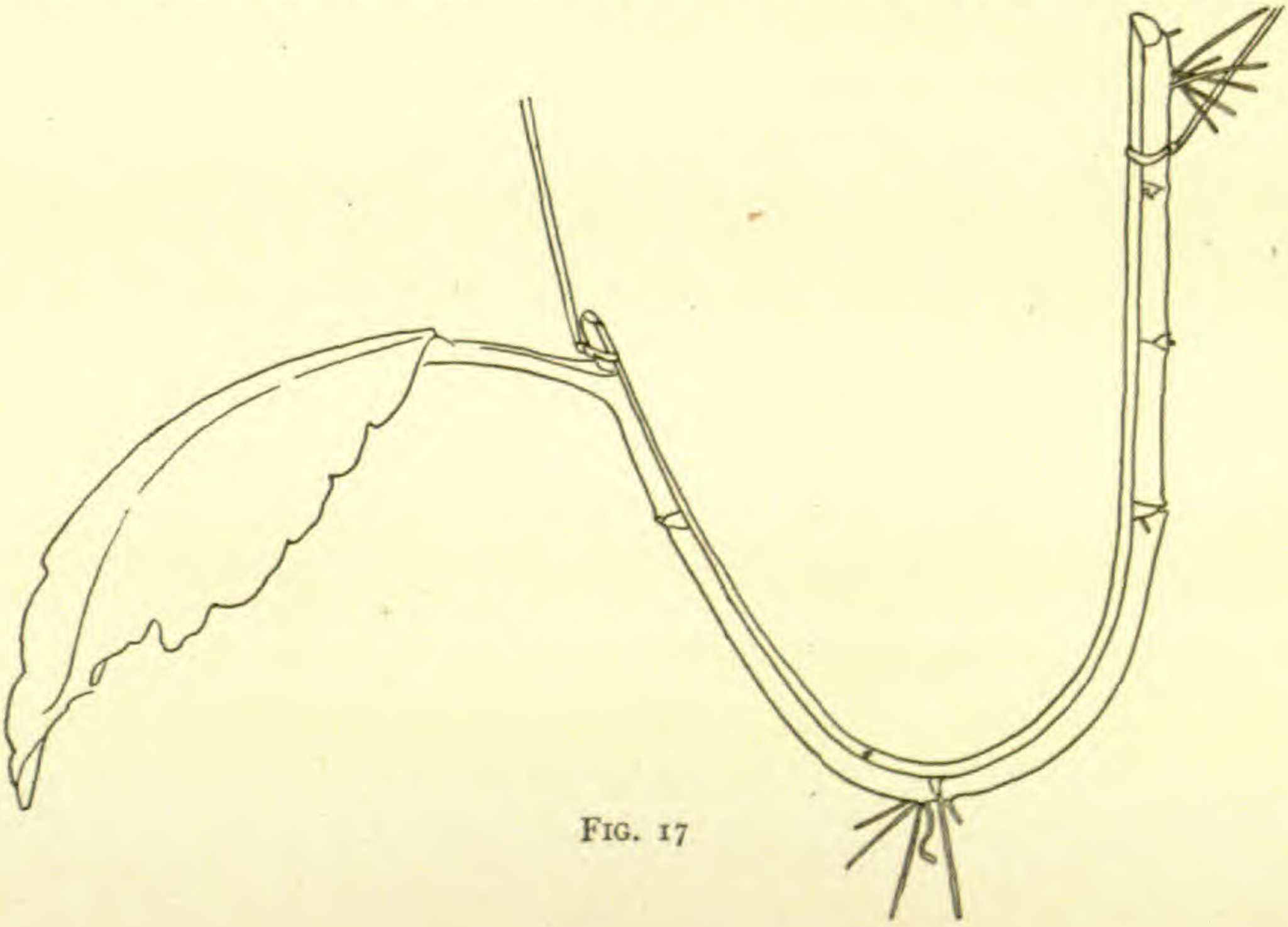

FIG. 17

where the curvature also occurs. Roots appear also at the basal nodes and sometimes at the cut basal surface of these

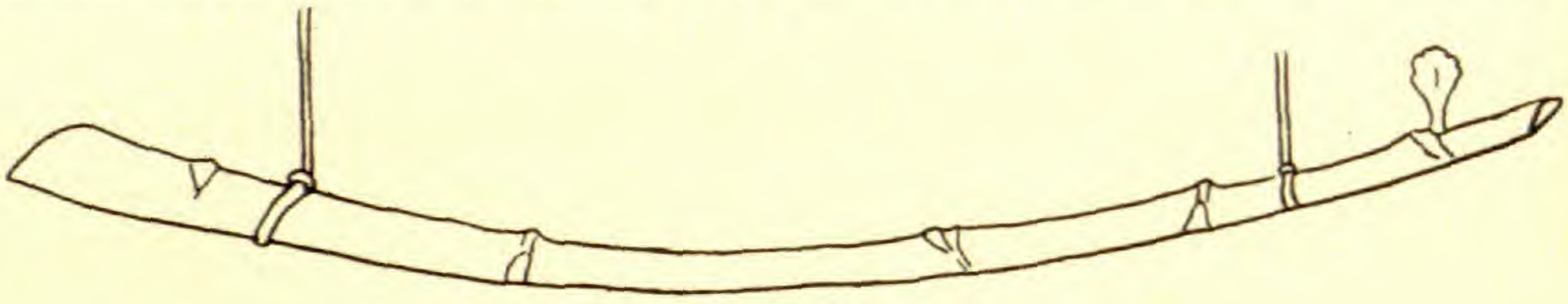

FIG. 18

stems. When the basal leaf is preserved and this leaf is below (fig. 15), no root formation takes place generally, or not for a long

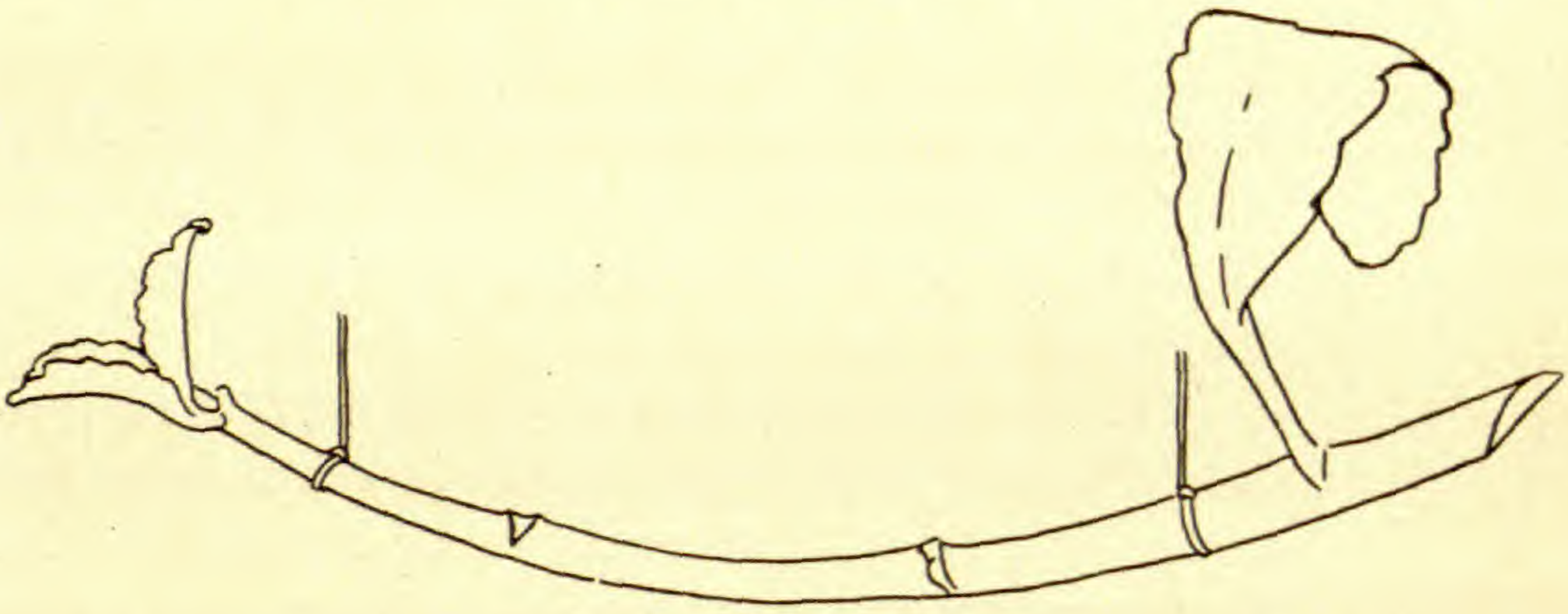

FIG. 19

time at least; while when the leaf is above (fig. 23), such root formation takes place in the basal node on the under side of the stem opposite the leaf. When the cortex is above and the leaf at the apex, root formation will occur, but chiefly or most rapidly in the basal node, and later in the next or the 2 nodes next to them (fig. 20). When the leaf is at the base or when the stem has no leaf, no root formation occurs in the cases where the cortex is above, at least for a long time.

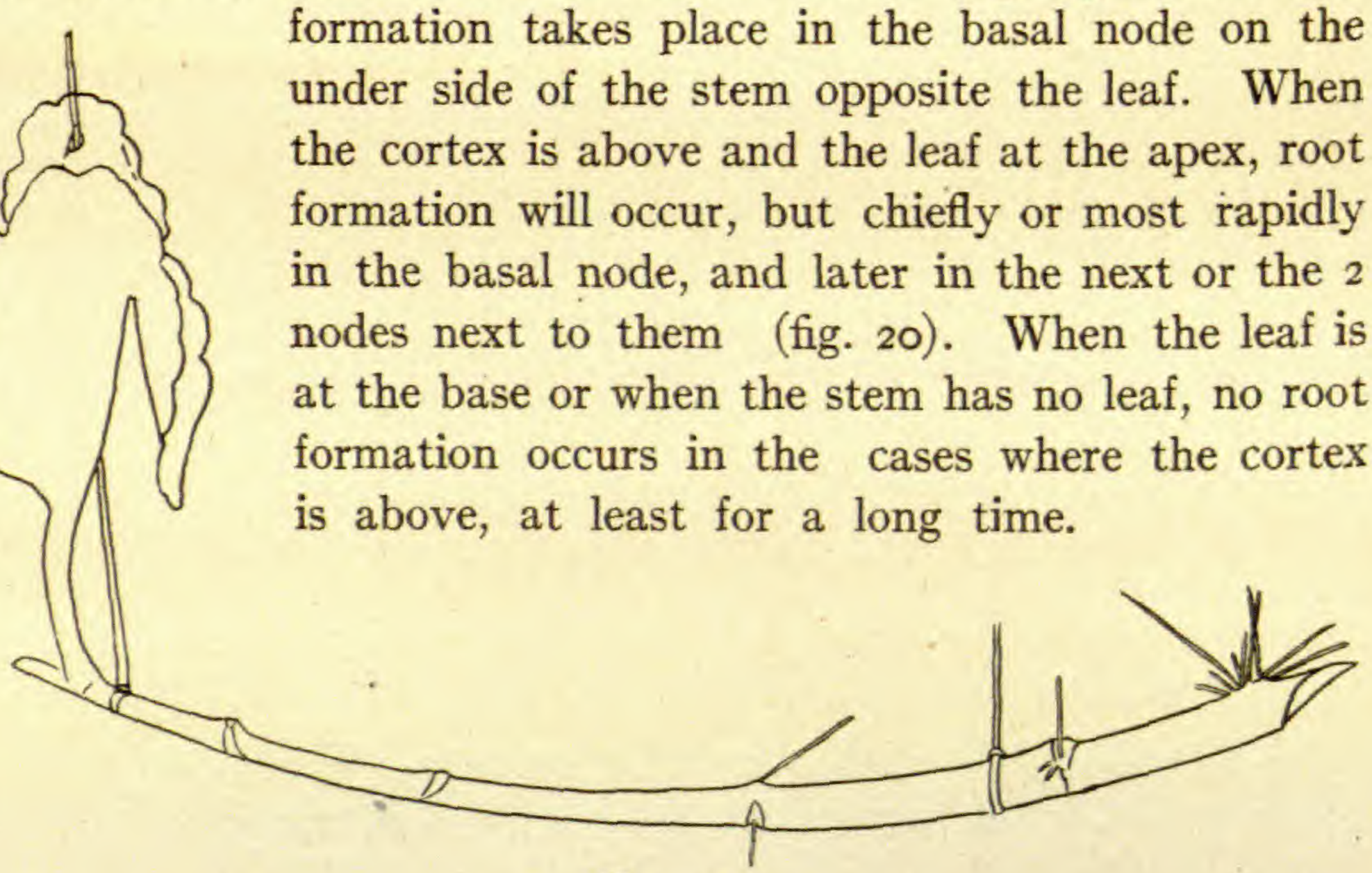

FIG. 20

IV. Mechanism of geotropic curvature in Bryophyllum calycinum

These experiments with split stems were used to obtain a more definite idea concerning the mechanism of the geotropic bending.

Immediately after the stems were split, marks were made with India ink on the cortex at a distance of 1 cm. from each other and then the stems were suspended horizontally, one-half of the split stems having their cortex below, the others having their cortex

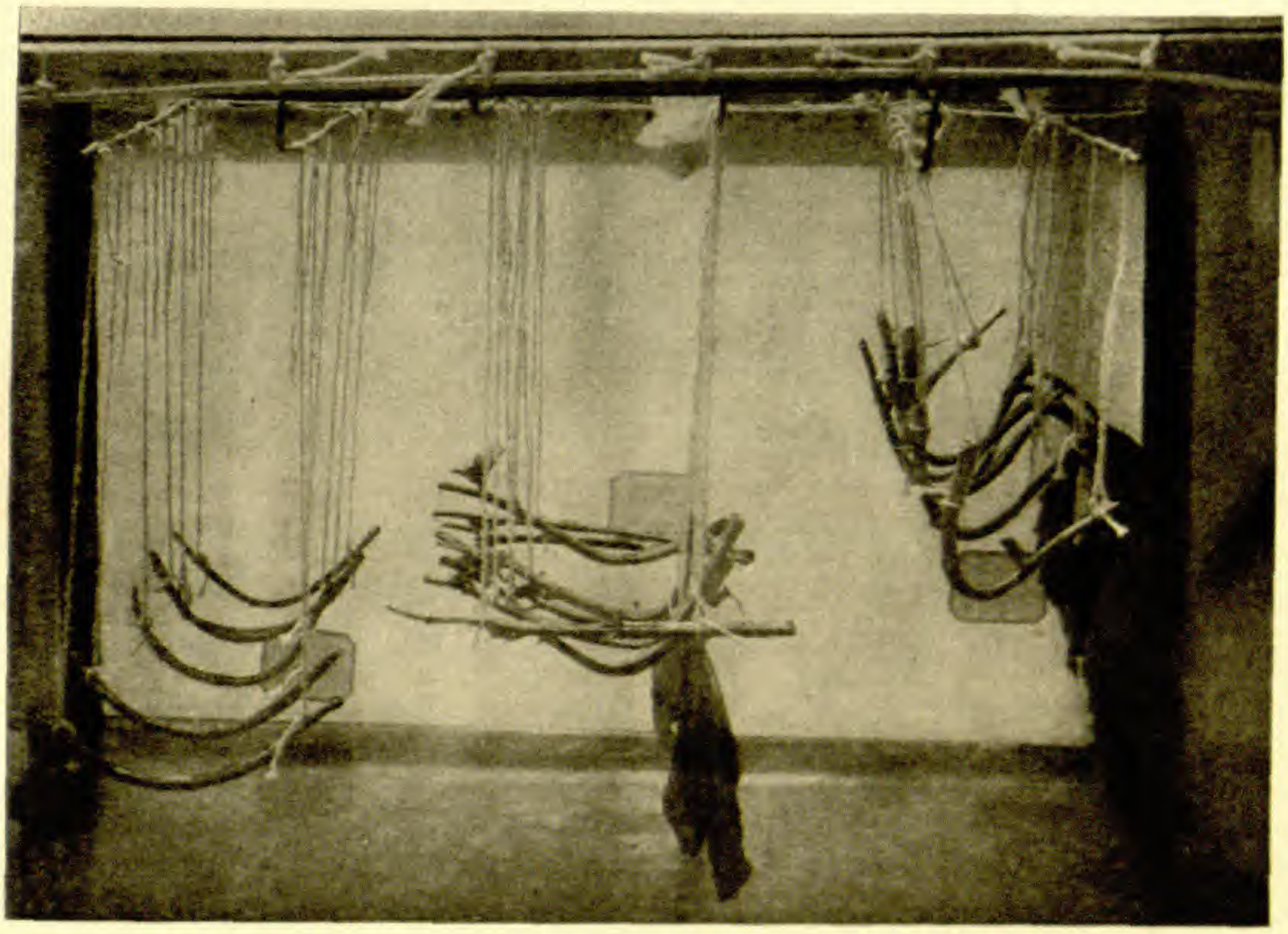

Fig. 21

above. Stems with an apical leaf were used for the purpose (like those in figs. 17, 20). After 10 days, when the halves with the cortex below had bent strongly, the displacement of the marks was ascertained. It was found that the marks on the halves in which the cortex was above and which had not bent were practically unchanged. The same was true of the marks in the non-bent regions of the other halves, where the cortex was below; while a growth of 15–20 per cent of the original length had taken place in the bent convex region of those stems having their cortex below.

Stems split lengthwise and with a leaf left at the most apical node were put horizontally into a jar saturated with water vapor. One-half of the stems were put with the cortex above (fig. 20), and one-half with the cortex below (fig. 17). Only the latter bent geotropically, the others showing only a slight concavity on the upper side, which may have been partly of a geotropic character, but which more likely was for the greater part, if not entirely, due

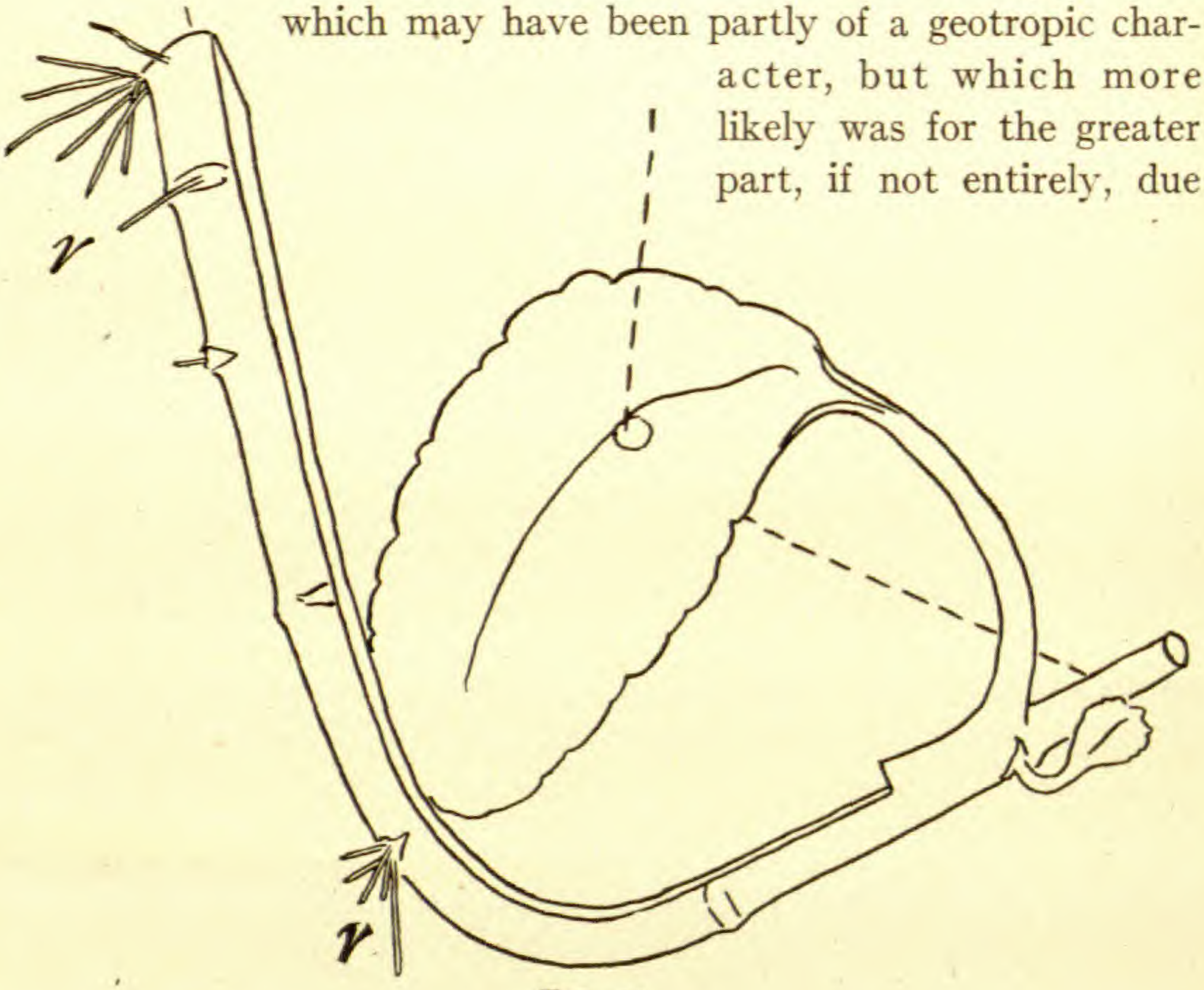

FIG. 22

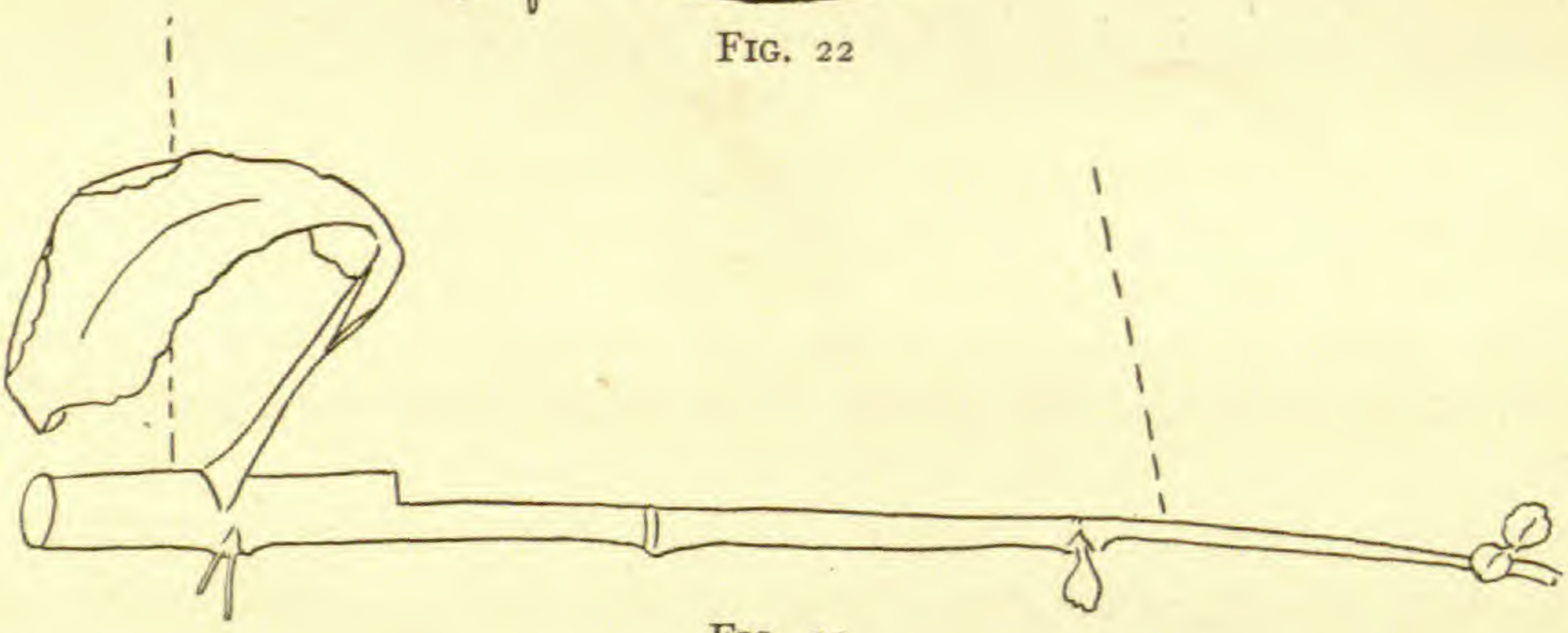

FIG. 23

to the tension of the cortex, which has a tendency to shorten in the longitudinal direction.

The experiment was begun June 21 and the measurements were taken on July 1. The original length of each piece of stem before the experiment was known from the India ink marks, the *final* length could be ascertained by direct measurement. First it was found that the length of the split stems which had been suspended with their cortex above was not altered, as shown in table I.

TABLE I

LENGTH OF SPLIT STEMS PLACED HORIZONTALLY WITH CORTEX ABOVE (IN CM.)

At beginning of experiment (June 20)	At end of experiment (July 1)
9.0	9.0
11.0	10.8
10.0	16.0
14.0	13.8

Obviously no growth had taken place in these halves; there may possibly have been a slight shortening, but if this was the case it was so small that it was within the limits of error of measurement.

An altogether different condition was found in the other halves of the stems which had been suspended horizontally with their cortex below. Here an increase in length was found in the bent part of the stem, while the apical and basal ends which had not bent were practically unaltered also in regard to length. We designate the apical unbent region *A*, the central bent region of the stem *B*, and the unbent basal region *C*. The measurements of 4 stems are given in table II (p. 40).

It is obvious that an increase in length of 15–20 per cent took place in 10 days in the bent central region of the stem (basally from or around the second node behind the apical leaf), while the unbent basal and apical regions showed no distinct alteration of length.

Fig. 24 is a photograph of marked *whole* stems 9 days after the beginning of the experiment. The stems had been suspended horizontally in the jar; all had one apical leaf left. That part of the cortex which was below had stretched, while the cortex above was shortened. The India ink marks were 1 cm. distant and were made at the beginning of the experiment. The photograph shows the change in the position of the marks on the convex and concave sides in the bent region of the stem.

It is highly probable, if not certain, that the increase in length on the lower side of the horizontally placed stem takes place primarily in the cortex of the bending region and not in the pith or wood. This follows from the behavior of these 2 parts when the cortex of a bent (split or whole) stem is removed, and the rigidity of the cortex is compared with that of the pith and wood taken out.

TABLE II

Region of stem measured	Stem I		Stem II		Stem III		Stem IV	
	Beginning of experiment	End of experiment	Beginning of experiment	End of experiment	Beginning of experiment	End of experiment	Beginning of experiment	End of experiment
A: non-bent apical part.....	3.0 cm.	3.2 cm.	3.0 cm.	3.0 cm.	4.0 cm.	4.0 cm.	4.0 cm.	4.1 cm.
B: bent central part.....	*4.0*	*4.9*	*5.0*	*5.7*	*6.0*	*7.0*	*4.0*	*4.85*
C: non-bent basal part....	*2.0*	*2.0*	*3.0*	*3.0*	*5.0*	*5.0*	*4.0*	*4.15*

If we remove the cortex on the lower (convex) side of a split geotropically bent stem, like that in fig. 17, we find that the rigidity of the cortex in the bent region is much greater than that of the wood or pith; the latter appears soft in comparison with the cortex of the bent region on the convex side of a geotropically bent stem. It is possible also that the increase in the rigidity of the cortex in this region may be due to a thickening of the cortex, a point which needs further investigation. Whatever the cause of this increase in rigidity may be, we reach the following conclusion regarding the mechanism of the geotropic bending of a horizontally suspended stem of *Bryophyllum calycinum*.

On the lower side of such a stem in a region the location of which depends upon the presence or absence, and, in the former case, upon the location of the leaf in the stem, the cortex begins to grow in length (and possibly in thickness). The wood, pith, and cortex on the upper side undergo no such growth. This increase in length (in one region) of the cortex on the lower side leads to a bending of the stem in which the lower side of a horizontally suspended stem becomes convex, the upper side concave.

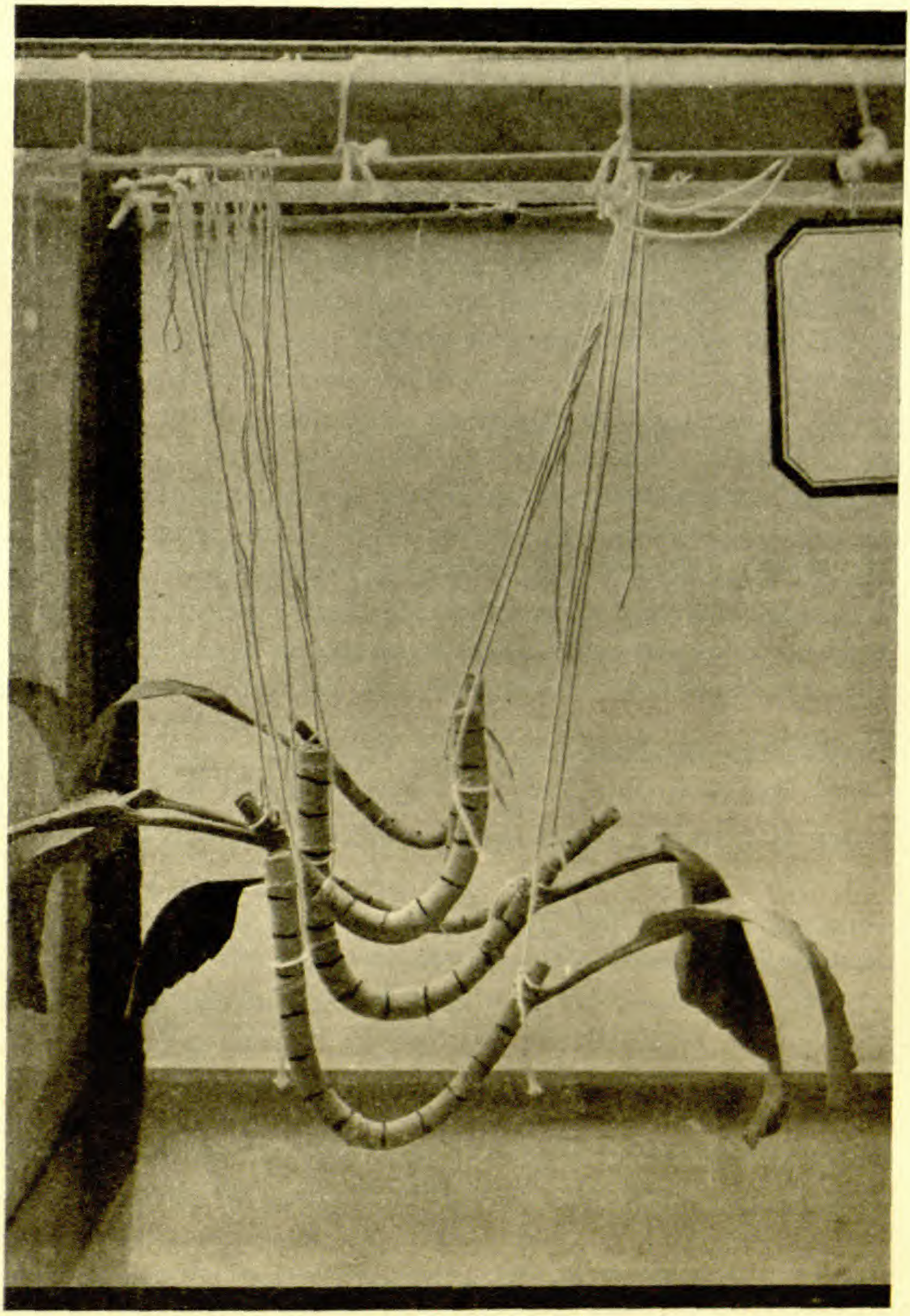

FIG. 24

Our investigation shows that this growing region of the cortex coincides with the region where early roots are formed. This suggests the possibility *that the geotropic growth of the cortex on the lower side of a horizontally suspended stem is due to a cause which is either closely associated or identical with the cause of root formation.*

If we assume with SACHS that there are specific root-forming substances, then the question presents itself whether we are not forced also to ascribe the geotropic curvature to the existence of specific geotropic substances or hormones; both substances having the tendency to collect on the lower side of a horizontally suspended stem; and both substances stimulating growth, the one of roots, the other of the cortex. On the basis of such an assumption we might understand why no or only an insignificant geotropic curvature takes place in a split stem when the cortex is on the upper side, the reason being that the geotropic substances settling at the lower side find no cortex which can grow and cause geotropic bending. This assumption will of course be a mere hypothesis until the existence of such hormones can be demonstrated directly.

V. FURTHER EXPERIMENTS ON THE INFLUENCE OF THE POSITION OF THE LEAF UPON THE GEOTROPIC BENDING OF A STEM

When we remove the cortex on the upper or lower side of a horizontally suspended stem of *Bryophyllum calycinum* (without removing the wood and pith), an extensive bending of the stem takes place instantly (fig. 25), the side on which the cortex is removed becoming convex. The mechanism of this phenomenon becomes clear on the assumption that the cortex is under a tension longitudinally which shortens the wood and pith. If this tension is removed on one side of the stem, the wood and pith on that side can stretch, while the wood and pith on the opposite side are held in check by the cortex. This leads to a considerable curvature whereby the side on which the cortex is preserved becomes concave (fig. 25). This curvature due to cortex tension is much stronger than the curvature which takes place instantly when we split a stem longitudinally. In this case not only the cortex but also the wood and pith are removed on one side of the stem, and hence the tendency of this side to stretch is considerably less than if only the cor-

tex is removed on one side. In the former case the stretching force of wood and pith on the side where the cortex is removed is lacking. Such stems show in a striking way the influence of the position of the leaf upon the geotropic curvature.

More than a dozen stems whose cortex was removed on the upper side were suspended horizontally (figs. 26, 27). Each stem had one leaf left, one-half of the stems having the leaf at the base (fig. 26), the other half having it at the apex (fig. 27). Only the latter bent geotropically, while the stems with the leaf at the basal end, not being able to overcome the resistance of the upper side of the pith and wood, did not undergo any geotropic bending. This shows that the geotropic growth of the cortex must be considerably less when the leaf is at the base than when it is at the apex.

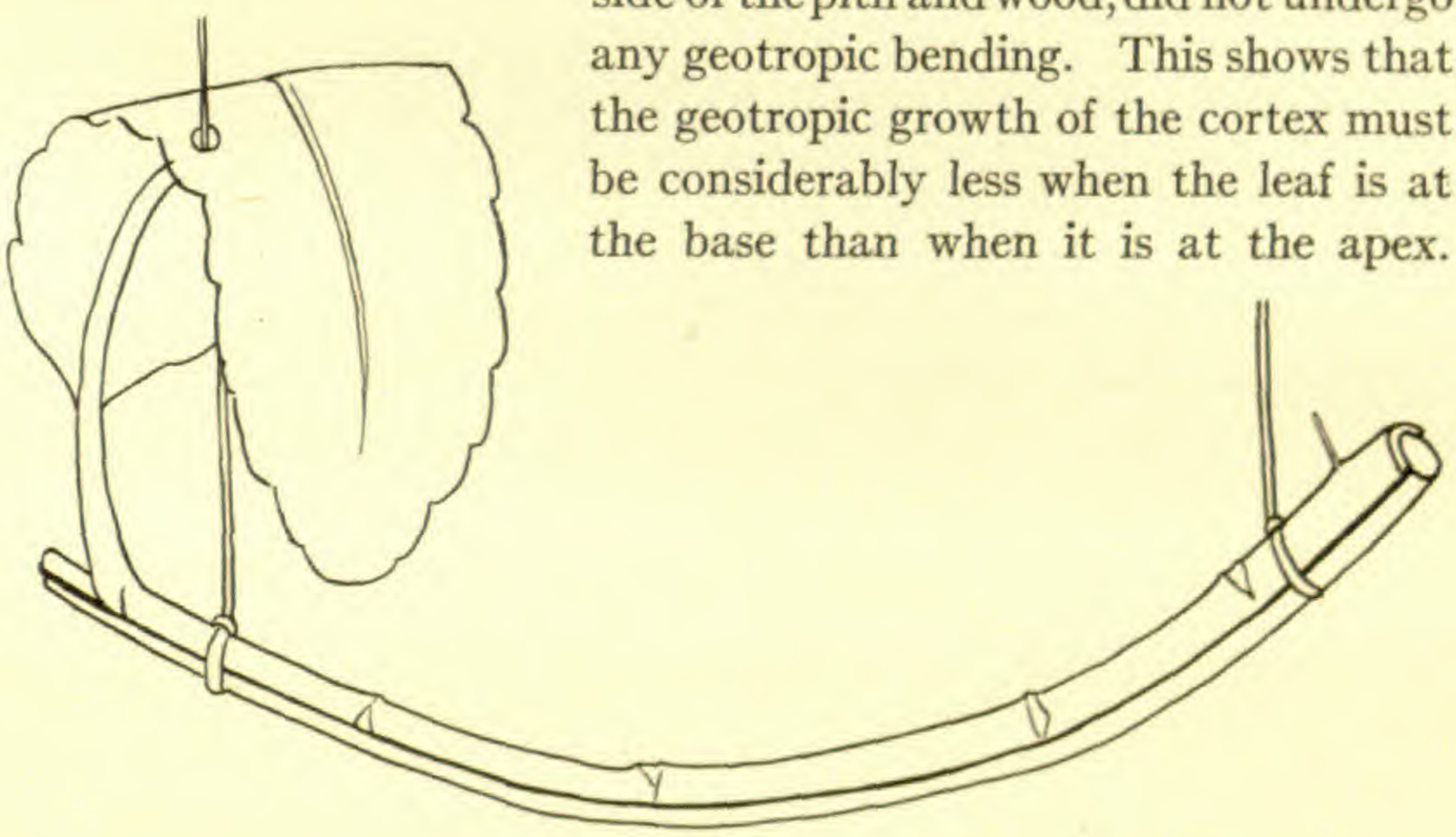

FIG. 25

When the cortex was above (fig. 25) and the leaf at the apex (or at the base), no or very little geotropic curvature ensued beyond the curvature due to the effect of the removal of the cortex on one side of the stem, which takes place instantly after the operation.

When the leaf is left at the apical end and the cortex below, as in fig. 27, the curvature occurs again in the region of the second node behind (basally from) the leaf; and in that node on the lower side the first roots develop (fig. 27). These drawings were made 9 days after the beginning of the experiment. If all the leaves are removed on such a stem it is no longer able to bend geotropically.

VI. FURTHER VARIATION OF THESE EXPERIMENTS

It is well known that the so-called geotropic "stimulus" goes around a corner, that is, around an incision. If we assume that the so-called "stimulus" is the flow of a liquid, we need not be surprised that it is able to go around a corner or around an incision in a stem. In a former paper (see footnote 1) we have shown that the "inhibition" of the stem upon the growth of the notches of a

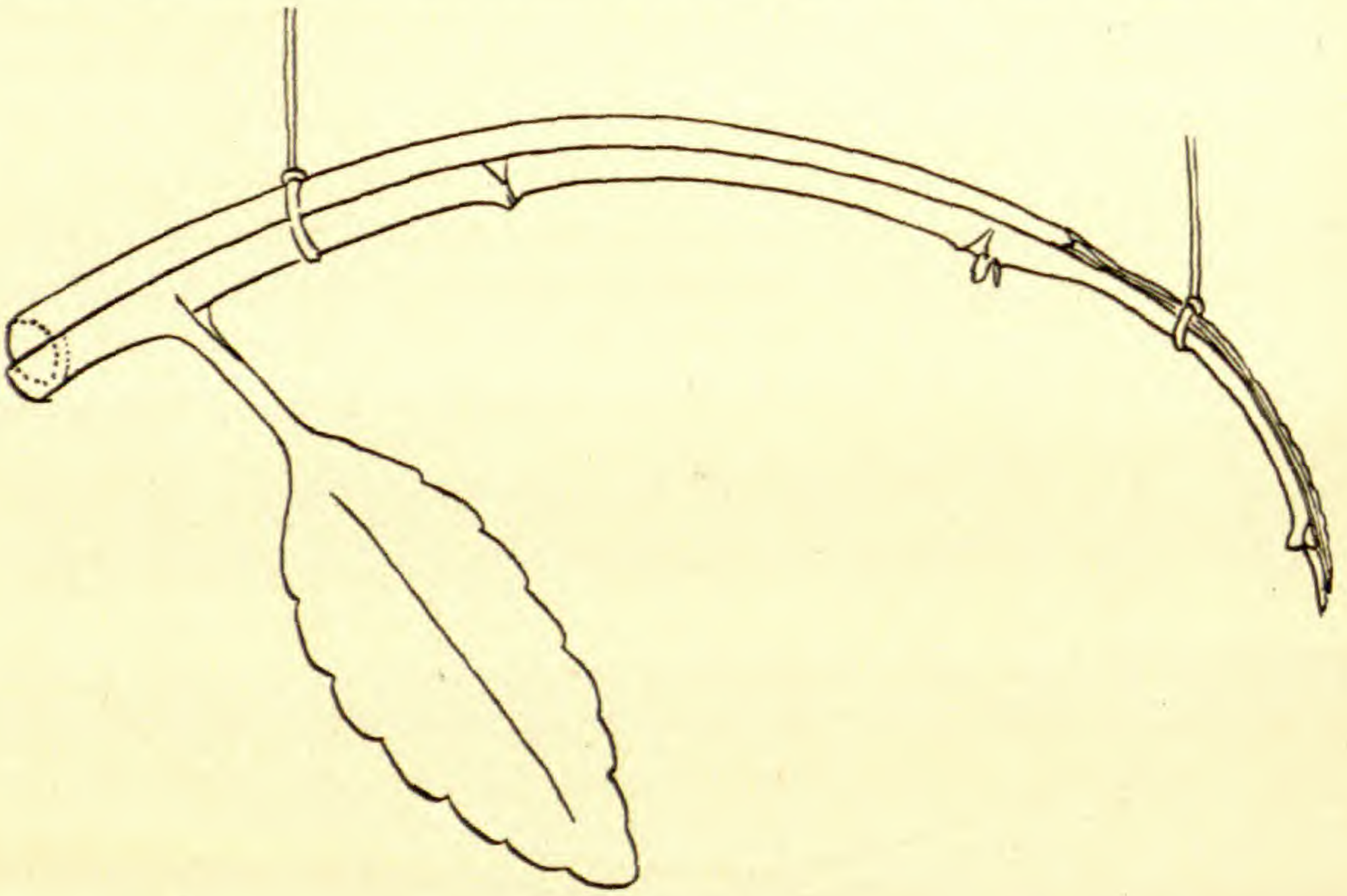

FIG. 26

leaf also goes around a corner in a leaf when incisions are made into such a leaf; and the mysterious character of the phenomenon disappeared with the recognition of the fact that the "inhibition" is the flow of certain substances (water or solutes) from the leaf into the stem through a system of interlinked channels which allows a flow in a zigzag around incisions. The same conception will explain in our opinion why a geotropic "stimulus" will flow around an incision in a stem, the "stimulus" like the "inhibition" being the flow of certain substances through the leaf or stem respectively.

Incisions were made into each internode of stems of *Bryophyllum calycinum*, at *a*, *b*, *c*, and *d* in figs. 28 and 29. The stems were suspended horizontally in a jar saturated with water vapor. Six

stems had one leaf at the apex and below (fig. 28), the other 6 had 2 leaves at the basal node (fig. 29). All of the stems with the leaf at the apex bent geotropically (though not as rapidly as stems without incisions), while those with the leaves at the base remained unbent. Figs. 28 and 29 show the difference after 10 days. In

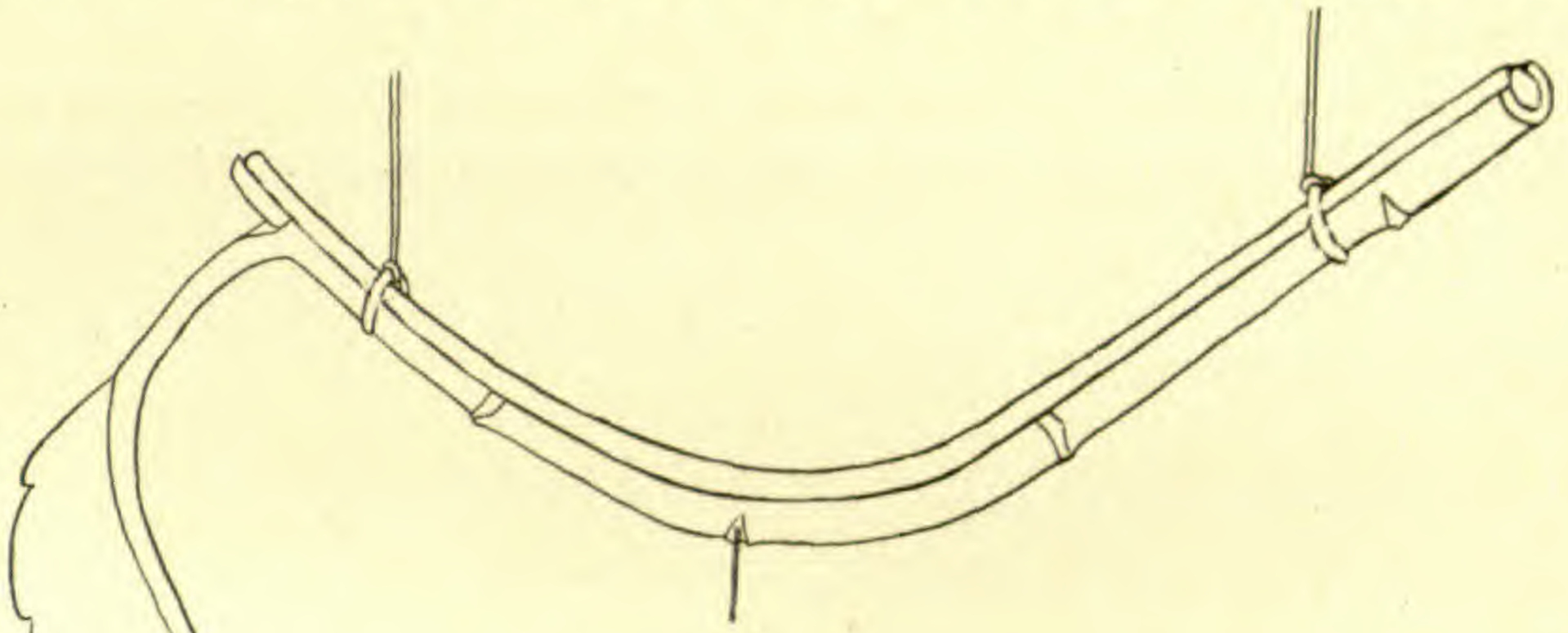

FIG. 27

time the bending of the stems with the leaf at the apex proceeded and the stems assumed the typical U-shape. The stems with the leaves at the base remained unbent. The stems with the leaf at the apex also formed roots (on the lower side of the stem); the stems with leaves at the base formed no roots or did not form them until much later.

VII. FORMATION OF ROOTS IN PASSIVELY BENT STEMS

We have seen that in stems suspended horizontally the roots have a tendency to form on the under side in the same region where the bending occurs. They form also at the basal nodes, both the upper and lower, but this fact does not concern us in this connection. The tendency of the roots to form on the lower side in that region which becomes convex might suggest the possibility that the root formation occurs in the convex region, not because it is the lower side, but because the convexity in itself might in some way favor root formation. The following experiment shows that the roots form on the lower side of a stem regardless of whether this lower side is concave or convex.

Stems were bent passively and fixed in this bent position by tying their ends to a piece of wood (fig. 30). Such pieces were then suspended in a jar saturated with water vapor. The experiments were made a year ago, before the writer was aware of the influence of the position of the leaf upon geotropic curvature and root formation, and in the expectation that passive bending of a stem would lead to the production of roots on the convex side of the stem. This was found not to be the case. Fig. 30 shows that numerous

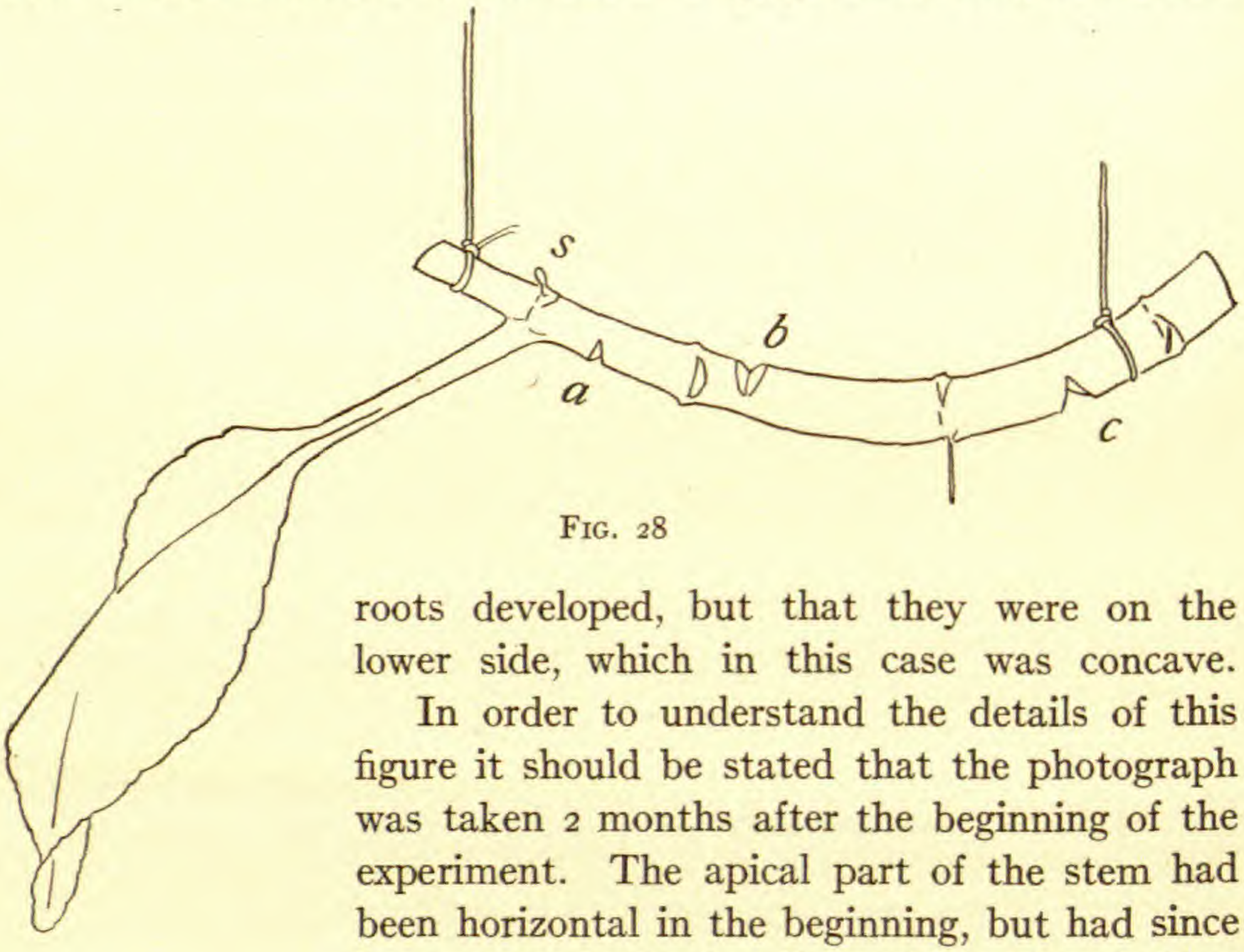

FIG. 28

roots developed, but that they were on the lower side, which in this case was concave.

In order to understand the details of this figure it should be stated that the photograph was taken 2 months after the beginning of the experiment. The apical part of the stem had been horizontal in the beginning, but had since bent upward, the bending taking place behind the second node. The side on which the roots were formed, therefore, had originally been the under side. The roots formed all along the lower side of that part of the stem which at first was in a horizontal position. Besides the small apical leaves, a large older leaf had been left on the stem, and from this leaf the stem was suspended. At the base of this large leaf and of the next 2 nodes a strong root formation took place. This is what we should expect on stems in which a leaf is left on the upper side.

Fig. 30 also illustrates in another way the influence of gravitation on root formation. The reader will notice that in the

horizontal region of the stem (basally from the large leaf from which the specimen was suspended) 2 large roots grew out from the internode. This occurs only after a long time and only on the lower side of a stem.

The experiment demonstrates, therefore, that roots will form on the concave side of a passively bent stem of *Bryophyllum calycinum* if this side is the under side of such a stem.

VIII. Theoretical remarks and summary

1. The theoretical remarks may be brief. We believe that these experiments show first that in *Bryophyllum calycinum* the substances which induce root formation have a tendency to collect on the lower side of a horizontally placed stem, although roots may appear also in nodes on the upper side (especially at the basal nodes), under special conditions which will be discussed in another paper. It is shown in this paper that a horizontally suspended stem of *Bryophyllum* will become concave on the upper side, and that this curvature, which will give such a piece a U-shape, is due to a longitudinal growth of the cortex on the under side of the horizontally suspended stem.

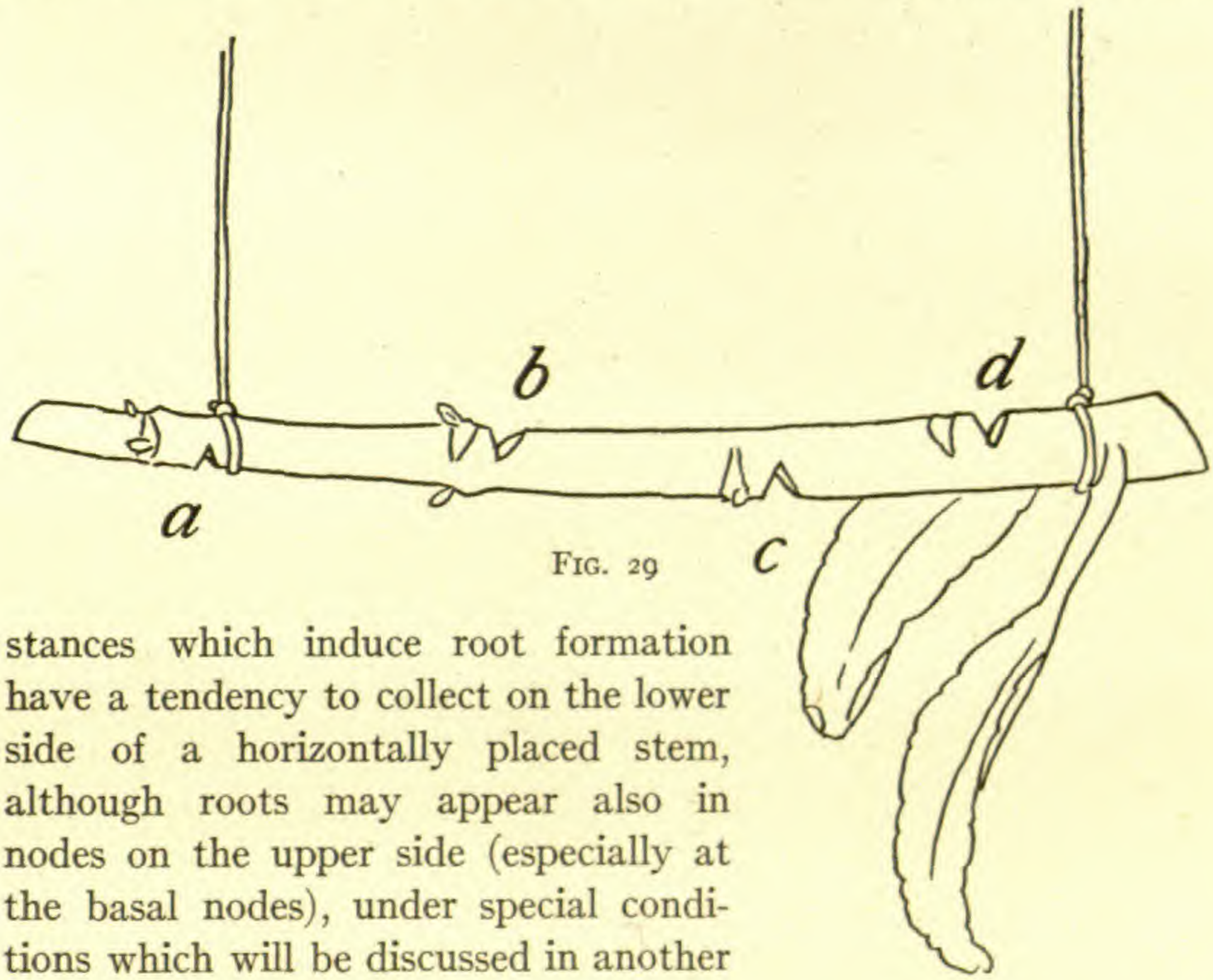

Fig. 29

2. We have seen that a leafless stem bends much more slowly than a stem in which one or more leaves are preserved; and we find

also that the roots form more slowly in a leafless stem than in a stem with leaves. We find also that in a general way the

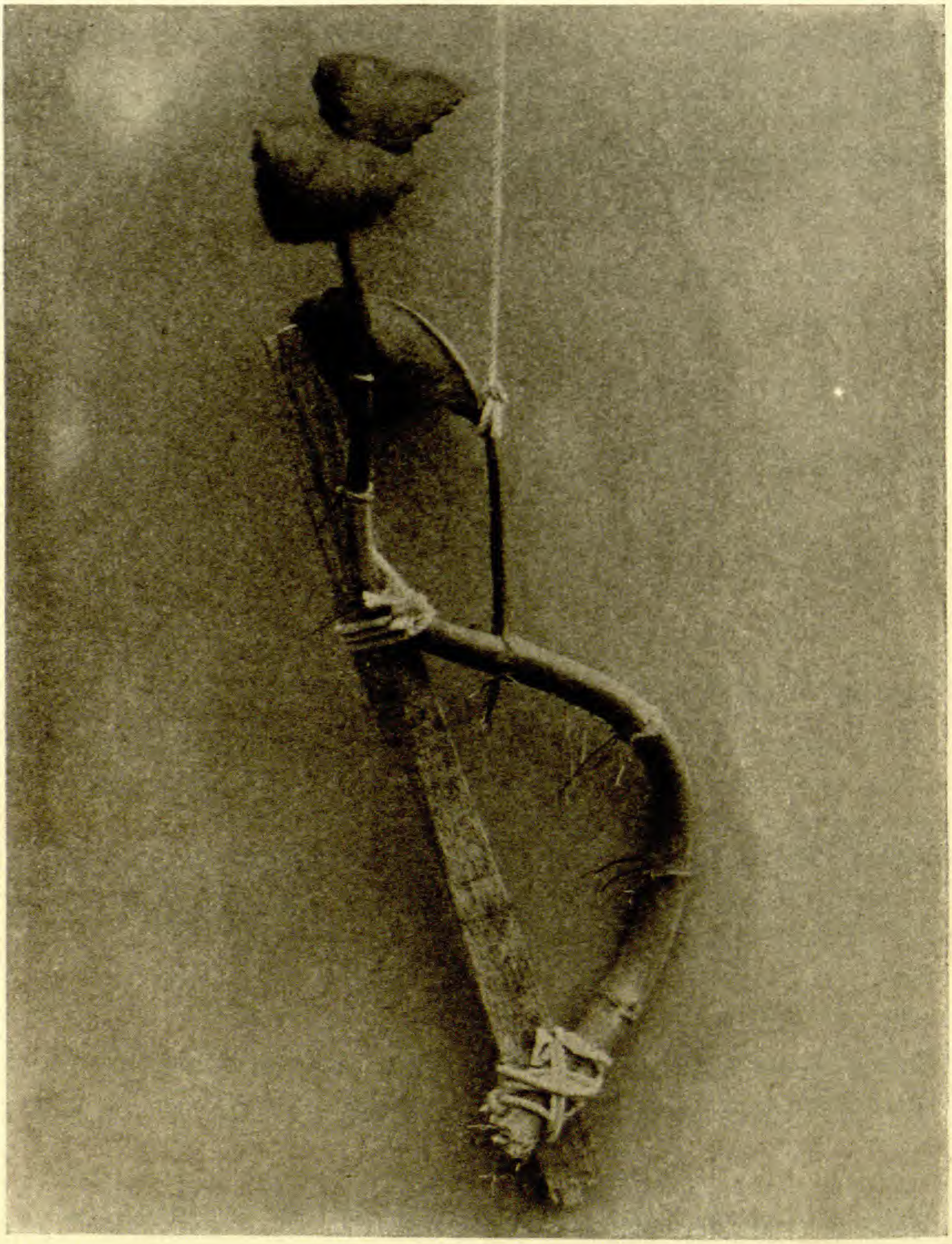

FIG. 30

amount of curvature and the amount of root formation vary in the same sense.

3. Both phenomena of geotropic curvature as well as of root formation depend in a striking way upon the position of the leaf on the stem. If in a horizontally suspended stem of *Bryophyllum* (in which the growing point is cut off) one leaf is preserved at the apex, and on the lower side of the stem, a rapid and very extensive geotropic curvature of the stem will take place, which is localized in the region basally from or around the second node from the leaf. The curvature is so extensive that the stem will assume the shape of a U with the concave side above. In such stems an extensive and rapid root formation will take place first in the second and fourth nodes behind the leaf on the lower side and also in the most basal nodes. The second and fourth nodes behind the leaf are therefore the centers of both kinds of growth in such a stem, namely, of the cortical growth which leads to the geotropic curvature and of the growth of roots. It should be pointed out also that in *Byrophyllum* the axes of successive nodes are always at right angles to each other, so that the favored nodes, the second and fourth behind the most apical one, all have the same orientation. It is quite possible that this structural peculiarity accounts for the fact that both root formation and geotropic curvature center around the second and fourth nodes behind a leaf left in the apex of an otherwise leafless stem.

4. If in a horizontally suspended stem only one leaf is left at the base of the stem (and on the lower side) the curvature is usually considerably less than in a stem with a leaf in the apex. The curvature in a stem with a basal leaf is confined to the region behind or around the leaf. It harmonizes with our previous statements that in such stems little or no root formation takes place, and that the root formation which occurs is confined to the node opposite the basal leaf and to the basal cut surface. When the piece of internode left behind the basal leaf is long, a more extensive curvature may occur than when the piece of internode left is short.

5. This difference in the influence of the apical and basal leaf can be made more striking when either the flow of substances in the stem is retarded (for example, by incisions in the stem) or when the resistance to the bending is made greater (by removing the cortex on the upper side of a horizontally placed stem whereby the

latter becomes concave on the lower side). In such cases geotropic curvature becomes possible only in stems with a leaf at the apex, but not in stems with a leaf at the base.

6. All these experiments become intelligible on the assumption that each leaf has a tendency to send shoot-forming substances toward the apex and root-forming substances toward the base of the stem. If it could be proved that in *Bryophyllum calycinum* a specific substance (hormone) is responsible for the geotropic growth (in the cortex of the lower side of a horizontally suspended stem), we might say that both substances show a tendency to collect on the lower side of a horizontally placed stem, and that the flow of both is influenced in the same way by the leaf. The apical leaf sends both substances toward the base of a stem, while the basal leaf acts as if it had a suction effect upon geotropic substances contained in the apical region. Such an idea suggests itself from the fact that a leafless stem has the center of its geotropic curvature in the middle (fig. 15), while a stem with a leaf at the base has either no curvature (fig. 16) or has it only in the region of the leaf.

While in *Bryophyllum* the hypothetical geotropic hormone is associated (or identical) with the root-forming hormone, in other plants the hypothetical geotropic substance might be associated with the shoot-forming hormone. This would explain the fact that in certain fir trees a horizontal branch next to the apex may suddenly become negatively geotropic when the apex is cut off. After the decapitation the (hypothetical) geotropic substance which before was flowing to the apex now can flow into the horizontal branches next to the apex, and the one which by chance gets a little more of the substance than the others will be the first to become vertical. After this the mechanical advantage due to the vertical position will favor the continued flow of these substances in this branch, which thus becomes the new apex.

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NEW YORK CITY

PROTHALLIA AND SPORELINGS OF THREE NEW ZEALAND SPECIES OF LYCOPODIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 222

CHARLES J. CHAMBERLAIN

(WITH PLATES II–III)

Few botanists have even seen the prothallia of *Lycopodium*, and most of those who have had such a privilege are indebted to one man, BRUCHMANN of Gotha; fewer still have made any investigation of the subject. The reason prothallia and sporelings of *Lycopodium* have not been so extensively studied as those of the ferns is not lack of interest, but difficulty in germinating the spores or finding prothallia growing naturally. In 1911 I collected adult plants of several New Zealand species of *Lycopodium* and made some effort to find prothallia, but my time was too limited for such slow, uncertain work. However, Professor A. P. W. THOMAS, at that time botanist of the University at Auckland, very kindly gave me prothallia and sporelings of 3 characteristic New Zealand species, *Lycopodium laterale*, *L. volubile*, and *L. scariosum*. Prothallia and sporelings are known in so few species that it seems worth while to give some account of this material.

Since the literature of the subject was examined with considerable care, all of the original papers cited being available, and since E. A. SPESSARD, a student of mine, is announcing in this issue of the BOTANICAL GAZETTE the first discovery of the prothallia and sporelings of several American species of *Lycopodium*, a historical résumé may be of service to those who are in favorable localities and who might wish to study the subject.

Historical

TREUB (7) introduced his classic account of the prothallium of *Lycopodium cernuum* with the remark that the history of the subject could be given in a few words, since it was necessary to cite only 3 or 4 investigators. SPRING (2), HOFMEISTER (3), DEBARY (4), FANKHOUSER (5), and BECK (6) are mentioned; but neither SPRING

nor HOFMEISTER ever saw any prothallia. DEBARY, and later BECK, germinated the spores of *L. inundatum*, and FANKHOUSER found a few prothallia of *L. annotinum*.

TREUB overlooked the English surgeon, JOHN LINDSAY (1), who nearly 100 years before had germinated the spores of the very species with which TREUB was dealing. LINDSAY was also the first to raise ferns from the spores. Having noted very young ferns growing in the open in Jamaica, he sowed the spores ("farina") and watched their development. It was in connection with this work that he tried *L. cernuum*. His own account is interesting: "I have very lately sown that fine farina or dust contained in the anthers of a species of the genus *Bryum*, namely, *Bryum caespititium*, or one very like it, and also the farina of *Lycopodium cernuum*. There is a vegetable growth taking place where they were sown which I hope will prove to be their young plants." Later, in a letter to Sir JOSEPH BANKS in regard to *Bryum caespititium* and *Lycopodium cernuum*, he states that he had repeatedy sown them both, and in proper situation found that they grew very readily. There were no further figures or descriptions of either the *Bryum* or *Lycopodium*.

SPRING'S failure to germinate spores brought him to the curious conclusion that *Lycopodium*, and also *Psilotum* and *Tmesipteris*, consist exclusively of male plants, the females having been destroyed in some geological catastrophe. At this time it was generally believed that the spores, if they should germinate, would develop directly into the leafy plants. A few years later, HOFMEISTER opposed this view and predicted that the spores would give rise to prothallia bearing antheridia and archegonia, and that the leafy plant would arise from the fertilized egg; but all attempts to prove his theory by germinating the spores resulted in failure.

DEBARY succeeded in germinating the spores of *L. inundatum*, and in 9 days obtained prothallia consisting of 7 cells; but repeated attempts failed to produce more advanced stages, except that one prothallium was observed which had reached the 11-cell stage. The prothallia soon died.

In 1873, FANKHOUSER, in Switzerland, found prothallia and sporelings of *L. annotinum* growing naturally. This fortunate find,

together with the results of DeBary, who had described early stages of *L. inundatum* from cultures, made it possible to make a general outline of the development from the germination of the spore up to the adult prothallium with sporelings attached.

Beck sowed the spores of various species under various conditions, but *L. inundatum* was the only one to germinate and the prothallia did not get beyond the 10-cell stage. He asserted that after 2 years the spores of *L. clavatum* showed chlorophyll and looked as if they were about to germinate, but no cell division occurred.

Since Fankhouser's paper appeared, 3 men have made large contributions to our knowledge of these peculiar prothallia. In 1884, 1886, 1888, and 1889, Treub (**7, 9, 11, 12**) published a splendid series of researches upon the prothallia of Javanese species. In 1885, Bruchmann (**8**) began his patient and persistent researches upon the difficult temperate species with subterranean prothallia which had baffled all previous investigators; and in 1887, Goebel (**10**) found prothallia of *L. inundatum*, so that, with the stages secured by DeBary, he was able to give a connected account of this species.

Treub began his series with an investigation of *L. cernuum*. He germinated the spores of this familiar tropical species and some of the prothallia reached the early antheridium stage before they died. However, he found abundant material growing wild, and so had a complete series from the germination of the spore to the adult prothallium with embryos and older sporelings.

The prothallia are green and grow on the surface of the substratum, the largest reaching a height of 2 mm. When the spore germinates, a more or less spherical body is formed, about 8 or 10 cells in diameter. From the top of this body, which Treub called the "primary tubercle," an alga-like filament then develops, at first consisting of a single row of cells, but soon dividing in all planes, so that a stout cylindrical body is formed more than twice the diameter of the primary tubercle and 5 or 6 times as long. The tip of the cylindrical portion is profusely branched, and at the base of the branches the antheridia and archegonia are borne. The embryogeny is particularly interesting, since the fertilized egg does

not develop directly into a leafy plant, but produces a protocorm with protophylls, resembling a miniature *Phylloglossum*.

TREUB (9) next dealt with *L. Phlegmaria*, another familiar tropical species, epiphytic upon trees. The prothallia are found on the tree trunks just below the surface of the humus, but they have no chlorophyll, being entirely saprophytic and abundantly supplied with an endophytic fungus. The main body is tuberous, about 2 mm. in diameter, more or less spherical or somewhat elongated, and has several branches extending in various directions. The branches vary from 1 to 6 mm. in length and bear antheridia or archegonia or reproductive bodies which TREUB called "propagula." The antheridia and archegonia are usually at the tips of the branches, not at the base as in *L. cernuum*. The propagula are of two general types, one consisting of scores or even hundreds of cells forming a flask-shaped body with a slender stalk of one or two cells; the other is much smaller, more or less spherical in shape, and consists of only a few cells, usually not more than 2 or 3, with the outer walls very much thickened. In the first type the propagula break off at the stalk and grow directly into new prothallia, while in the second type there is a more or less prolonged resting period. The first type seems to correspond to the gemmae of liverworts and mosses, and the second type seems to correspond to the brown bulbils of mosses.

TREUB was not able to germinate the spores, and he believed that most of the prothallia found in nature come from propagula, prothallia from spores being comparatively rare.

The accounts of the development of antheridia, archegonia, and embryo are very complete, but the vascular anatomy of the sporeling is not described.

The prothallia of *L. salakense* are aerial, green, have no endophytic fungus, and are the only ones which TREUB succeeded in raising from the spore to the adult stage with antheridia and archegonia. In a few days the prothallia developed to the primary tubercle stage, then rested for several months, and finally resumed their growth and completed the life history. As in *L. cernuum*, a simple filament appears at the top of the primary tubercle and then forms a cylindrical body several cells in thickness, but other fila-

ments then develop and behave in the same manner, so that there are several branches. The antheridia and archegonia are formed at the tops of these branches, there being no leaflike organs as in *L. cernuum* and *L. inundatum*.

The prothallia of *L. carinatum*, *L. Hippuris*, and *L. nummulariforme* are all of the *L. Phlegmaria* type, those of *L. carinatum* bearing such a close resemblance that TREUB (**11**) warns prospective collectors against collecting in localities where both species occur, since it is impossible to distinguish either the prothallia or the embryos. The prothallia of *L. Hippuris* are similar, but are more vigorous and the branches are thicker. TREUB was not able to disentangle complete prothallia of *L. nummulariforme* from the substratum and so had to write his account from fragments. He did not find any endophytic fungus.

The final paper in TREUB'S (**12**) series dealt with the embryo of *L. cernuum*. The series of stages was very complete, from embryos consisting of a few cells, through the protocorm stages, and up to sporelings with a few leaves. After the embryo has developed a protocorm with protophylls resembling a small *Phylloglossum*, a definite growing point is organized which develops into a leafy axis and at the same time the first root appears. TREUB indicated the course of the vascular bundles, but did not make any further study of the anatomy. He regarded the protocorm as a recapitulation of a *Phylloglossum* stage in the ancestry of *Lycopodium*.

In 1884, the same year that TREUB (**7**) began his research upon tropical forms, BRUCHMANN (**8**) found 3 prothallia of *L. annotinum*, and thus began a series of researches which extended over 25 years and resulted in clearing up the life-histories of the much more difficult temperate species. BRUCHMANN'S first paper appeared in 1885, but TREUB'S first account, although dated 1884, appeared at about the same time, so that neither knew the other was working upon prothallia. BRUCHMANN'S (**13**) most extensive work, which gained him the prize of the Paris Academy of Science, appeared in 1898, and contained descriptions of *L. clavatum*, *L. annotinum*, *L. complanatum*, and *L. Selago*. All were found growing in the Thuringer forest near Gotha, but the germination of the spores and earliest stages in the development were lacking. The development

of antheridia, archegonia, and embryos are very clearly described. Ten years later this account was supplemented by a very complete description of *L. complanatum*. Although BRUCHMANN (**15**) had made repeated efforts to germinate the spores of various species, he met only the failures which had discouraged other botanists; but finally his perseverance was rewarded and he was able to give a complete account of the germination and early development of *L. clavatum*, *L. annotinum*, and *L. Selago*. The surprising feature is the long-delayed germination. The spores of *L. Selago* germinated in 3–5 years, and the development of antheridia and archegonia was complete in 6–8 years; *L. clavatum* and *L. annotinum* were even slower, germinating in 6–7 years and requiring 12–15 years to complete the development of antheridia and archegonia. BRUCHMANN suggests that possibly the periods might be shortened artificially if the proper stimuli could be discovered. All the species reported in BRUCHMANN'S various papers are subterranean and saprophytic, but the spores germinate independently and develop to the 4 or 5-cell stage, and at this stage the fungus must enter or there will be no further development.

L. salakense, which TREUB (**11**) succeeded in keeping in cultures throughout the whole life history, is green, aerial, has no fungus, and germination takes place in a few days. *L. cernuum* is also aerial and green and germinates with equal promptness, but it does not develop beyond the primary tubercle stages unless the fungus enters. *L. inundatum* in DEBARY'S cultures developed to an early primary tubercle stage with some chlorophyll and then died. The subsequent work of GOEBEL (**10**), who found prothallia growing naturally, proved that this species also has an aerial, green prothallium with an endophytic fungus. In *L. cernuum* and *L. inundatum*, however, the fungus infection is much slighter than in the saprophytic species.

So far as I have been able to determine, there is only one paper which makes any mention of the prothallia of New Zealand species of *Lycopodium*, and this paper by HOLLOWAY (**16**) deals primarily with the anatomy of the sporophyte. The investigation, both in the field and in the laboratory, is of such high grade that we hope HOLLOWAY will sometime give us an extended account of the pro-

thallium and the anatomy of the sporeling. The varied species, ranging from epiphytes to ground forms, with prothallia ranging from the green, leafy aerial type to the deepest subterranean tuberous type, make New Zealand an ideal place for such a study.

Material and observations

The epiphytic type is not represented in the species at my disposal. *Lycopodium laterale* has a stout creeping rhizome, with numerous erect branches, and cones borne laterally; *L. scariosum* has a somewhat similar habit, except that the cones are terminal; *L. volubile* is the most beautiful species of the genus, bearing a striking resemblance to *Selaginella* as it trails along the ground or over bushes; but, unlike *Selaginella*, it keeps well after being gathered and is much used for table decoration.

PROTHALLIA

L. LATERALE.—The only reference I have been able to find in regard to the prothallium of this species is in HOLLOWAY'S (**16**) paper. He says "in the case of *L. laterale* prothallial plants were found in two localities, growing on recently overturned marshy soil. The prothallus of this species corresponds to the type of *L. cernuum*, is small and short-lived, and is situated at the surface of the ground."

I had at my disposal 3 prothallia with protocorms attached and one older protocorm entirely free from the prothallium. In the first 3, each of the protocorms bore 2 fully grown protophylls; the older protocorm bore 10 protophylls. Two of the prothallia with their young plants are shown in figs. 1 and 2, the exact size being indicated in fig. 1*a*. The older protocorm with its 10 protophylls is shown in fig. 3. In fig. 1 the particles of sand and soil are not represented.

The upper half of the prothallium projects above the surface of the soil. There is a more or less spreading crown of leafy lobes, abundantly supplied with chlorophyll, and at the base of the inner face of these lobes the antheridia and archegonia are borne. It seems evident that the base of the prothallium was first to develop, but no sharply differentiated primary tubercle, like that shown in

TREUB's figures of *L. cernuum*, was found in these specimens. However, the base of the prothallium is more pointed than in *L. cernuum*, and this pointed base may represent the primary tubercle.

In proportion to the size of the prothallium, the protocorm is much more massive than in *L. cernuum*. There is no single, definitely organized growing point giving rise to all the protophylls, but rather a series of points, each giving rise to a protophyll. Stomata are abundant almost to the base of the protophyll; they are of the simplest type and open into a loose parenchyma with large air spaces. The transverse section is circular and shows a single weak vascular strand extending a short distance into the protocorm and ending blindly, without uniting with the strands of neighboring protophylls. The protuberance shown in front of the large protophyll in fig. 2 might be mistaken for the growing point from which the leafy axis is to be developed, but that point is formed much later, after several protophylls have appeared. The prothallium and protocorm shown in fig. 2 are similar, but indicate that there is considerable variation in both structures. Outlined against the protocorm is a second embryo.

The much older protocorm (fig. 3) indicates that the protophylls arise at irregular points, although there is a general progression, so that the protocorm resembles a very short horizontal rhizome. The 2 protophylls in the foreground are evidently the first ones formed, and the 3 much smaller ones at the left are the latest. The leafy axis of the permanent plant has not yet appeared. This specimen and also those shown in figs. 1 and 2 were sectioned, but the soil prevented satisfactory results. However, the sections showed the position of sex organs, the distribution of the fungus, and the relation of the protocorm to the prothallium. These features are shown, in a very diagrammatic way, in fig. 1*b*.

L. laterale belongs definitely to the type represented by *L. cernuum* and *L. inundatum*, since it has a short-lived green prothallium and an ephemeral protocorm with protophylls preceding the permanent leafy plant. *L. salakense* also belongs here, since the prothallium is green, and in its earlier stages behaves like that of *L. cernuum;* but it differs from the other 3 in having no endophytic

fungus. Whether it has a protocorm stage is not known. TREUB failed to find sporelings when he made his first investigation; later, in his work on the embryogeny of *L. cernuum*, he figures a protocorm stage in *L. salakense*.

It is interesting to note that the protocorm stage has been found only in *L. cernuum*, *L. salakense*, *L. inundatum*, and *L. laterale*, all of which have spores which germinate, giving rise to green short-lived prothallia. The spore-bearing plants of all 3 species, as well as that of *L. salakense*, grow upon the ground. No green prothallium or a protocorm phase in the embryogeny has yet been reported for any epiphytic *Lycopodium*.

L. VOLUBILE.—The only reference to the prothallium of this species is by HOLLOWAY (**16**). He says "the prothallus is large, firm, and long-lived. Healthy prothalli were seen still attached to sporelings which were as much as 10 cm. in length. Generally the prothalli are subterranean, being buried 1–4 cm. in depth; in several instances, however, they were observed growing on the surface of the ground, and the upper portion of the prothallus was then well supplied with chlorophyll."

The material at my disposal included 9 prothallia and 2 sporelings, one of them still attached to the prothallium (figs. 4–9). All belong to the subterranean tuberous type, and 4 of them (figs. 4, 5, 8, and 9) show a primary tubercle. Although the material is somewhat limited, it is evident that there is considerable variation in size and form.

The endophytic fungus is most abundant midway between the center and the surface, and is entirely lacking in the crown, in the upper part of the depression within the crown, and in the axis of the prothallium. Cells with considerable fungus abut directly upon those with none at all, making a sharp contrast (fig 12). A detail is shown in fig. 13.

The crown is differentiated into two regions in some places, only the inner one of which bears archegonia and antheridia, as shown in figs. 5 and 6; but even in these 2 prothallia some portions of the crown show no such differentiation, and the prothallia shown in figs. 7 and 8 have uniformly rounded crowns with no indication of two regions. While most of the sex organs are on the swollen rim

of the crown, they are not confined to this region, but occur in scattered patches within the rim on any part of the depressed region. A sectional view of a typical distribution of archegonia, antheridia, and the fungus region is shown in fig. 14.

The antheridia vary in size, shape, and output of sperms. They form hemispherical projections, with a nearly spherical mass of sperms; or they project scarcely at all, in which case the mass of sperms is not quite so regular. In a few cases, the sperm mass was elongated, making the topography bear some resemblance to that of an archegonium. In all cases, only one layer of cells separates the sperms from the surface, so that the essential course of development is uniform. A typical view is shown in fig. 15.

The foot of the sporophyte is strongly haustorial, and the cells surrounding it have some starch but very little protoplasm or other visible contents; consequently, the food supply must come largely from the fungus region and must be in a liquid condition even at a considerable distance from the foot. This is quite different from the condition in some gymnosperms, where only a single layer of cells may separate the haustorial cells of the embryo from those containing an abundance of food material in solid form. The foot is small and the vascular strand does not extend into it, but extends in an unbroken line from the shoot into the root, which is very late in developing. However, a few elongated cells, which do not become lignified, bend away from the main axis and point toward the foot.

L. SCARIOSUM.—The only description of this species is that given by HOLLOWAY (**16**), who says "the prothallus of *L. scariosum* was discovered in two localities. Like that of *L. volubile*, it appears to correspond to the *L. clavatum* type. It is large, firm, and long-lived, and in every case was found deeply buried (2–6 cm.)." Three specimens of this species were available and all had reached maturity, one bearing a young plant 18 mm. long, and the other two showing the foot and base of younger sporophytes which had broken off. Both prothallium and sporeling are larger and coarser than in *L. volubile*, as can be seen by comparing figs. 9 and 11, which are drawn to the same scale. The prothallium is densely infested by the fungus, which has about the same distribution as in *L. volubile*.

ORIGIN OF THE SUBTERRANEAN HABIT.—That the green leafy prothallia represent the original type from which the subterranean forms have diverged can scarcely be doubted. The species with green, leafy prothallia (*L. cernuum*, *L. inundatum*, *L. salakense*, and *L. laterale*) have spores which, in the first 3 species, are known to germinate immediately; while in all those with subterranean prothallia the spores germinate only after a long resting period. It would seem that some change has occurred in the spore which has delayed the germination; and then only such spores as reached a protected situation would survive to germinate at all. Germinating in protected situations, with little or no light, the prothallia naturally would assume the forms of subterranean, dependent structures. That this has been the order of regression is indicated by the fact that the leafy crown has not been lost altogether, but only modified. In *L. annotinum*, as described by BRUCHMANN, the prothallium is subterranean and saprophytic, but still retains some of the leafy appearance; in *L. laterale* the crown is sometimes broken up into separate fleshy cushions which may represent leafy lobes; in more extreme cases, there is merely a swollen, fleshy rim to represent the leafy structure. The position of antheridia and archegonia is about the same as in the green, leafy forms.

If those who are expert in hastening the germination of seeds which normally have a long resting period, could find some way to make the spores of *L. annotinum*, or some such species, germinate immediately, it would not be surprising if green, leafy prothallia should appear.

ANATOMY OF THE SPOROPHYTE

In the vascular structure of the adult sporophyte *Lycopodium* still presents some difficult problems, although investigations like those of HILL and others have cleared up some of the phases. However, it seems likely that the final solution will come through a comparative study of sporelings, intermediate stages, and adult plants. TREUB (7), BRUCHMANN (13), Miss WIGGLESWORTH (14), and HOLLOWAY (16) have figured and described a few sections; but material has been scanty or other features of the problem have so engrossed the attention that this important feature has received little attention.

It would be dangerous to draw any serious conclusions from a study of 2 or 3 sporelings, all of which had reached the leafy stage; but, in the present condition of the subject, it seems worth while to describe a few features. The study was made from the sporelings shown in figs. 9–11.

In *L. volubile* the foot is quite small, and although somewhat larger in *L. scariosum*, no vascular strand extends into it, but a few cells, not lignified, point in its direction. The vascular strand extends in an unbroken line from the tip of the stem to the tip of the root, which in both species is late in appearing.

The sporeling of *L. volubile* shown in fig. 9, and that of *L. scariosum* shown in fig. 10, were sectioned transversely down to the crown of the prothallium, and the portion below the crown was then cut longitudinally. It would have been much better if transverse sections had been continued throughout. In both species the leaves are surprisingly like the protophylls of *L. laterale*. Throughout a considerable portion of their length the transverse section is circular, and even in the broader middle region the leaves are thick and spongy, consisting almost entirely of very loose parenchyma with large intercellular spaces and a single vascular strand. Stomata are irregularly scattered over the entire surface (fig. 16). The adult leaves in both species are rather thin.

In *L. scariosum* the shifting topography of the stele is a conspicuous feature, especially in the upper, leafy region; in the lower half of the sporeling, where there are only a few scale leaves with no leaf traces, the arrangement is more uniform. Near the middle of the leafy portion, a hexarch, pentarch, and tetrarch condition occurs within a vertical distance of 1 mm. Throughout the lower one-third of this specimen the stele is rather constantly tetrarch; but, just above the foot a few sections show a triarch and even a diarch stele. That the leaf traces connect with the protoxylem points is evident at a glance; but whether the leaf traces determine the topography is not so clear. However, it is significant that the stele is more complex in the leafy region and that it attains its greatest complexity in mature plants with larger leaves and vigorous leaf traces. In various places there are indications of the banded arrangement characteristic of the adult stele.

The differentiation of the vascular tissues is interesting. A short distance below the meristem the large cells which are to form the largest tracheids are easily recognized, and some of the cells of the points of the radial structure can be distinguished, although lignification has not yet begun. Very soon the points of the radial structure begin to lignify and are then marked off very sharply from the surrounding tissues (fig. 17). These patches of lignified tissue consist almost exclusively of coarsely pitted tracheids. It is possible that there are some spiral vessels, but it looks as if practically all of the spirally marked cells belong to the leaf traces. If protoxylem is to be identified by spiral and annular markings, very little of the tissue which becomes lignified at this early stage would satisfy such a criterion; but the tissue is so well defined and becomes lignified so far in advance of the rest of the xylem, and is so sharply marked off from the large cells which in longitudinal view have the typical scalariform marking, that it may very properly be called the protoxylem. It should be recalled that in the cycads spiral vessels in the protoxylem are largely confined to the seedling, the protoxylem of the adult plant consisting almost exclusively of tracheids.

The study of the root was not satisfactory. Near the tip the bundle is C-shaped and diarch with the phloem in the sinus.

The sporeling of *L. volubile* is comparatively slender and in every way more delicate than that of *L. scariosum*. In the upper leafy portion the stele is quite regularly radial and tetrarch; but from the secondary root (*r* in fig. 9) down to the prothallium the structure is regularly or irregularly triarch. In connection with the more uniform topography of this stele, it should be noted that the leaves and their single vascular strand are not nearly so robust as in *L. scariosum*. The adult stele has the banded arrangement. The differentiation of the tissues of the stele proceeds as described for *L. scariosum*.

Summary

1. *Lycopodium laterale* has a green, leafy prothallium, and there is a protocorm-protophyll stage in the embryogeny. *L. volubile* and *L. scariosum* have subterranean prothallia with no protocorm stage, but the early leaves have the structure of protophylls.

2. In *L. scariosum* and *L. volubile* the sporeling has a radial stele. The adult plants have a banded stele.

3. The outer part of the ray of the radial structure consists almost exclusively of pitted tracheids with scarcely any spiral vessels, but becomes lignified long in advance of the large tracheids of the metaxylem, and should be regarded as the protoxylem.

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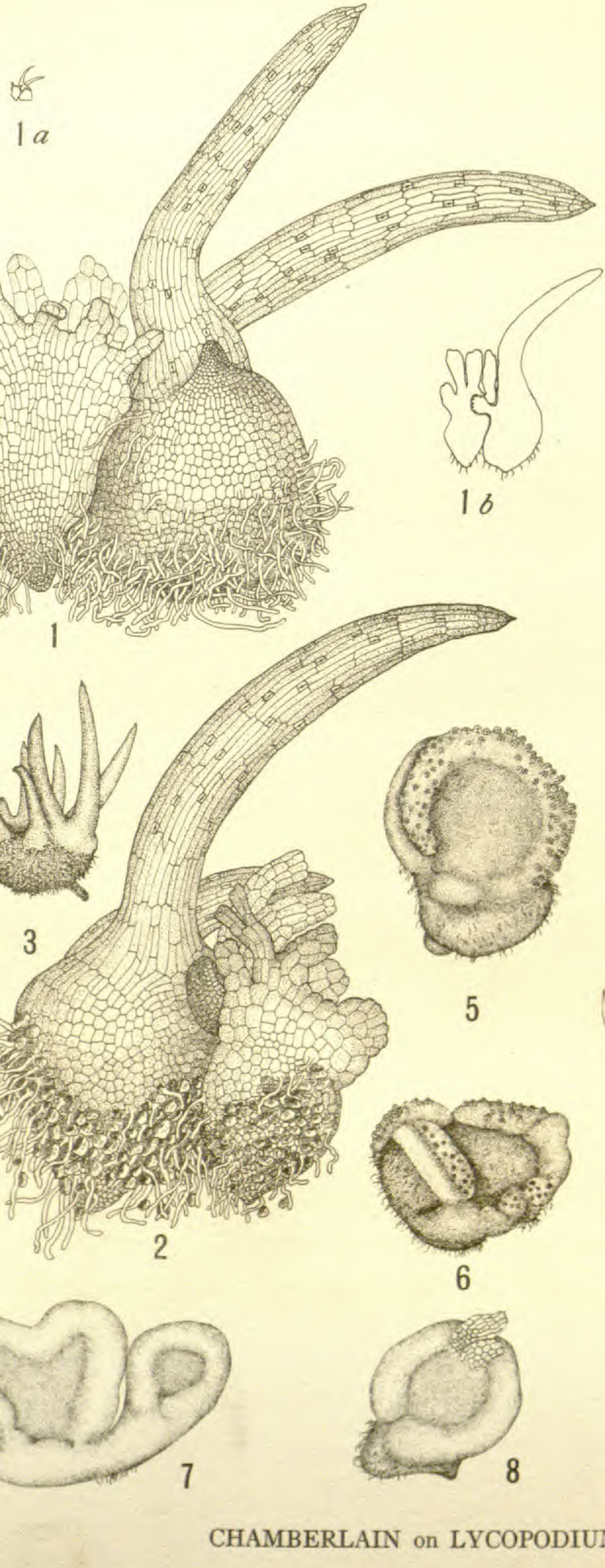
LITERATURE CITED

1. LINDSAY, JOHN, Account of the germination and raising of ferns from the seed. Trans. Linn. Soc. **2**:93–100. *pl. 18*. 1794.
2. SPRING, A. F., Monographie de la famille des Lycopodiacées. 1842.
3. HOFMEISTER, W., Vergleichende Untersuchungen höherer Kryptogamen. 1851.
4. DEBARY, A., Sur la germination des Lycopodées. Ann. Sci. Nat. Bot. IV. **9**:30–36. *pl. 4*. 1858 (published almost simultaneously in other places).
5. FANKHOUSER, L., Über den Vorkeim von *Lycopodium*. Bot. Zeit. **31**:1–6. *pl. 1*. 1873.
6. BECK, G., Einige Bemerkungen über den Vorkeim von *Lycopodium*. Oesterreich. Bot. Zeit. **30**:341–344. 1880.
7. TREUB, M., Études sur les Lycopodiacées. I. Le prothalle du *Lycopodium cernuum* L. Ann. Jard. Buitenzorg **4**:107–138. *pls. 9–17*. 1884.
8. BRUCHMANN, H., Das Prothallium von *Lycopodium*. Bot. Centralbl. **21**:23–28, 309–313. *pl. 1*. 1885.
9. TREUB, M., Études sur les Lycopodiacées. II. Le prothalle du *Lycopodium Phlegmaria* L. Ann. Jard. Buitenzorg **5**:87–114. *pls. 11–22*. 1886; III. Le développement de l'embryo chez *L. Phlegmaria*. **5**:115–139. *pls. 23–31*. 1886.
10. GOEBEL, K., Über Prothallien und Keimpflanzen von *Lycopodium inundatum*. Bot. Zeit. **45**:161–168. *pl. 2*. 1887.
11. TREUB, M., Études sur les Lycopodiacées. IV. Le prothalle du *Lycopodium salakense*. Ann. Jard. Buitenzorg **7**:141–146. *pls. 16–18*. 1888; V. Les prothalles des *Lycopodium carinatum, nummulariforme*, et *Hippuris*. *ibid.*, 146–150. *pl. 19*.
12. ———, Études sur les Lycopodiacées. VI. L'embryon et la plantule du *Lycopodium cernuum* L. Ann. Jard. Buitenzorg **8**:1–14. *pls. 1–5*. 1890; VII. Les tubercles radicaux du *Lycopodium cernuum* L. *ibid.* 15–22. *pls. 6–12;* VIII. Considerations théoretiques. *ibid.* 23–37.
13. BRUCHMANN, H., Über die Prothallien und die Keimpflanzen mehrerer Europäischer Lycopodien. pp. 119. *pls. 1–8*. 1898.



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CHAMBERLAIN on LYCOPODIUM

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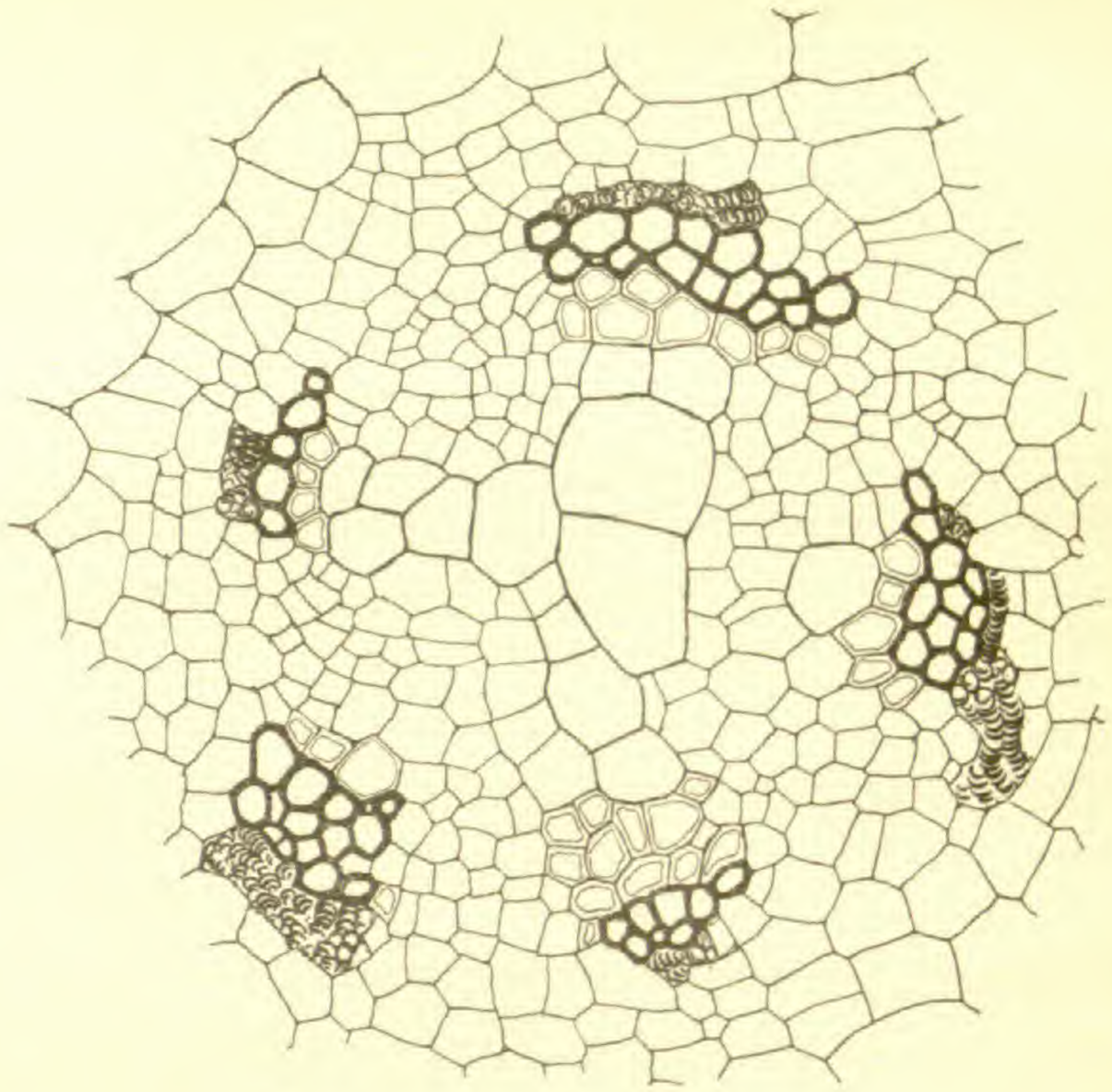

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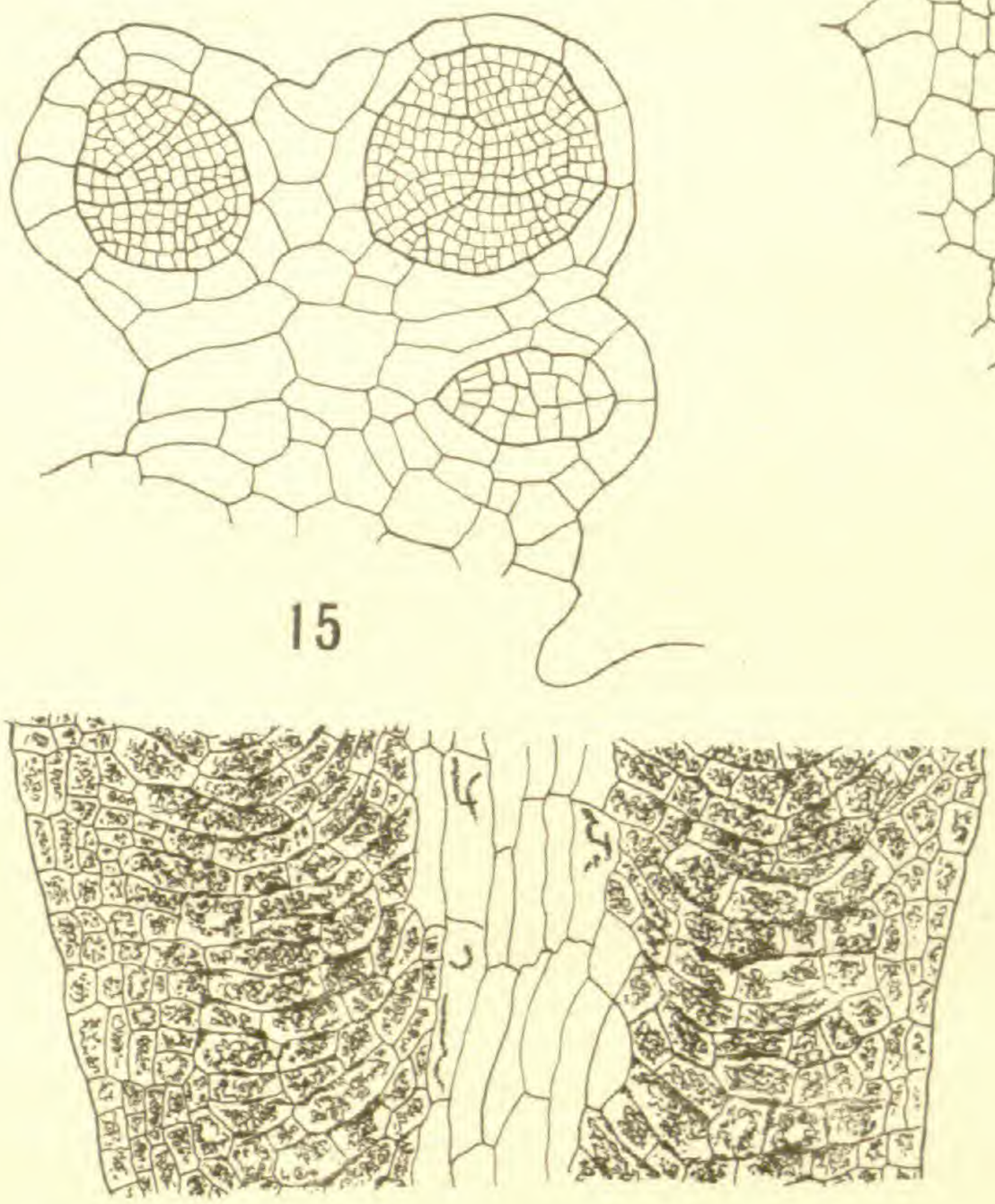

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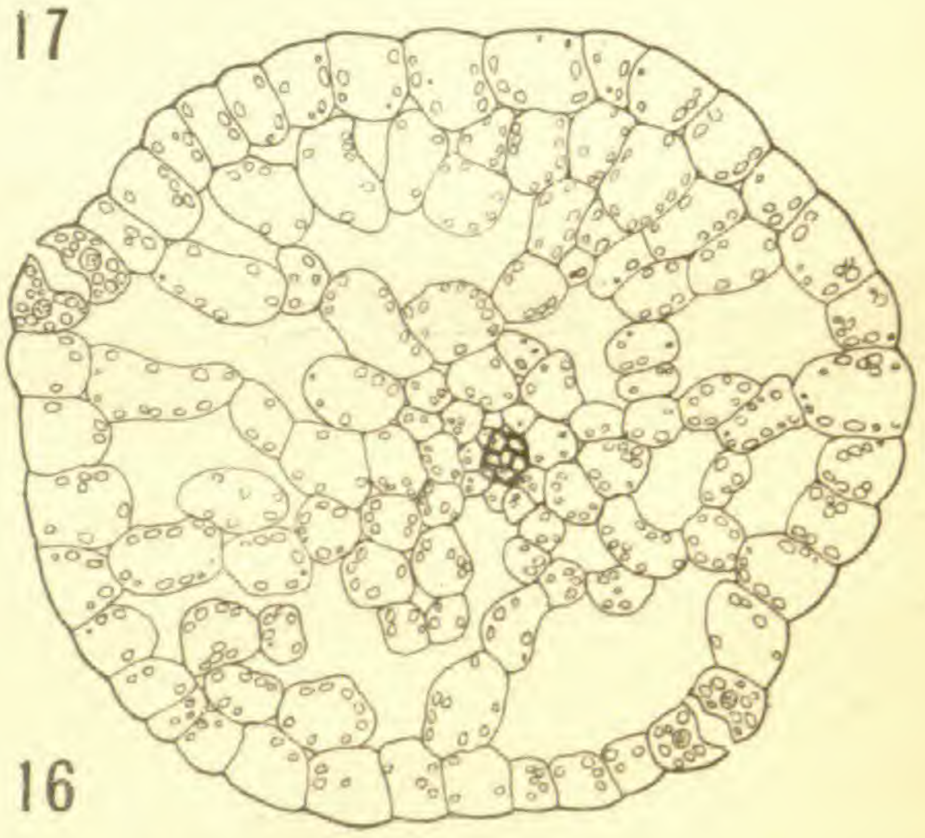

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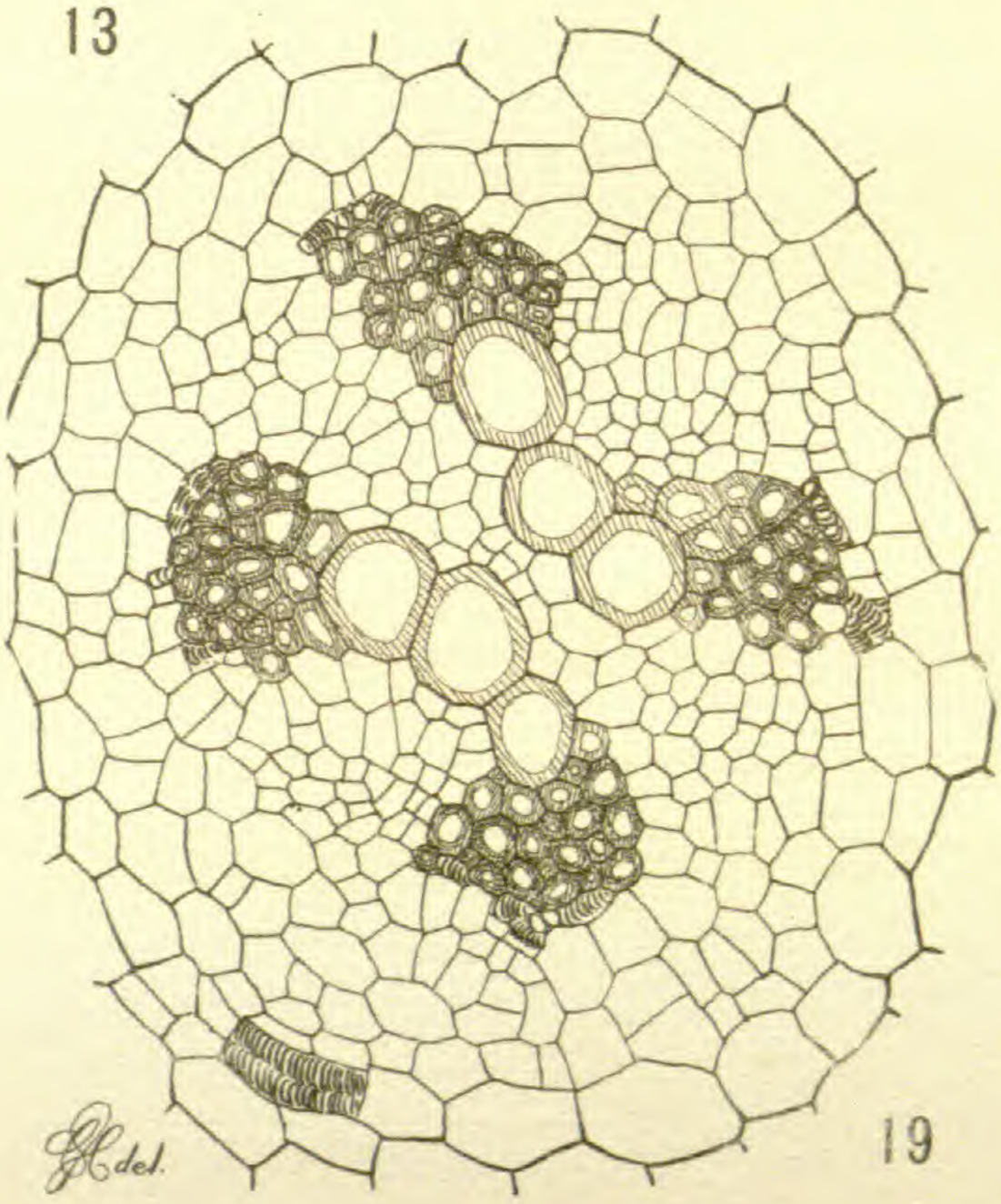

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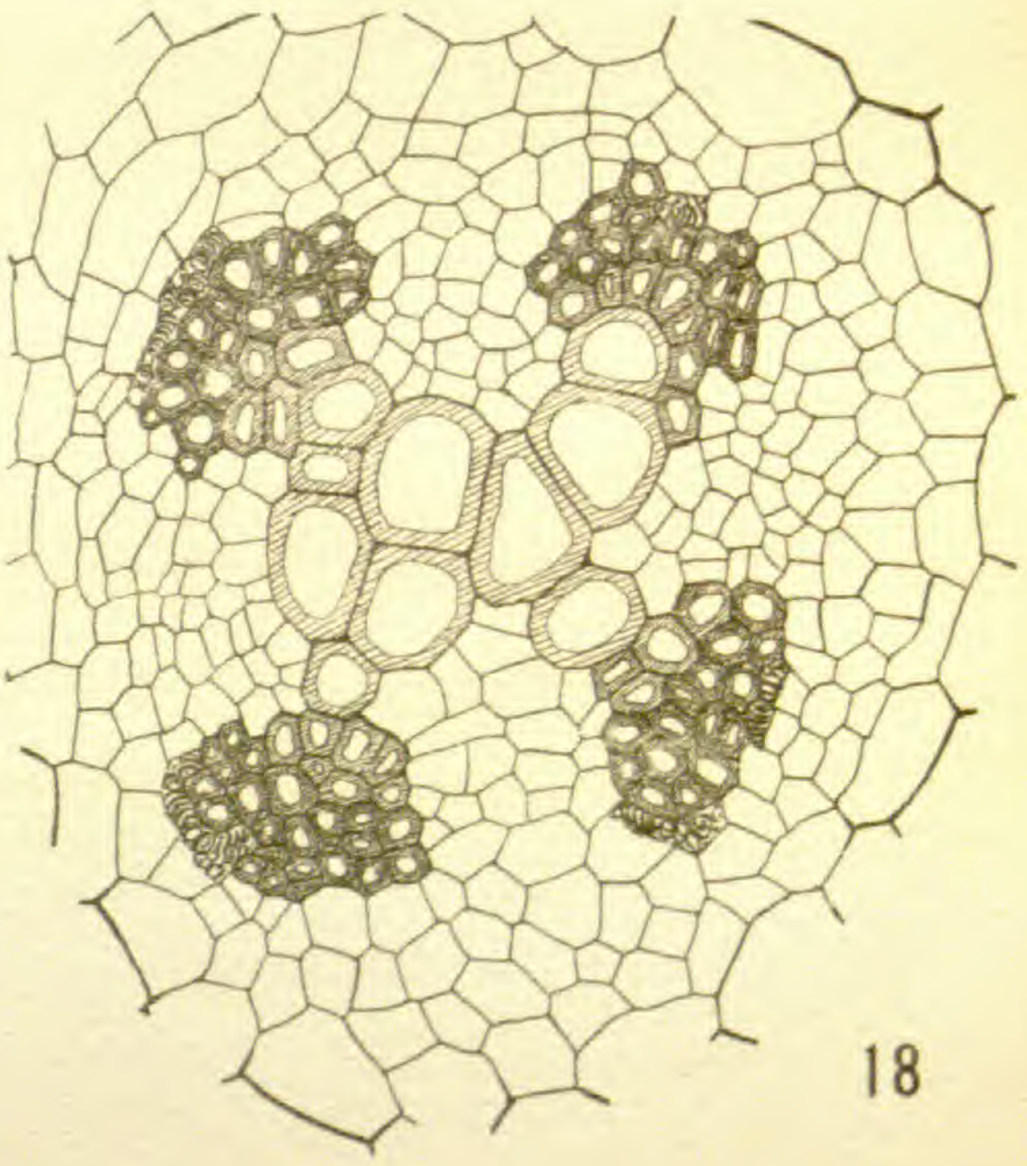


18

CHAMBERLAIN on LYCOPODIUM

14. WIGGLESWORTH, GRACE, The young sporophytes of *Lycopodium complanatum* and *L. clavatum*. Ann. Botany **21**:211–234. *pl. 22*. 1907.
15. BRUCHMANN, H., Die Keimung der Sporen und die Entwickelung der Prothallien von *Lycopodium clavatum*, *L. annotinum*, und *L. Selago*. Flora **101**:220–267. *figs. 35*. 1910.
16. HOLLOWAY, J. E., A comparative study of the anatomy of six New Zealand species of *Lycopodium*. Trans. New Zealand Inst. **42**:356–370. *pls. 31–34*. 1910.

EXPLANATION OF PLATES II–III

FIG. 1.—*Lycopodium laterale:* prothallium with leafy crown at left and bearing, at right, a protocorm with 2 fully grown protophylls and one young protophyll; ×20 (fig. 1*a* shows this plant natural size; fig. 1*b* is sectional view).

FIG. 2.—*L. laterale:* prothallium, at right, bearing a protocorm with 2 protophylls and also a second embryo; the soil and sand have not been removed; ×20.

FIG. 3.—*L. laterale:* protocorm with 10 protophylls; ×6.

FIG. 4.—*L. volubile:* prothallium showing primary tubercle at base; the crown is very even; ×10.

FIG. 5.—*L. volubile:* prothallium showing crown with numerous archegonia and antheridia; primary tubercle at base; ×10.

FIG. 6.—*L. volubile:* prothallium with archegonia and antheridia; the crown is lobed; ×10.

FIG. 7.—*L. volubile:* large, irregular prothallium; ×10.

FIG. 8.—*L. volubile:* prothallium with 2 young sporophytes; ×10.

FIG. 9.—*L. volubile:* prothallium with sporeling; a secondary root is shown at *r;* ×4.

FIG. 10.—*L. scariosum:* prothallium with sporeling; ×4.

FIG. 11.—*L. scariosum:* lower part of a young sporeling showing roots and foot; ×4.

FIG. 12.—*L. volubile:* diagrammatic view of prothallium with foot and lower part of sporeling.

FIG. 13.—*L. volubile:* detail of prothallium.

FIG. 14.—*L. volubile:* diagrammatic sectional view of prothallium showing archegonia, antheridia, and distribution of fungus, the latter indicated by shading.

FIG. 15.—*L. volubile:* portion of prothallium showing antheridia.

FIG. 16.—*L. volubile:* transverse section of leaf.

FIG. 17.—*L. scariosum:* transverse section of stem of sporeling before the large tracheids become lignified; *px*, protoxylem; *p*, phloem.

FIG. 18.—*L. scariosum:* same sporeling lower down; large tracheids have become lignified.

FIG. 19.—*L. scariosum:* same sporeling showing indication of banded arrangement.

PROTHALLIA OF LYCOPODIUM IN AMERICA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 223

EARLE AUGUSTUS SPESSARD

(WITH TWENTY-ONE FIGURES)

Since BRUCHMANN'S great work upon the prothallia of the European species of *Lycopodium* appeared in 1898, and probably before that date, numerous attempts have been made to find prothallia in America; but, so far as the writer has been able to determine, no successful searches have been reported.

While taking a correspondence course in botany with Professor CHARLES J. CHAMBERLAIN, he suggested that I avail myself of the opportunities afforded by my location in a *Lycopodium* region and make a thorough search for prothallia. In accordance with his suggestions and directions, the work was undertaken and has resulted in the finding of 21 prothallia and over 50 sporelings, representing 5 species: *L. clavatum*, *L. complanatum*, *L. annotinum*, *L. obscurum*, and *L. lucidulum*. It is also a pleasure to acknowledge my indebtedness to Dr. W. J. G. LAND for valuable suggestions.

The first specimen, that of *L. complanatum*, was found May 22, 1916. During the same month and in September of the same year, 6 more of this species, 8 of *L. clavatum*, 3 of *L. obscurum*, 2 of *L. annotinum*, and 1 of *L. lucidulum* were dug up, making 21 in all. Although the first sporeling bearing a foot, which indicated that a prothallium was recently present, was found on April 10, 1915, it was not until May 20, 1916, that a second was found 10 miles from it. During this interval approximately 150 days were spent crawling over the ground among and around the dense beds of adult sporophytes.

At first these tedious and futile efforts would seem to indicate that prothallia are rare. Yet, when it is noted that 17 of the 21 specimens were obtained from an area not more than 10 m. square, only about one-fourth of which was actually dug up; and furthermore, that as many as 6 were yielded by one small space 4 cm.

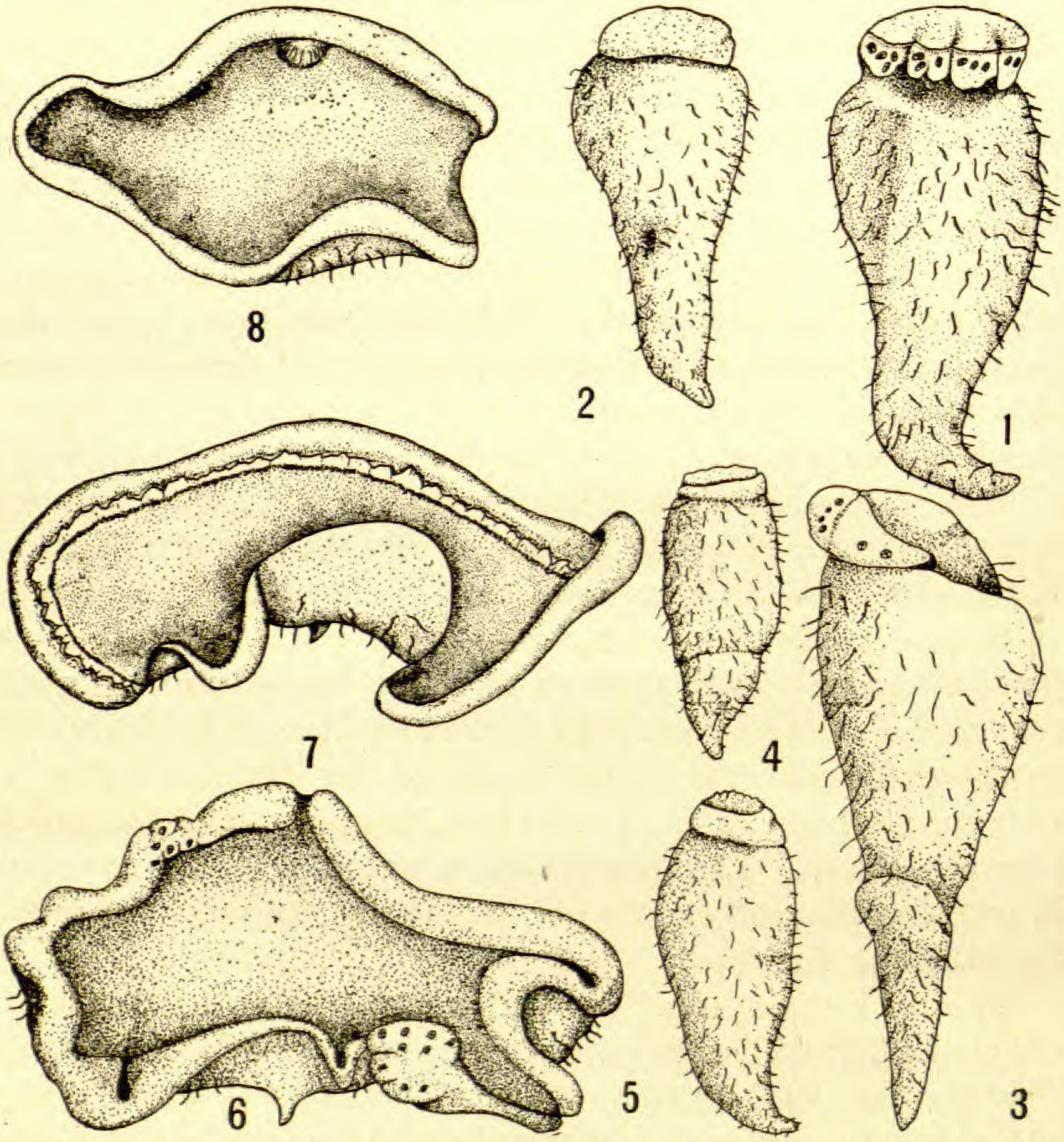
square, it seems probable that prothallia are rather abundant. The probability is even greater since the discoverer of these 21 prothallia was almost absolutely ignorant of the appearance of *Lycopodium* prothallia.

The discovery of the original locality was almost an accident. It is a place where one would least expect to find *Lycopodium* prothallia, at least those of *L. complanatum*, *L. clavatum*, and *L. annotinum*, since it is almost completely bare of adult sporophytes of these 3 species. I was wandering about looking for *Morchella*, when by chance I caught sight of an old sporeling of *L. clavatum*, growing in an exposed position between some wintergreen plants. An examination of the soil showed no prothallium. No adult sporophytes were within sight; but a sporeling is a good sign, and this encouragement led me to make the final successful search two days later.

Lying to the northwest of Marquette, Michigan, are a brewery and the remains of an old pesthouse. To the east of this pesthouse, about 700 m., is an open space bordering an open pasture on the north, a small wooded swamp on the east, and a wood composed of second growth poplar and maple on the west. A path runs to the northwest from the road which leads from Marquette to the pesthouse. Beside this path, at the distance indicated from the pesthouse, 18 of the 21 prothallia and most of the sporelings were found. The other prothallia and sporelings were found in two separate stations, the one half a mile, the other two miles from this one described.

The soil is mainly sand, covered in places by a very thin layer of humus; that within the edge of the wood is a black sandy loam. The specimens of *L. annotinum* and *L. lucidulum* shown in figs. 9 and 10 are the only ones which were found in the sandy loam where the ground is constantly shaded in summer. This specimen of *L. annotinum* is also enormously larger than the other prothallia, while that of *L. lucidulum* is decidedly the smallest. All the other specimens and most of the 50 sporelings were found in open, exposed, sandy places. The topography is uneven, rocks jutting up here and there between water-logged regions. Scattered about on the elevated regions are numerous small sandy knolls covered

partly by *Polytrichum*, and sometimes by a species of grass. In many spots these knolls are absolutely bare save for a few plants of *Polytrichum*. It was in these knolls that all but 2 of the 21 prothallia were found, as well as most of the sporelings.



FIGS. 1–8.—Fig. 1, prothallium of *L. complanatum*, first specimen found, ×5; figs. 2, 3, prothallia of *L. complanatum* (?), showing openings to the antheridia which form the lobes entirely, ×5; figs. 4, 5, prothallia of *L. obscurum* (?), crown is double, ×5; figs. 6–8, prothallia of *L. clavatum;* in fig. 6 the antheridial lobes are marked as in figs. 1 and 2; in fig. 7 the lobed edge is double; ×10.

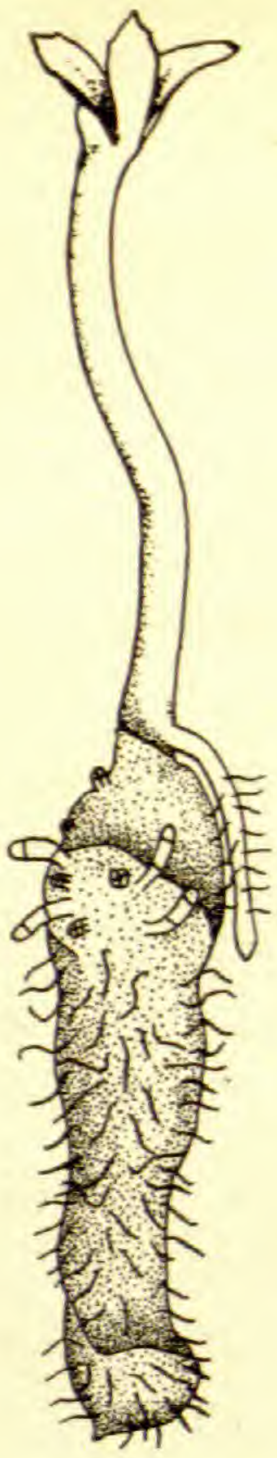
The frequent occurrence of sporelings and prothallia on these small, bare, exposed elevations suggests the idea that those spores which fall in such localities are first of all beaten into the ground

by the frequent rains; later, they are covered over by the shifting surface sand, and are finally conveyed to a favorable depth by the percolating soil water. No experiments were made to see whether this is the correct interpretation; but it was this idea which led me to investigate the knolls. This observation was heeded in all searches subsequent to the first successful one, and all yielded one or more prothallia.

So far as the elevation of the knolls is concerned, too much importance should not be placed upon it. The fact that they are elevated somewhat and exposed as they are to the air currents merely causes them to be covered by leaves less frequently than the lower or more wooded regions near them, and consequently they offer a more receptive landing to the spores which are transported thither. This idea is corroborated by the fact that many sporelings of *L. lucidulum* and *L. annotinum*, as well as 3 prothallia, were found in the middle of a trail which is frequently used by hunters. In these instances the spores were clearly trampled into the soil until they found the depth required for their growth into prothallia. I suspect that future observations may show that the prothallia of *L. annotinum* and *L. lucidulum* require more moisture for their development than the other species mentioned. The 3 specimens I found of these 2 species, although the surface indications were similar, grew in decidedly wetter places.

In searching for prothallia, soil was removed with forceps. This method was not very satisfactory, however, and 4 of the prothallia were broken or pierced by the forceps before they were seen. It would be better to remove the soil to a depth of 10 cm. and wash it through a coffee sieve. This would not only avoid the danger of damaging prothallia, but would increase the probability of finding young stages. The specimens grew at depths varying between 1 and 7 cm. The species growing nearest the surface is *L. lucidulum;* the one growing deepest is *L. obscurum*. In one hole 2 kinds, *L. clavatum* and *L. obscurum*, were seen within 2 cm. of each other.

The prothallia of *L. clavatum* and *L. annotinum* grow with the wrinkled side toward the surface, and the primary tubercle pointed downward; that of *L. lucidulum* grows erect and the sporeling



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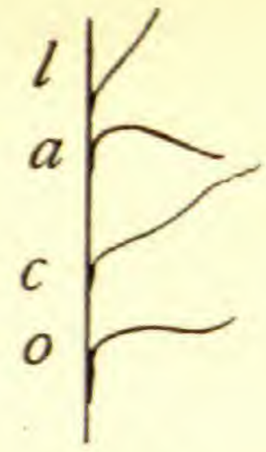

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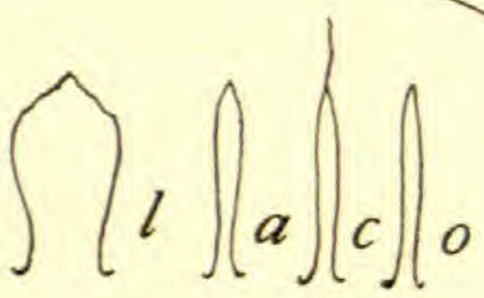

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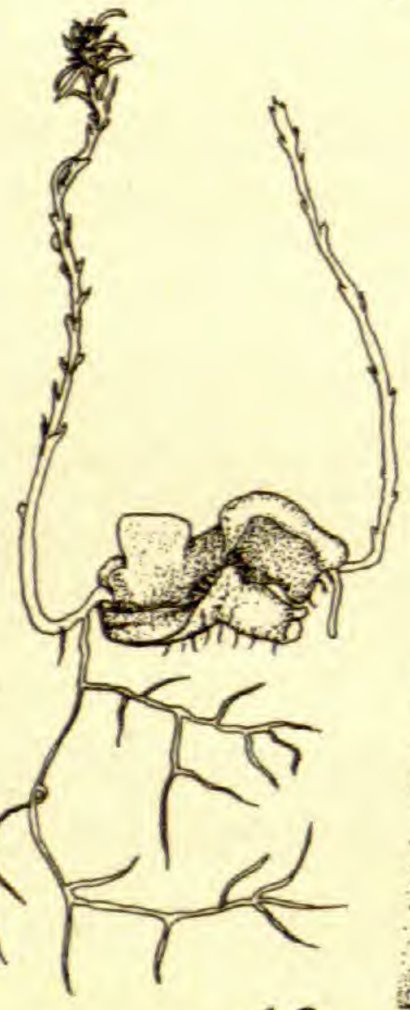

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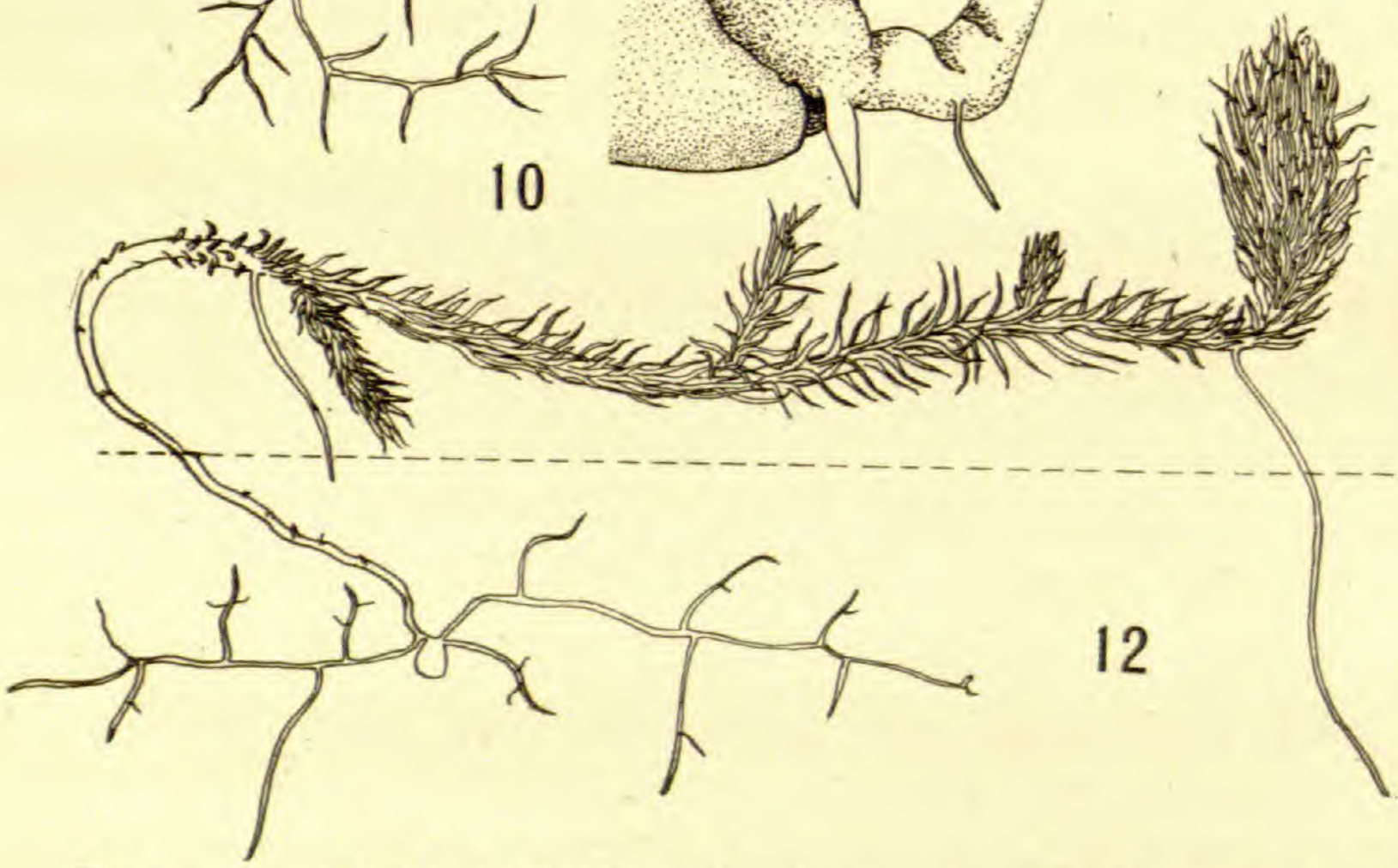

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FIGS. 9–15.—Fig. 9, prothallium bearing a sporeling of *L. lucidulum*, the only specimen found, showing 4 paraphyses, archegonia, primary tubercle, rhizoids, and an enlargement made by foot of sporeling, ×6; fig. 10, prothallium of *L. annotinum* bearing 3 sporelings, smallest one just emerging from upper tip of lower lobe of gametophyte, natural size; fig. 11, detailed drawing of largest sporeling shown in fig. 10, ×3; fig. 12, old sporeling of *L. clavatum* with foot still present, having lost erect habit of younger sporelings and assumed trailing habit of adult, ×2; fig. 13, sporeling and portion of gametophyte of *L. obscurum*, rest of gametophyte being like corresponding portions of fig. 5, ×4.25; fig. 14, diagram showing leaf contour of sporelings of *L. lucidulum* (*l*), *L. annotinum* (*a*), *L. clavatum* (*c*), and *L. obscurum* (*o*); fig. 15, diagram showing general profile of leaves of sporelings, the leaf being directed upward in *L. lucidulum* (*l*), drooping in *L. annotinum* (*a*), directed upward but bearing bristle in *L. clavatum* (*c*), bending downward then upward in *L. obscurum* (*o*), leaving the stem almost at a right angle.

arises straight out of the end lying immediately beneath the surface of the soil. The habit of growth cannot be stated definitely for *L. obscurum* and *L. complanatum*. The specimen of *L. complanatum* shown in fig. 3 was found in the same position as the specimen of *L. obscurum* shown in fig. 13. All the other specimens of *L. obscurum* and *L. complanatum* which I collected were disturbed before I saw them, and consequently their exact position was not determined, except the one in fig. 1 which grew vertically. In size, the prothallia of figs. 4 and 5, are very similar to that of fig. 13. This resemblance in size, together with a common external contour, are my only reasons for assuming that these 3 specimens belong to the same species. But here arises a difficulty. Although the prothallium in fig. 3 is about twice as large as the adult prothallium of *L. obscurum* (fig. 13) bearing a sporeling, we find the two growing in exactly the same position. This may mean two things; either the prothallia I have classed with *L. complanatum*, except no. 1, might as readily belong to *L. obscurum*, or the prothallia of *L. complanatum* grow in various positions in regard to the directions of their axes. If the axis of a prothallium of *L. complanatum* can be shown to grow always in a vertical position, and if that of *L. obscurum* can be shown to grow always in a horizontal position, the identification of the two species will then become a very simple matter if care be taken while hunting for them. I can only regret that this important point must be left for future observation to settle.[1]

Adult sporophytes of *L. clavatum* and *L. annotinum* were rare in this immediate locality, only one small patch of each having been found. A few plants of *L. complanatum* were found. *L. obscurum* and *L. lucidulum* grow in small clumps throughout the region.

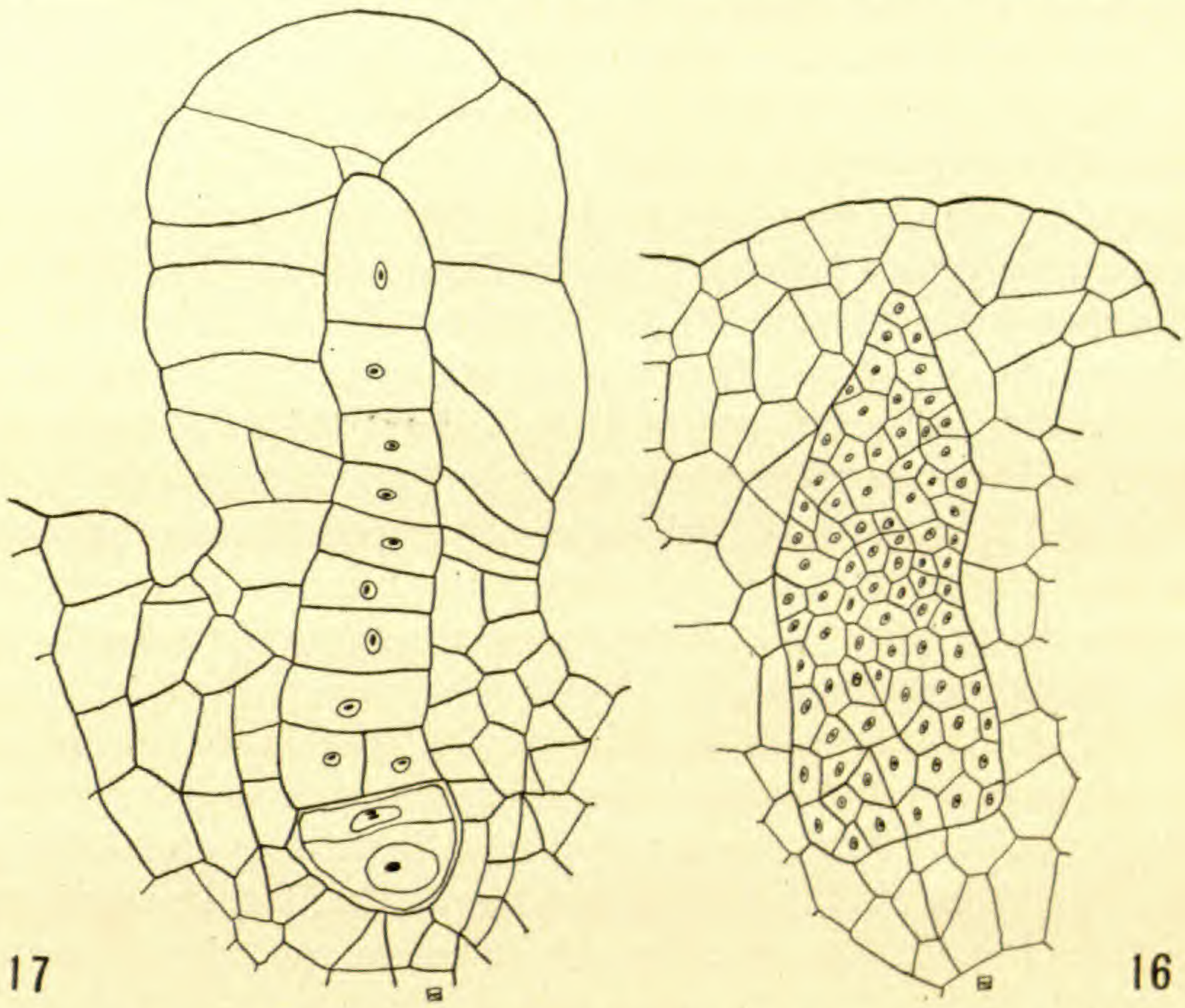
The 50 sporelings belong mainly to *L. clavatum*, but a few were found of all the other species mentioned in this paper except *L. complanatum*. Since the sporeling is the first guide in the

[1]On November 5, 1916, after this paper had gone to press, the writer found a large specimen of *L. obscurum* with sporeling attached. It was of the same color and shape as and grew horizontally like the prothallium labeled *L. complanatum* in fig. 3. This bears out the suggestion that all the prothallia classed with *L. complanatum*, except no. 1, very probably belong to *L. obscurum*.

search for prothallia as well as in their identification, I have given 2 diagrams in figs. 14 and 15 to show the specific differences between the juvenile leaves of the 4 species of sporelings which I obtained. If the descriptions of these 4 species as given in GRAY's *Manual* are followed carefully, the specific differences may readily be determined. The leaves of *L. clavatum* terminate in a bristle even in a stage as young as that sporeling of *L. annotinum* shown in figs. 10 and 11. I have not been able to separate sporelings of these 2 species at such a young stage or younger by any other character than by the presence or absence of the terminal bristle on the leaf. At such a stage the leaves have not yet assumed such very definite directions of growth as they do a little later, and as indicated in *a* and *c* of fig. 15. The sporeling of *L. obscurum* is easily identified by the 6 rows of leaves which early assume the characters peculiar to this species. Of course in the sporeling stage the leaves are fewer and more spreading than in the adult, so that a hurried examination would scarcely show the relationship between the two. I actually thought I was digging for a prothallium of *L. annotinum* while removing the soil from around the sporeling shown in fig. 13, and it was not until all the sand had been removed from among the rhizoids of the prothallium, and after the leaves had been examined under a lens, that I discovered my error. The sporeling of *L. lucidulum* cannot be confused in any way with the sporelings of any of the species I have mentioned. However, one may very readily believe that he is digging up a genuine one only to find that peculiar winged bud at the bottom of it. Yet, although I have experienced this disappointment a hundred or more times, through it I have observed that the sporelings bearing a foot grow just a trifle deeper in the ground. Generally the bud lies nearly or wholly on the surface, and unless it is rotted away, is readily seen. The sporeling of *L. lucidulum* looks exactly like one of the plants which grows from a bud, except that it has a distinct foot. Both bear leaves like an adult plant. Some of the plants in the vicinity are *Morchella*, *Polytrichum Acer*, *Populus*, *Pteris*, *Gaultheria*, *Rhus*, and *Polygala*.

The largest specimen of *L. annotinum* (fig. 10) measured 10 ×7 mm. It bore 3 sporophytes, one of which reached only 0.5

cm. above the ground, and another had just emerged from the prothallium. In color it resembled closely the gametophyte of *Botrychium virginianum*. The specimens of *L. clavatum* were much smaller and almost white in color. They varied in size from 6.5×5 mm. to 5×3 mm. The largest fragment measured 7×5 mm., and the smallest 2×1 mm. Figs. 6, 7, and 8 show the entire specimens. The fragments were more twisted and wrinkled than



FIGS. 16, 17.—Fig. 16, antheridium of *L. clavatum* in sperm mother cell stage, ×365; fig. 17, archegonium of *L. complanatum* immediately before neck canal cells break down, the lowest neck canal cell being double, ×365.

these, but both the fragments and the entire specimens showed a distinct crown on the border, which in one case (fig. 7) was double, the inner crown having an irregular outline. Each of the entire prothallia showed the primary tubercle very distinctly, near the middle, on the ventral side.

The prothallia of *L. complanatum* were all entire except one which had the lower portion broken off. Seven of these prothallia,

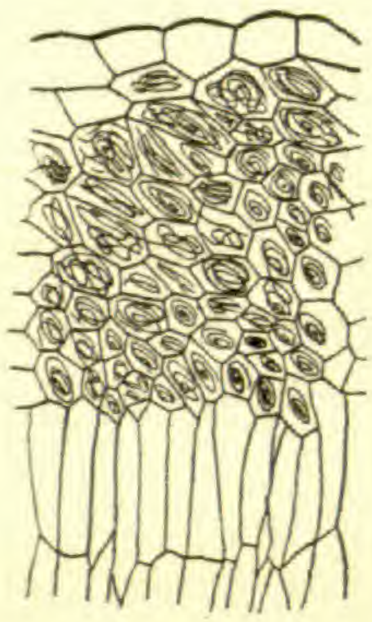
illustrated by figs. 1, 2, and 3, bore a single crown which was more or less lobed. In section (fig. 19) these lobes proved to be masses of antheridia. The crown on each of the 3 prothallia of *L. obscurum* (figs. 4, 5) was double. An accident to the sections prevented a study of the relation between the lobes and the location of the sex organs in these prothallia. The crown was unequal in every specimen of *L. complanatum* and *L. obscurum*, and the lobes appeared only at one side, as shown in fig. 3.

The one prothallium of my collection which may cause some readers to question is that of *L. lucidulum*. I have found 10 sporelings and only one prothallium, but this single prothallium fortunately has a sporeling attached. This fact alone would not serve to convince a botanist in doubt of its genuineness. However, there are 5 reasons why I am convinced of its nature. Fig. 9 shows these 5 points. They are (1) archegonia, (2) paraphyses, (3) rhizoids, (4) a primary tubercle, and (5) the sporeling arises from it like the sporeling of any recognized gametophyte; there is a foot, and a primary root originates at the point where the stem breaks through. Certainly all these features cannot be connected with a young plant of *L. lucidulum* of asexual origin or with an associated fungus growth.

This prothallium of *L. lucidulum*, which, like that of *L. obscurum*, I believe is new to botanists, shows some very interesting evolutionary points. The body is somewhat cylindrical, but not entirely so. It is somewhat more flattened longitudinally than the figure shows. In this figure the flat side is turned toward the reader. Near the middle it makes a short twist toward the left. In general outline it looks like a prothallium of *L. complanatum* in the making, but on the upper lobed region are 4 paraphyses. Such a feature suggests a transition stage between the *Phlegmaria* type of prothallium, as represented by *L. Selago*, and such a form as *L. complanatum*. The specimen contained no chlorophyll so far as I was able to determine.

Among the 50 sporelings gathered there was a variation in age between 1 and 5 years. Each season's growth above the soil could be determined definitely by the alternate regions of longer and shorter leaves on the species *L. clavatum* and *L. annotinum*.

Fig. 11 shows in detail the largest sporeling of *L. annotinum* growing from the prothallium represented in fig. 10. The sporeling of *L. clavatum* shown in Fig. 12 was one of the largest, and serves to indicate the size one may obtain still bearing the foot. It is very possible, even for one who has studied carefully the figures of *Lycopodium* prothallia given in the various papers upon the subject, to mistake other forms of tubers for them. The sex organs may be indistinct or undeveloped, so that identification by means of



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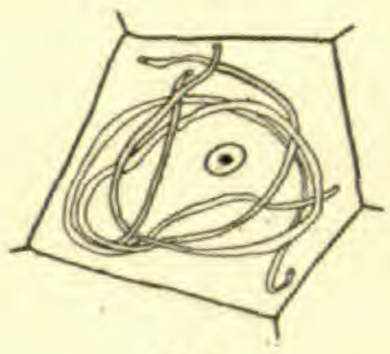

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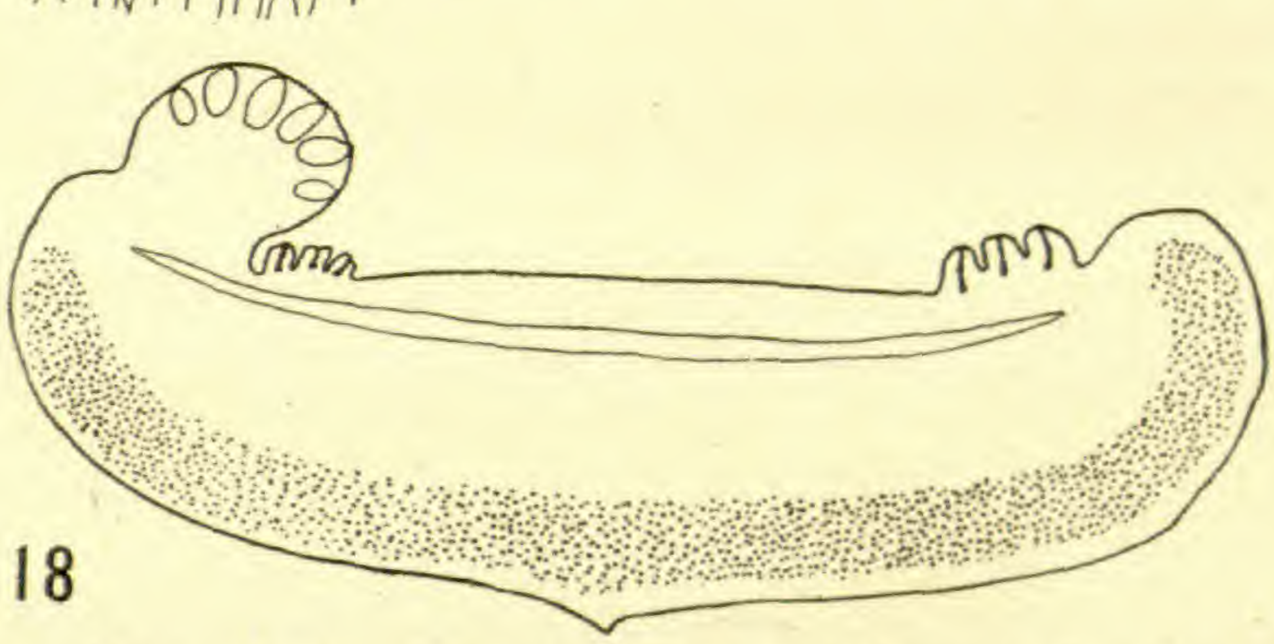

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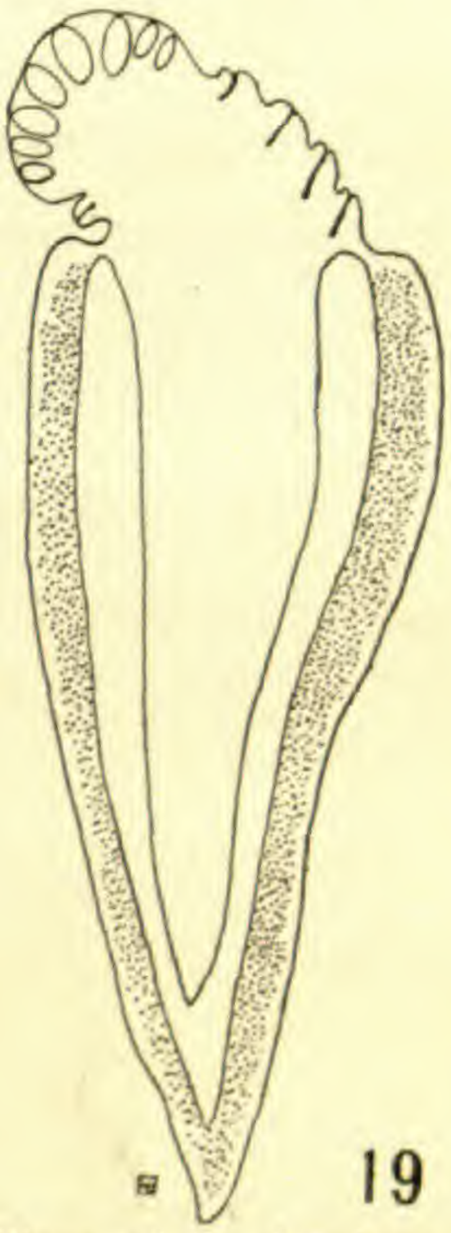

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FIGS. 18–21. Fig. 18, diagram of tissue regions in prothallium of *L. clavatum* (antheridia and archegonia indicated); fig. 19, diagram of median region of prothallium of *L. complanatum*, the fungus-infected region being indicated in this and fig. 18 by dotted shading; fig. 20, detailed sketch of fungus-infected region, showing its location beneath the epidermal tissue, ×75; fig. 21, single cell with endophytic fungus coiled within (fungus passes freely from one cell to another by piercing the cell wall), ×750.

them is impossible in such an instance. The number and variety of small tubers which grow in the soil of a wood are both large and confusing. Some of them are surprisingly similar in all outward appearances to small prothallia of *L. complanatum*. I do not doubt that I threw away some genuine specimens because they appeared to be tubers. I know that I retained some tubers and even sectioned them in paraffine because I thought they were prothallia.

To establish definitely whether the forms that I have collected are genuine prothallia of *Lycopodium*, therefore, I have drawn figs. 16, 17, 20, and 21 to show an antheridium, an archegonium in which the lowest of the neck canal cells is double, and 2 detailed sketches of the fungus-infected region. The diagrams in figs. 18 and 19 indicate the tissue regions shown by sections of 12 prothallia.

This article seeks to establish the fact that the prothallia of *Lycopodium* have been found in America; to make known to those botanists who may later desire to find prothallia for themselves, what the conditions were under which the specimens collected were found; and to announce the discovery of 2 new species of *Lycopodium* prothallia, namely, *L. obscurum* and *L. lucidulum*. Concerning the development and structure of the American *Lycopodium* gametophytes, the writer hopes to deal in a later paper.

MARQUETTE, MICH.

SIMILARITY IN THE EFFECTS OF POTASSIUM CYANIDE AND OF ETHER

W. J. V. OSTERHOUT

(WITH ONE FIGURE)

The writer has pointed out that typical anesthetics, such as ether, chloroform, and alcohol, produce a temporary decrease in permeability.[1] In view of the fact that anesthesia is looked upon by some as a form of asphyxiation, it seems desirable to investigate the manner in which permeability is affected by KCN, which not only acts as an anesthetic, but also inhibits oxidation to a remarkable degree.

The experiments here described were made in 1912, in connection with a series of experiments on anesthetics, of which a brief announcement has already appeared. Since then a paper by KREHAN[2] has been published which states that KCN produces a transitory increase of permeability which soon disappears. The writer is unable to confirm this statement, as will appear from the following account.

The experiments were made on tissues of *Laminaria Agardhii*. The permeability was measured by determining the electrical resistance in the manner described in previous publications.[3] The KCN employed was Kahlbaum's best, and the distilled water was prepared with especial care. A solution of KCN of the same conductivity as the sea water (about 0.381M) was prepared. This was added to the sea water and its effect on the tissues was observed. The following experiment will serve to illustrate the procedure.

A lot of tissue which had in sea water a resistance of 1140 ohms was placed in sea water to which had been added a solution of KCN 0.381M in sufficient quantity to make the concentration

[1] OSTERHOUT, W. J. V., The effect of anesthetics upon permeability. Science N.S. **37**: 111–112. 1913.

[2] Internat. Zeit. f. Phys. Chem. Biol. **1**: 189. 1914.

[3] OSTERHOUT, W. J. V., The permeability of protoplasm to ions and the theory of antagonism. Science N.S. **35**: 112–115. 1912.

0.01M.[4] The resistance rose rapidly to 1170 ohms, where it remained for 10 minutes, after which it began to fall. The results are given in table I and fig. 1.

TABLE I

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in minutes	In KCN 0.01M in sea water	In sea water
0	1140	1080
10	1160	0
20	1160	0
30	1150	1070
110	1010	0
200	910	0
300	810	0
400	710	1070

All readings were taken at 14° C. or corrected to this temperature.

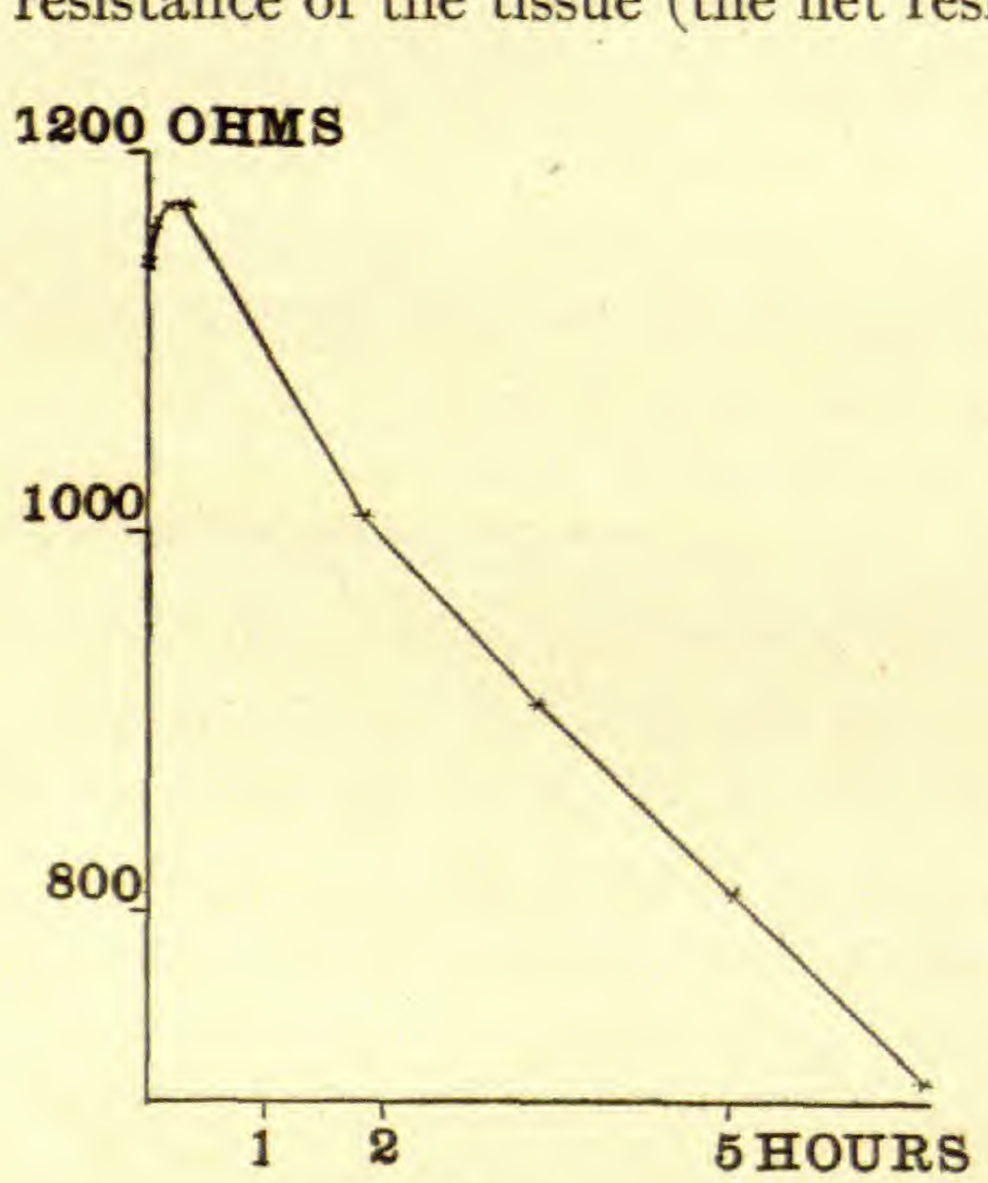

FIG. 1

The resistance of the apparatus was 250 ohms; hence the resistance of the tissue (the net resistance) at the start was 1140—250=890 ohms. We may put the permeability as equal to the conductivity, or, for convenience, as equal to the conductance; hence the permeability was 1÷890=0.001124. The maximum net resistance was 1170—250=920 ohms, and the permeability was 1÷920=0.001087. Hence the loss of permeability was (0.001124—0.001087)÷0.001124=3.3 per cent.

Similar experiments were made with other concentrations from 0.002M up to 0.381M (solution of KCN without sea water). The results were irregular, and it is not possible to say

[4] This mixture had the conductivity of sea water. The sea water after the addition of the KCN was slightly alkaline to litmus. The hydrogen ion concentration of 0.01M KCN in sea water was 1.4×10^{-10} as determined by the gas chain. The alkalinity tends to make the rise of resistance less pronounced.

without numerous additional experiments at exactly what concentration the maximum decrease of permeability occurs. It seems doubtful whether it amounts to much more than 3 or 4 per cent at any concentration.

In KCN 0.381M[5] (without sea water) there was in some cases a rise in resistance, followed by a rapid fall, and in other cases the resistance did not rise, but fell from the start. It is probable, however, that in these cases there was a transitory rise which disappeared before the end of the first minute, at which time the first measurement was taken.

The experiments demonstrate that there is a temporary decrease of permeability instead of a temporary increase as described by KREHAN. At no concentration was a temporary increase of permeability observed. Whenever the permeability began to increase, it continued to increase steadily until the tissue was dead. The concentrations employed ranged from 0.002M to 0.381M. It may be added that the method of plasmolysis, which was employed by KREHAN, cannot be relied upon to give as accurate measurements of permeability as the determination of electrical resistance.

If tissue be allowed to remain in KCN until the resistance has fallen about 100 ohms, it will often completely regain its original resistance on being transferred to sea water. But if the resistance be allowed to fall much beyond this, recovery is usually incomplete and the greater the fall of resistance (beyond the point where complete recovery is possible) the less the recovery.

The concentrations of KCN necessary to produce a decrease of permeability are very much smaller than the corresponding concentrations of ether, chloroform, and alcohol. This accords with the fact that it also takes less KCN to produce narcosis. The period of decreased permeability cannot be prolonged as much by means of KCN as by means of the other anesthetics mentioned. This agrees with the fact that organisms can be kept longer under

[5] The hydrogen ion concentration was 7×10^{-13} as measured by the gas chain. This is sufficiently alkaline to cause a considerable fall in resistance (cf. Jour. Biol. Chem. **19**: 335. 1914). The concentration of KCN was determined by weighing out the requisite amount, but owing to the presence of alkali it was really less than 0.381M.

narcosis without injury by means of ether, chloroform, and alcohol than by means of KCN.

The fact that KCN resembles typical anesthetics (such as ether and chloroform) in producing a temporary decrease in permeability does not, in the opinion of the writer, show that anesthesia is a form of asphyxiation. It seems quite probable that the decrease of permeability and the anesthesia produced by KCN have connection with its effect on oxidation.

LABORATORY OF PLANT PHYSIOLOGY
HARVARD UNIVERSITY

CURRENT LITERATURE

NOTES FOR STUDENTS

Oenothera genetics.—HERIBERT-NILSSON[1] discusses the data from his studies of *Oenothera Lamarckiana*, suggesting what he calls a Mendelian interpretation of the mutating tendency of this species. The character with which he worked was the red pigmentation found in the leaf nerves of some of his plants and absent in others. He concludes that the red-nerved and white-nerved plants form a distinct discontinuous variation; that the white-nerved plants are pure recessives and when selfed or intercrossed produce only white-nerved plants; that a homozygous dominant is not formed, and that therefore a strain of pure red-nerved plants cannot be produced, but all red-nerved plants when selfed or intercrossed will produce some white-nerved plants. Finding the average ratio of red-nerved to white-nerved plants in *O. Lamarckiana* and most of its "mutants" to be 2.68:1, or nearly 3:1, instead of 2:1 as would be expected when no positive homozygotes are formed, he adopts the explanation proposed by WILSON in explaining the work of CUÉNOT with yellow mice. According to this explanation, most of those positive female gametes which would normally be fertilized by positive male gametes, but which for some reason cannot be so fertilized, are fertilized by recessive male gametes. This would produce an average ratio of the red-nerved to white-nerved plants a little lower than would be the case under normal genetic behavior, thus accounting for a ratio of 2.68:1 instead of 3:1. It should not be forgotten, however, that the work of CASTLE removed the necessity for this interpretation in the case of yellow mice, and thus lessened its value as an interpretation of this sort of deviation from expected ratios.

In "*gigantea*" (the *gigas* type) the author interprets the observed ratios as modifications of 3:1, 15:1, 63:1, and 255:1, and concludes that in this type the red-nervedness is probably produced by any one of four factors. He also finds that the factor or factors for red leaf nerves affects other morphological and physiological characters of the plant.

Having thus striven for a Mendelian interpretation of the behavior of red vs. white nerves, the author presents his observations on the mutation ratios of *O. Lamarckiana* and its mutants, or, as he calls them, "Kombinante," and suggests the following explanation of the mutating tendency of this species. *O. Lamarckiana* is dependent upon a number of groups of multiple factors, the majority of which cannot be produced in a homozygous dominant condition,

[1] HERIBERT-NILSSON, N., Die Spaltungserscheinungen der *Oenothera Lamarckiana*. Lunds Univ. Årsskrift 12:4–131. 1915.

and the various mutants are plants which result when one or more of these groups are in a homozygous recessive condition. This might be represented graphically thus: *O. Lamarckiana*=*Aa Aa Aa aa; Bb Bb bb bb; Cc Cc cc cc*, etc., where in every group at least one of the factors would be present in the positive condition. A mutant=*aa aa aa aa; Bb Bb bb bb; Cc Cc cc cc*, etc., where in at least one of the groups none of the factors are present in the positive condition. This interpretation is thought to explain the occurrence of different ratios of mutation, for if there were 4 such independent multiple factors for the *Lamarckiana* character, a given mutant dependent upon the absence of all these positive factors would occur in the following percentage: 1.2 per cent when all of the 4 factors are heterozygous; 3.7 per cent when only 3 of the 4 factors are heterozygous; 11.1 per cent when only 2 of the 4 factors are heterozygous; 33.3 per cent when only 1 of the 4 factors is heterozygous.

At several points in his paper the author points out that since different strains of *O. Lamarckiana* yield different series of mutants it cannot be an elementary species, as DEVRIES claims, but must be a group of elementary species, the free intercrossing of which makes possible the production of the mutants by ordinary Mendelian segregations. The assumption of extensive linkage of characters, of the occurrence of heterogamy (that is, the transmission of hereditary characters through the sperms differing from those possessed by the eggs of the same individual), and the assumption that one sort of sperm may hinder the activities of another sort of sperm, are not in strict accord with the author's claim that he has given a Mendelian interpretation of *Oenothera* genetics.—BEN C. HELMICK.

Mutation in Matthiola annua, a "Mendelizing" species.—In a preliminary paper under the foregoing title FROST[2] has published certain conclusions in regard to the origin of Mendelian dominants which are sure to arouse no little interest. Until the full account appears it will be impossible to judge of the validity of FROST's interpretation of his discoveries, but the discoveries themselves are obviously of prime importance, interpret them however we may. According to his own view, he has observed the origin by mutation of 8 different dominant Mendelian varieties from a single strain of stocks. To show that this would be a discovery of the highest theoretical significance, it is only needful to point out that similar evidence is extremely meager, and in practically every case not as well attested as one might wish. The list of new dominants which have arisen by mutation is practically exhausted when we have mentioned KEEBLE's giant *Primula* and COLLINS' albinistic maize, for the case of GATES' *Oenothera rubricalyx* is still in dispute.

FROST states that the individual mutations of his *Matthiola* cultures obviously are not extracted recessives, but heterozygous dominants; that they seem to be due to definite changes in the germ plasm distinct from the recombi-

[2] FROST, HOWARD B., Mutation in *Matthiola annua*, a "Mendelizing" species. Amer. Jour. Bot. 3:377–383. 1916.

nations involved in ordinary Mendelian phenomena; that the mutative changes concern various characteristics of the plant, but that the factor for each new type is regularly inherited as a unit, sometimes showing linkage with another factor pair, so that we may suppose, in some cases at least, that the essential change is limited to a portion of one chromosome. The very first test of these conclusions would demand that the mutations reproduce the mutational type in 75 per cent of their progeny in the first generation, and that 25 per cent of the progeny be homozygous dominants. This condition apparently is satisfied in the case of only 1 mutation of the 8, and until the data appear we have no basis for an independent judgment as to whether the progenies of the second generation were large enough to prove the point at issue. Except from this one mutation, no homozygous mutational type has segregated from any of the supposed heterozygous dominants. In the mind of one who is familiar with the group of the evening primroses a suspicion naturally arises that FROST's mutations are not Mendelian at all, but that they show the type of behavior familiar in *Oenothera lata* DeVries, and recently discovered in mutations from *O. stenomeres* and *O. pratincola*. These mutations always give progenies consisting of a mixture of the parental and mutational types. In the case of *O. lata* the cytological explanation is now so well known as hardly to require comment; it certainly suggests that a cytological examination of the *Matthiola* mutations would not be amiss. Reciprocal crosses between the mutational and parental types might also throw light on the possible analogy between the evening primroses and stocks, for in such types as *Oenothera lata* mutational characters are carried only by part of the female gametes, and by none of the male gametes. All that FROST tells about the *Matthiola* mutations so exactly parallels what is found in *Oenothera* that one can hardly refrain from suggesting, in the absence of data supporting his own interpretation, that instead of discovering new Mendelian dominants he has found in a widely distant group some of the perplexing phenomena which critics of the mutation theory persist in regarding as peculiar to *Oenothera*. More and more facts are coming to light in groups other than *Oenothera* which do not fall into line according to Mendelian expectations. As an example of what looks like mutation in the DeVriesian sense, one thinks of the rogues of peas, investigated by BATESON; as an example of matroclinic, non-segregating hybrids, quite comparable to those of *Oenothera*, we have the cases in *Primula*, recently reported by PELLEW and DURHAM. If the type of heredity shown by *Oenothera lata* were found to apply to the mutations of *Matthiola*, it would be almost as interesting as the discovery of new Mendelian dominants.—H. H. BARTLETT.

Respiration in succulents.—That succulent plants exhibit peculiarities in their respiratory processes and periodic changes in acidity with light and darkness has been known for a long time. RICHARDS[3] has investigated these

[3] RICHARDS, HERBERT M., Acidity and gas interchange in cacti. Carnegie Inst., Washington, Publication no. 209. pp. 107. 1915.

periodic acidity changes and the respiratory ratios in cacti, a group heretofore not sufficiently studied. Extensive work has been done, principally with *Opuntia versicolor*, with results in general agreement with what is already known regarding respiration in succulents. The paper presents a large mass of data, and considers the influence of light, temperature, oxygen supply, and wounding on the acidity of the tissues, and devotes considerable space to the relation of acidity, light, temperature, oxygen, etc., to the rate and ratio of gas interchanges. The production of the acid, chiefly malic acid in cacti, is thought to be due to lack of oxygen in the tissues, owing to anatomical structures which, to restrict transpiration, restrict the other gas exchanges as well. During the night the acid accumulates, because the chief factors capable of causing deacidification, namely, light, high temperature, prolonged darkness, and unusually high oxygen pressures, are absent.

The true respiratory quotient for cacti is low, and can be measured accurately only when acidity is stationary or rising. For during falling acidity, the approach of the ratio to the typical ratio, unity, is not real, because the increased CO_2 is furnished merely by the decomposition of the acid, which is not considered a respiratory process. Some of the minor points brought out are that while CO_2 production closely parallels rise and fall of temperature, it lags behind by about an hour, maximum and minimum CO_2 production being reached about an hour later than maximum and minimum temperature; and that total acidity increases more rapidly than the acid concentration of the juice. This is reasonably traced to greater hydration of the colloids in the presence of the acids, and to an increased osmotic pressure in the cell sap leading to greater turgidity.

The main point of interest to physiologists is the interpretation of the phenomena, which differs somewhat from that of NATHANSOHN, who looked upon the breaking down of the acids by day as a completion of the respiratory process at a time when CO_2 could be used in photosynthesis. This view makes the CO_2 production during deacidification a source of respiratory energy, and at the same time of great biological significance in conserving the raw materials for photosynthesis. RICHARDS considers the acid the end product of respiration rather than an intermediate product. The breaking down of the acid by day is due chiefly to light, aided by the accompanying high temperature. The reaction is photolytic and not respiratory, probably takes place in the cell sap, and therefore probably yields its energy not in connection with the living protoplasm. He points out that CO_2 production during deacidification may be so rapid as to exceed photosynthetic use of the gas, and states that "whatever of energy there may be from the final oxidation of the acid outside the sphere of protoplasmic activity is simply the result of anatomical peculiarities of the plant, the advantages of which may well outweigh this loss."

The whole problem of acidity and gas exchange under life conditions is necessarily a very complex one because so many variable factors are involved,

and a careful reading of the paper emphasizes this fact. Conclusions must therefore be drawn with considerable care.—CHARLES A. SHULL.

Insects and plant diseases.—Although both botanists and entomologists have realized for a long time that insects are carriers of organisms of plant diseases, very little attention has been given to the study of the subject. However, there is now a tendency to take up this line of investigation. Four papers have come to the reviewer's desk recently.

A paper by RAND[4] on the dissemination of the bacterial wilt of cucurbits follows out a suggestion given by ERWIN F. SMITH and produces evidence indicating that this leaf-eating cucumber beetle (*Diabrotica vittata*) is both the summer and the winter carrier of the *Bacillus tracheiplilus* which causes the wilt of cucumbers and other cucurbits.

In a later paper by RAND and ENLOWS,[5] the authors not only confirm the conclusions given by RAND in the first paper, but also include the 12-spotted cucumber beetle (*D. duodecimpunctata*) as an important summer carrier of this organism. In experiments by the same authors, the squash bug (*Anasa tristis*), the flea beetle (*Crepidodera cucumeris*), the melon aphis (*Aphis gossypii*), and the 12-spotted lady beetle (*Epilachna borealis*) did not transmit the disease.

Another paper by HYSLOP[6] on *Triphleps insidiosus* and corn rots gives conclusive evidence that this insect is the carrier of the fungi causing ear rots. In view of the fact that this insect has been considered beneficial since about 1881, HYSLOP's studies are of more than ordinary interest.

A fourth paper by STEWART and LEONARD[7] records their results with a number of experiments and comes to the conclusion "that all of the sucking bugs found in the nursery are of more or less importance in producing fire blight infections and must be considered *tout ensemble*. The relative importance of each species is difficult to determine. By virtue of their method of feeding and prevalence during each season, certain species are undoubtedly more destructive than others. On the other hand, under special conditions when a certain species is found in large numbers it may become of considerable importance. Usually the tarnished plant bug is more injurious than the leaf-hopper from the fact that the greater percentage of leaf-hopper punctures occur in the leaf tissue."—MEL T. COOK.

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[4] RAND, F. V., Dissemination of bacterial wilt of cucurbits. Jour. Agric. Research **5**:257–260. 1915.

[5] RAND, F. V., and ENLOWS, ELLA, M., Transmission and control of bacterial wilts of cucurbits. Jour. Agric. Research **6**:417–434. 1916.

[6] HYSLOP, J. A., *Triphleps insidiosus* as the probable transmitter of corn ear rot (*Diplodia* sp. *Fusarium*). Jour. Econ. Entomology **9**:435–437. 1916.

[7] STEWART, V. B., and LEONARD, M. D., Further studies on the rôle of insects in the dissemination of fire blight bacteria. Phytopath. **6**:152–158. 1916.

Taxonomic notes.—BAILEY[8] has published in advance some of the changes in nomenclature that will appear in the *Standard Cyclopedia of Horticulture*. The changes selected for publication involve the names of 100 species and varieties, and some of the changes affect North American species. For example, the retention of *Malus* in *Pyrus* involves changes in 24 names; while a new interpretation of *Statice* as contrasted with *Limonium* calls for changes in 43 names. The author pays his respects to a certain type of taxonomic work as follows: "It has been the desire, in the compilation of the cyclopedia, to accept new generic limitations with caution. The temper of the present times is to find differences, as opposed to the tendency of the immediately preceding workers to find agreements. The analytic intention is the mark of systematic work in this generation, as the synthetic intention was the mark of the past generation. There is reason to expect a return from the method of disunion to the method of relationships; and as a work designed for the use of horticulturalists, who cannot be skilled in bibliography and pedantry, should be conservative, I have thought it best, so far as possible, to avoid unnecessary and fantastic sub-divisions."

CONARD[9] has revived the discussion concerning certain generic names of our water lilies. With the help of even the more conservative manuals, we were accustoming ourselves to say *Castalia* when we thought of *Nymphaea*, and to say *Nymphaea* when we thought of *Nuphar*. Now CONARD has shown that the valid generic name for the white water lilies is *Nymphaea* after all, and for the yellow pond lilies is *Nuphar*.

FERNALD[10] has discussed the species of *Sabatia* usually recognized as occurring in New England, and has described a new species (*S. Kennedyana*) occurring in Massachusetts and Rhode Island.—J. M. C.

Life cycles of bacteria.—LÖHNIS and SMITH,[11] in a preliminary communication, present some of their conclusions from a study of 42 strains of bacteria. All of these strains showed life cycles "not less complicated than those of other microorganisms"; and the authors are inclined to believe that this may be true of all species of bacteria. The forms studied live alternately in an organized and in an amorphous stage, the latter being called a "symplastic" stage, because in this stage the separate cells undergo "a thorough mixing." From this "symplasm" new individual cells arise in various ways. In all cases what are called "regenerative units" become visible, which increase in size, and

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[8] BAILEY, L. H., Nomenclatorial transfers. Rhodora **18**:152–160. 1916.

[9] CONARD, HENRY S., *Nymphaea* and *Nuphar* again. Rhodora **18**:161–164. 1916.

[10] FERNALD, M. L., The genus *Sabatia* in New England. Rhodora **18**:145–152. *pl. 121*. 1916.

[11] LÖHNIS, F., and SMITH, N. R., Life cycles of the bacteria. Jour. Agric. Research **6**:675–702. *pls. 1–7*. *fig. 1*. 1916.

later "either by germination or by stretching become cells of normal shape." A direct union of two or more individual cells was also observed, the significance of which was not studied. The authors state that "the life cycle of each species of bacteria studied is composed of several subcycles, showing wide morphological and physiological differences. They are connected with each other by the symplastic stage. Direct changes from one subcycle into another occur, but they are rather rare exceptions." It is obvious that if such life cycles are established for bacteria in general, a new field is opened up in bacteriology.—J. M. C.

Cane sugar and translocation.—BOYSEN-JENSEN[12] concludes that cane sugar plays an important rôle in the germination of pea seeds. In the first stages of germination the cane sugar present in the ungerminated seed is used both as building and respiratory material, as is evident from its reduction in amount during the first few days of the process. In the second stage of germination cane sugar is the translocation form of the starch, as is shown by the following facts: (1) there is a higher concentration in the cotyledons than in the axillary organs; (2) the concentration rises with time in the isolated cotyledons and falls in the isolated axillary organs; (3) only inconsiderable amounts of reducing sugars are present in the cotyledons. The author cites several investigations showing the frequent appearance of cane sugar as the translocation form of starch, and concludes by saying that either monosaccharides or disaccharides may be translocation forms of starch, depending upon the character of the plant part.—WILLIAM CROCKER.

Cabbage yellows.—GILMAN[13] has investigated this disease and the relation of temperature to its occurrence. It is a wilt disease caused by *Fusarium conglutinans*, which is a facultative parasite living in the soil, and under certain conditions becoming destructive to cabbage. It has a high optimum temperature and is very resistant to drying. Inoculation experiments were largely successful in inducing the disease, the failures being due obviously to variations in the virulence of the cultures and in the susceptibility of the host. Control plants remained entirely free from the disease. The appearance of the characteristics symptoms is dependent upon a temperature of 17–22° C. or above, lower temperatures preventing the occurrence of the disease. Field observations through three seasons confirmed this relation between the occurrence of the disease and high temperature.—J. M. C.

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[12] BOYSEN-JENSEN, P., Vorkommen, Bedeutung, und Bildung des Rohrzuckers bei der Keimung von *Pisum sativum*. Jahrb. Wiss. Bot. **56**:431–446. 1915. PFEFFER'S Festschrift.

[13] GILMAN, J. C., Cabbage yellows and the relation of temperature to its occurrence. Ann. Mo. Bot. Gard. **3**:25–84. *pls. 2. figs. 21.* 1916.

Rot of potato tubers.—HAWKINS,[14] continuing his studies on the effect of various fungi on their hosts, has investigated the effect of *Fusarium oxysporum*, *F. radicicola*, and *F. coeruleum* on the sugar content, both sucrose and reducing sugar, pentosans, methyl pentosans, galactans, dry matter, starch, and crude fiber of the potato tuber. The crude fiber content of the tubers was not reduced, starch and methyl pentosans were not affected appreciably, while the content of the other substances was reduced. It is interesting from the point of view of resistance to fungus invasion that the least digestible forms occur in greatest proportions in the skin and cortical regions of the tuber. *Fusarium oxysporum* and *F. radicicola* were found to secrete sucrase, maltase, xylanase, and diastase. The diastase, like the malt diastase that BROWN and MORRIS worked with, is incapable of attacking ungelatinized potato starch.—GEORGE K. K. LINK.

Phytoplankton of the oriental tropics.—OSTENFELD[15] has published a list of the phytoplankton of one of the straits of the Malay Archipelago. The list is based chiefly upon a large collection of drawings made by P. TH. JUSTESEN in 1909 and 1910, while residing at one of the small military stations in the Dutch Indies. The list includes 100 species, the largest group being the diatoms, with 56 species representing 23 genera. The Peridiniales constitute the other large group, including 40 species in 11 genera, the largest genera being *Ceratium* with 17 species, and *Peridinium* with 12 species. The general character of the plankton is said to be that of a "tropical neritic plankton," very much like the plankton examined by CLEVE and OSTENFELD from the Malay Archipelago and the Gulf of Siam.—J. M. C.

Branched prothallia.—Miss WUIST[16] has investigated the early stages of the gametophytes of the Polypodiaceae in reference to branching, subjecting them to various culture conditions. She observed branching in cultures of 15 species representing 9 genera. Branching, which was both dichotomous and monopodial, was not a response to any one type of culture medium, but appeared on distilled water, on soil, and on various nutrient solutions. Branches did not appear at any definite period in the life history of the gametophyte, but were formed by any cell of the filament, by divisions of the last cell of the filament, and from the margin and apex of the expanded portion of the prothallium. The author has concluded that a definite relation exists between branching and nutrition.—J. M. C.

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[14] HAWKINS, LON A., Effect of certain species of *Fusarium* on the composition of the potato tuber. Jour. Agric. Research **6**:184–196. 1916.

[15] OSTENFELD, C. H., A list of phytoplankton from the Boeten Strait, Celebes. Dansk Bot. Arkiv **2**:no. 4. pp. 18. *figs. 10.* 1915.

[16] WUIST, ELIZABETH D., Branched prothallia in the Polypodiaceae. Bull. Torr. Bot. Club **43**:365–383. *figs. 15.* 1916.

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A COMPARATIVE STUDY OF WINTER AND SUMMER LEAVES OF VARIOUS HERBS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 224

J. P. STOBER

Introduction

The structure of most plants varies with the habitat and even with the varying conditions of the same habitat. This has been emphasized by GREVILLIUS,[1] CHERMEZON,[2] COWLES,[3] STARR,[4] and others. GREVILLIUS made an extensive comparative study of vegetation growing on the island Öland. He compared the plants of a dry, rocky, treeless plain (alvar) with the same species growing in favorable regions. The former he calls alvar forms; the latter, normal forms. The alvar forms, in general, were more hairy and had a more highly cutinized and thicker epidermal wall, a more compact palisade parenchyma, and more closely crowded stomata than the normal forms.

These structural peculiarities due to environmental changes may be observed readily in almost any plastic plant. *Oenothera*

[1] GREVILLIUS, A. Y., Morphologisch-anatomische Studien über die xerophile Phanerogamenvegetation der Insel Öland. Bot. Jahrb. **23**:24–108. 1897.

[2] CHERMEZON, H., Recherches anatomiques sur les plantes littorales. Ann. Sci. Nat. Bot. **12**:117–313. 1910.

[3] COWLES, H. C., The ecological relation of the vegetation of the sand dunes of Lake Michigan. BOT. GAZ. **27**:95–117, 167–202, 281–308, 361–391. 1899.

[4] STARR, ANNA M., Comparative anatomy of dune plants. BOT. GAZ. **54**: 265–305. 1912.

biennis, for example, when growing in a dry, sterile soil and exposed to strong wind and maximum sunlight, is found to have smaller and thicker leaves, more perfectly developed palisade parenchyma, a more hairy and a more densely cutinized epidermis, and, in general, a more xerophytic structure than the same species growing under more favorable conditions.

A similar structural difference is apparent in summer and winter leaves, or in stem and rosette leaves. Winter leaves, as the name implies, exist during the winter, which, in our latitude, is the most unfavorable season of the year. During the winter transpiration becomes relatively excessive because of the reduced rate of absorption, and plants are thus put to the severest test. Sometimes for days at a time the ground is frozen and absorption is practically zero; while during the warmest part of the day considerable transpiration may take place. The plant is thus exposed to the danger of desiccation. Moreover, during the night the most exposed leaves may freeze hard. Toward noon of the following day they may thaw out, presenting a wilted condition as if killed by scalding. However, it is surprising how quickly such leaves will revive as conditions again become more favorable. No sooner is the absorption of soil water resumed than the leaves once more become turgid and resume their wonted appearance, apparently none the worse for the ordeal through which they have passed.

Since winter leaves are exposed to such severe conditions, it would be natural to suppose that they must be quite xerophytic in structure. While this is true to a certain extent, in some respects they are less exposed to unfavorable conditions than stem leaves. This is especially true of winter leaves occurring in rosettes. In rosettes the internodes are extremely short and the leaves thus become closely crowded and overlapping. Since epinasty prevails during the winter, these overlapping leaves lie almost flat on the ground, thus affording maximum protection for each other from sudden changes of temperature, as well as from high winds and excessive transpiration. It is seldom that winter leaves die as the direct result of freezing, and when it is borne in mind that such leaves have a low water content and a high osmotic pressure, thus insuring easier absorption of soil water, the protection would seem

ample, regardless of any special protective structures. The stem leaves, on the other hand, are usually borne some distance above the ground and exposed to greater intensity of light, stronger winds, and greater extremes in temperature, humidity, and transpiration.

Method of study

Most of the plants used in this comparative study were collected in the region about Chicago; the remainder, in eastern Pennsylvania. In order to simplify matters, all winter leaves, whether produced in typical rosettes, on prostrate runners, or on basal shoots, will be designated as rosette leaves, and summer leaves will be designated as cauline or stem leaves.

Leaves for study were killed, fixed, and preserved in a 4 per cent solution of formaldehyde in 50 per cent alcohol. Delafield's haematoxylin was used as a general staining reagent. Sections were also treated with chloriodide of zinc, the cellulose wall turning blue, while the cuticle and cutinized portions of the epidermal wall turned yellow. Alcannin tincture imparts a pink color to cutin, but is much slower in its action than chloriodide of zinc.

Unless otherwise specified, all observations were made on the middle of the leaf, from the midrib to the margin. All observations and measurements were made with $\frac{2}{3}$, $\frac{1}{6}$, and $\frac{1}{12}$ in. (oil immersion) objectives, and with a 1 in. micrometer eyepiece with divisions of 0.1 mm. Camera lucida drawings were made of portions of the epidermis for measurement and comparison of epidermal cells and stomata. Chloral hydrate was used as a clearing agent for leaves to facilitate the study of air spaces and packing of mesophyll tissues. Most measurements and counts represent an average of 5–20, depending upon the degree of variability of the objects measured. Measurements are expressed in microns, and counts represent the number in the field under low or high power, which is indicated in each case. Different plants of the same species (in cases where the plants could readily be secured) were studied at different times and the results compared. These results varied only slightly when the plants came from the same habitat, but usually differed considerably in plants from different habitats.

With such a tendency to variation in plants, few measurements and counts can be regarded as absolutely fixed, but the final results in any case do not materially affect the principles involved.

Epidermal hairs

There is considerable variation in the kind, number, size, and distribution of epidermal hairs, not only in different plants of the same species, but also on different leaves of the same plant, or even on different parts of the same leaf. Some plants, such as *Oenothera biennis*, vary greatly when grown under different physical conditions. In a low, moist, and comparatively shady habitat the leaves of *Oenothera* are thin, and the hairs rather weak and comparatively few and scattered. On a dry slope or bank along the roadside, the leaves are decidedly thicker, and the hairs stouter and very much more abundant; while under intermediate conditions corresponding variations have been observed.

Oenothera is an extremely plastic plant, responding readily to changed conditions of environment. *Leonurus Cardiaca*, *Lepidium virginicum*, *Capsella Bursa-pastoris*, and others also show some variations, but not to the same extent as *Oenothera*. *Verbascum Blattaria*, on the other hand, is glabrous no matter under what physical conditions it may be growing. Occasionally, when growing on a dry bank along a dusty roadside, a few hairs may be found on the ventral side of the midrib of the lower stem leaves and upper rosette leaves. This plant is extremely rigid and does not at all, or but slightly, yield to changing conditions of environment. It is perhaps a good illustration of a congenital mesophyte.

In studying the number and distribution of hairs, *Oenothera biennis*, *O. rhombipetala*, *Leonurus Cardiaca*, *Lepidium virginicum*, *Capsella Bursa-pastoris*, and *Hieracium paniculatum* were selected as types. Care was taken to collect both the stem and rosette plant of each species in the same or as nearly the same habitat as possible. Five plants of each species were studied, and the counts for each particular kind of hairs were averaged and tabulated. The field of the low power of the microscope was adopted as the unit area of observation, and the average of 5 or more counts was taken as the number for each area under observation.

From the tabulated results of observations made on these species of plants and a careful study of a number of other species, the following conclusions can be formulated. (1) Epidermal hairs are most abundant on the upper stem leaves, and decrease, as a rule, to the lowest stem leaves, and from the upper to the lowest rosette leaves. On the basal leaves of both stem and rosette are found the smallest number of hairs. (2) Hairs are also more abundant on the lower than on the upper surface of the leaf, usually being most abundant on the ribs, veins, and margin of the leaf. (3) Hairs are most abundant toward the base of leaves, although in basal stem and rosette leaves the reverse is usually the case. (4) Young leaves are more hairy than older ones. This may be due partly to the fact that in young immature leaves the epidermal cells have not yet reached their mature size and therefore the hairs will of necessity be more crowded than in a mature leaf. This diminished hairiness in older leaves also may be due in part to the fact that hairs in the course of time may break off, or for some reason or other drop off, and thus reduce the number per unit area of surface. (5) Exposure to sun, wind, and other desiccating influences tends to increase the hairiness in the upper stem leaves. Transpiration, wind, moisture, and character of soil are undoubtedly potent factors in determining hair production, but that these are not the only factors is clearly shown by the fact that young leaves just emerging from buds, and therefore most protected, are usually most hairy, sometimes even tomentose.

As stated before, some leaves are most hairy toward the base, where the leaf is most protected from those influences that would ordinarily tend to produce hairiness. It is difficult also to see why *Verbascum Thapsus* and *V. Blattaria* should grow side by side, the one glabrous and the other extremely hairy. So far as hairiness is concerned, it would seem that the former is a congenital xerophyte while the latter is a congenital mesophyte.

It is difficult also to see that hairiness is beneficial to plants, and that these epidermal outgrowths protect the plant against excessive transpiration, against the ravages of animals and parasites of various kinds, against excessive sunlight, etc., when *Verbascum Blattaria*, entirely devoid of hairs and with only a slightly

thicker epidermal wall, is fully as successful in the struggle for existence as *V. Thapsus* growing by its side, so thoroughly protected by an abundance of epidermal hairs. It is not difficult to see that the woolly coating may be advantageous to young leaves, just emerging from the bud; but it is extremely difficult to find any advantage in the few simple and stellate hairs scattered over the leaves of *Lepidium* and *Capsella*.

Stomata

In over two-thirds of all the plants studied the stomata were found to be more abundant on stem than on rosette leaves. Sometimes this difference in number is only slight, but sometimes, as in *Mitella diphylla*, *Lepidium virginicum*, *Monarda punctata*, *Aquilegia canadensis*, *Campanula rotundifolia*, *Capsella Bursa-pastoris*, and *Geum album*, this difference is considerable. Stomata are also most abundant on the lower side of the leaf. This is true of about 80 per cent of all the plants studied. This difference is most pronounced in leaves that have their upper and lower sides well developed, such as the broad mesophytic rosette leaves. Narrow, xerophytic stem leaves, such as have both sides almost equally exposed to light and air, have approximately the same number of stomata on both sides. The more xerophytic the leaves, the greater are the number of stomata as compared with the corresponding mesophytic leaves. As a rule, the size of stomata is correlated with the number. The larger the number of stomata on a given leaf surface the smaller they are. This was found to be true in over 60 per cent of the specimens compared. Broad mesophytic rosette leaves have fewer but larger stomata on a given surface than the corresponding narrower, more xerophytic stem leaves. In these there is a larger number of stomata per unit surface, but the stomata are decidedly smaller in size.

There also seems to be a correlation between the number and size of stomata, and the size of epidermal cells. The broad rosette leaves have, as a rule, larger epidermal cells. With these larger cells are associated fewer but larger stomata.

Anterior-posterior orientation of stomata is noticeable in the stem leaves of *Campanula rotundifolia*, *Linaria canadensis*, *Arabis*

lyrata, *A. brachycarpa*, *A. laevigata*, and *Satureja glabra;* and in both stem and rosette leaves of *Artemisia caudata* and *Lechea villosa*. All these leaves are linear or oblong. Not all linear or oblong leaves have their stomata longitudinally oriented, but such orientation is characteristic of linear and oblong leaves, especially if the epidermal cells are longitudinally elongated.

The stomata of the species investigated are not sunken below the surface in either stem or rosette leaves, except in the sand dune xerophytes, *Artemisia canadensis* and *A. caudata*, where they are depressed about half the thickness of the epidermis. In a few instances the stomata seemed even to be elevated slightly above the surface. In *Mitella diphylla*, *Leonurus Cardiaca*, *Aquilegia canadensis*, and *Chelidonium majus*, the stomata are confined to the ventral surface of the leaf.

Rosette stomata are not only larger but also more elongated than stem stomata. Stem stomata are not only smaller but also more nearly round than rosette stomata. Perhaps the number of stomata ought to be correlated with the mass of the chlorenchyma. The smaller number of stomata in the broad, thin (frequently thicker than stem leaves), mesophytic rosette leaves, when compared with the smaller mass of chlorenchyma to be aerated, may be relatively as abundant as the larger number per unit surface in the long, narrow, thick xerophytic stem leaves, where a larger mass of chlorenchyma must be aerated through a given surface area; that is, the number of stomata is correlated with the amount of chlorenchyma to be aerated, and not with the mere surface area of the leaf. The number of stomata also seems to be correlated with the thickness of the cuticle and cutinized outer wall of the epidermis. The greater the thickness, the less is the possibility of gases passing through, and the greater is the need for stomata. It is probably for these two reasons, the greater mass of chlorenchyma per leaf surface and the greater thickness of the cuticle and cutinized outer wall of the epidermal cells, that xerophytic leaves have an increased number of stomata in a given surface area. The relatively thinner and frequently more shaded rosette leaves are broader and have a thinner cuticle, a thinner outer epidermal wall, and a greater development of air

lacunae. Such leaves need fewer and are provided with a smaller number of stomata. Stomata are not needed for transpiration, since transpiration is believed to be a necessary evil. It seems strange, therefore, that in xerophytic leaves, where there is effected the greatest protection against the loss of water by the development of a thick cuticle and a thick outer epidermal wall, there should be the development of a large number of stomata, thus facilitating the loss of water from the plant through stomatal transpiration.

In leaves whose outer epidermal wall and cuticle are thin, there is less need of stomata to facilitate exchange of gases in photosynthesis and respiration, since under such circumstances considerable interchange of gases can take place through the epidermis. There is no doubt that mesophytic rosette leaves with a reduced number of stomata have an ample supply of stomata to meet their needs. Moreover, rosette leaves are close to the soil and are therefore more advantageously situated for the intake of carbon dioxide than are stem leaves. In stem leaves the pressure of CO_2 cannot accumulate beyond 0.0003 A, or about 0.22 mm. Hg, since above this pressure it diffuses outward. But in rosette leaves close to the ground, where the exhalation of CO_2 from the soil often increases the CO_2 to 10 or more times the normal amount, a much higher pressure of CO_2 may accumulate. This increased amount of CO_2 in rosette leaves is available for carbohydrate synthesis in all cases where the leaves are not too much shaded. But since plants under normal conditions receive much more energy of sunlight (about 4 or 5 times as much) than is necessary to synthesize the small amount of available CO_2, rosette leaves are most advantageously situated for photosynthesis in spite of the reduced number of stomata and the diminished amount of light. These facts have an important bearing upon the development of chlorenchyma and air spaces in rosette leaves.

Epidermal cells

In monocotyledonous plants the epidermal cells are usually elongated. In dicotyledonous plants they are generally elongated along the ribs and larger veins, but elsewhere they may be polygonal and nearly isodiametric in outline, or entirely irregular. The

shape of the leaf, to a certain extent, determines the shape of the epidermal cells. In narrow and elongated, or linear, leaves, such as those of the stems of *Arabis brachycarpa*, *A. lyrata*, *Linaria villosa*, and *Artemisia caudata*, the epidermal cells also are elongated or linear. In such elongated or linear cells the lateral walls are quite regular. The upper epidermal cells, however, are usually more regular than the lower, except in such stem or rosette leaves as are almost equally exposed to light. Such leaves have both surfaces almost equally exposed to desiccating influences, hence the shape and size of the epidermal cells on both sides of the leaf are practically the same. This is very apparent in such xerophytic stem leaves as those of *Linaria villosa*, *Arabis lyrata*, *A. brachycarpa*, *A. laevigata*, and *Campanula rotundifolia*.

The shape and size of epidermal cells vary greatly, not only in different species and in individuals of the same species, but even in stem and rosette leaves of the same individual. There may even occur considerable variation in different parts of the same leaf. Thus in *Leonurus Cardiaca* the sinuosity of the lateral walls increases slightly from the lower to the upper stem leaves. In *Geum album* the sinuosity seems to increase from the upper to the lower rosette leaves. In *Lepidium virginicum* the sinuosity is practically the same from the upper stem leaves to the lowest rosette leaves. However, the sinuosity in the lower epidermis, in the case of *Lepidium*, is greater than in the upper epidermis. The lateral walls of the lower epidermal cells are, as a rule, more sinuous than those of the upper epidermis, and in the majority of instances (70 per cent) the sinuosity is greater in rosette than in stem leaves. Sinuosity of the lateral wall culminates under the most mesophytic conditions. Increased transpiration tends to produce relatively straight lateral walls. Hence we find the epidermal walls of the stem leaves less sinuous than those of rosette leaves, and those of the upper surface of both stem and rosette leaves less sinuous than those of the lower surface, since stem leaves are more xerophytic than rosette leaves, and the upper side of leaves more xerophytic than the lower side. Sinuosity of lateral epidermal walls is not known to be of special significance to plants. It may add a little to the strength of the epidermis and afford a larger diffusion surface

for substances passing from cell to cell. No chloroplasts are present in epidermal cells except in guard cells, and, to a slight extent, in winter leaves of *Leonurus Cardiaca*.

As to size, the upper epidermal cells are larger than the lower, and, with few exceptions, the epidermal cells of rosette leaves are larger than those of stem leaves. In 80 per cent of all observations made the epidermal cells of rosette leaves were found to be larger than those of stem leaves. The size of epidermal cells is somewhat correlated with the size of leaves, the larger leaves having the larger epidermal cells; but there are so many exceptions to this that such a general statement is not warranted.

The vertical diameter of epidermal cells is greater, as a rule, in rosette than in stem leaves (true of 80 per cent of cases), in the upper than in the lower epidermis, and usually increases from the apex toward the base of the leaf. In the middle rosette leaves the maximum diameter is usually found in the middle of the leaf. In *Capsella* there is a gradual increase from the upper stem to the lowest rosette leaves. As a rule, the maximum diameter is attained in both the middle stem and rosette leaves.

Blade, epidermal wall, and cuticle

The blade decreases in thickness from the apex to the base of the leaf. It also decreases from the upper to the basal leaves. This is less apparent in middle leaves, where the leaf sometimes increases in thickness from apex to base, or where the maximum thickness of the blade occurs in the middle of the leaf. Those leaves or parts of leaves most shaded are usually thinnest. Rosette leaves are thicker than stem leaves, owing to a greater development of spongy parenchyma. This is true more particularly of the middle and basal stem and rosette leaves. The upper stem leaves, especially the apical portions of those leaves, are frequently thicker than the corresponding portions of rosette leaves. The blade, in most instances, also appears thicker than the blades of stem leaves. Notable exceptions are *Arabis lyrata*, *A. laevigata*, *Linaria canadensis*, *Leonurus Cardiaca*, *Campanula rotundifolia*, and *Monarda punctata*. All these species, except *Leonurus Cardiaca*, have either linear or oblong lanceolate stem leaves, while the basal leaves

are broad and thin. These are plants that have almost a typical xerophytic shoot and a mesophytic rosette.

The outer epidermal wall is decidedly thicker in stem than in rosette leaves. In each of the 3 types considered, *Lepidium virginicum*, *Capsella Bursa-pastoris*, and *Chrysanthemum Leucanthemum*, the stem leaves are borne considerably above the ground and rather widely separated from each other, thus exposing them freely to air, sunlight, and desiccating winds. The rosette leaves, on the other hand, are close to the ground and considerably shaded; hence we should naturally expect this difference in thickness of epidermal cell walls. There is a slight tendency for the wall to diminish in thickness from the apex to the base of the leaf. The maximum thickness is usually reached in middle stem and apical rosette leaves, while the maximum thinness is probably to be found in the lowest rosette leaves. The outer epidermal wall on the upper surface of the stem leaves is but slightly thicker than that of the lower, especially in those upper stem leaves that grow obliquely upward so as to expose both surfaces almost equally. In the lower stem and rosette leaves this difference is much greater, the epidermal wall on the lower side being considerably thinner. The thickness of the cuticle varies with the thickness of the outer epidermal wall, the thickest wall having the thickest cuticle. The cuticle of the stem leaves of the types treated is decidedly thicker than that in the rosette leaves. It is thicker on the upper than on the lower surface of the leaf, except in the upper stem leaves, where both surfaces are about equally exposed. Here the lower cuticle is almost as well developed as the upper. The greatest decrease in thickness of cuticle is observable in the basal rosette leaves.

In interpreting the facts set forth it must be borne in mind that only middle stem leaves are compared with middle rosette leaves, and that whatever conclusions may be deduced must rest upon this comparison. Most plants have their rosettes better protected than their shoots. In 83 per cent of 30 plants observed, the cuticle is thicker in rosette than in stem leaves. In at least 75 per cent of the number the epidermal wall is also thicker in rosette than in stem leaves. Thus it seems that when the effective means of

protection of the middle stem leaves and the middle rosette leaves are compared, the preponderance of protection is in favor of the rosette leaves. However, it must be borne in mind that the difference in thickness of wall and cuticle in a number of instances is so slight as to be almost negligible. Moreover, in notable instances the stem leaves have a decidedly thicker wall and cuticle. This is true of *Chrysanthemum Leucanthemum*, *Capsella Bursa-pastoris*, *Artemisia caudata*, *Satureja glabra*, *Scutellaria parvula*, and others. *Chrysanthemum* has broad, spatulate rosette leaves on long slender petioles, while the stem leaves are oblong or oblanceolate, and have a decidedly xerophytic form and structure. The stem leaves of *Lepidium*, *Capsella*, *Satureja*, and, to a certain extent, *Scutellaria*, in like manner have a decidedly xerophytic form and structure as compared with their corresponding rosette leaves. *Artemisia* is one of those sand dune xerophytes whose stem and rosette leaves are finely dissected and almost equally exposed, and hence almost equally xerophytic in form and structure. In such mesoxerophytes as *Verbascum Thapsus*, whose leaves are thoroughly protected by a woolly coat of branching multicellular hairs, the difference in protection of stem and rosette leaves is also slight. Some plants, therefore, seem to have xerophytic shoots and mesophytic rosettes; others show a tendency to xerophytic rosettes and mesophytic shoots; while in still others the distinction is not evident.

Chlorenchyma

The apical, middle, and basal stem and rosette leaves of certain plants were studied with a view to determining the similarities and differences of the chlorenchyma of the corresponding regions of the stem and rosette leaves of the same plant. For example, an apical stem leaf and an apical rosette leaf would be selected for comparative study. Sections through the apical region of the stem leaf were then studied and the results compared with those obtained from a similar study of corresponding sections of the rosette leaf. Sections through the middle and basal regions of the leaves were similarly studied and compared. After the apical leaves were thus studied, the middle stem and middle rosette leaves, as well as the basal stem and basal rosette leaves, were similarly

studied. The thickness of the leaf, the thickness of the palisade parenchyma, the thickness of the spongy parenchyma, the number of the palisade layers of cells, and the average size of the cells of each layer, together with the size, shape, and arrangement of the cells of the spongy parenchyma, were the leading points of observation in this comparative study. All measurements are expressed in microns, and were made approximately 800 μ from the midrib of the leaf. For want of space the tabulated results cannot be given; a general summary in each case must suffice.

LEPIDIUM VIRGINICUM.—In the upper stem leaves the palisade parenchyma is almost equally developed in both the upper and lower side of the leaf. This may be due to the fact that the leaves stand at a very acute angle to the stem and are almost equally illuminated. In the middle stem leaves a lower palisade tissue is found only in the apical region of the leaf. No lower palisade layers are found in the lower stem leaves or in any of the rosette leaves. The palisade layers of the upper stem leaves are quite compact. The cells reach a maximum length in the middle stem leaves. In the basal stem leaves the cells become larger and more rounded, the layers are less closely packed and less definitely organized. The palisade cells of the rosette leaves are larger, having a decidedly greater diameter, and on the whole are less compactly arranged than in stem leaves. The upper and middle stem leaves have the thickest outer epidermal wall and cuticle. This is also true of the upper rosette leaves when compared with the lower. The difference, however, between the thickness of the epidermal wall and cuticle of the apical and basal leaves is much greater in rosette than in stem leaves.

CAPSELLA BURSA-PASTORIS.—The outer epidermal wall of stem leaves is thicker in the upper than in the basal leaves, attaining a maximum in the middle leaves. The cuticle is proportionally thickest in the upper leaves and thinnest in the proximal part of the basal leaves. Similar conditions obtain in the rosette leaves, except that the contrast between apical and basal cells is less pronounced. Palisade tissue is best developed in both upper stem and upper rosette leaves. Palisade cells are slightly longer and decidedly thicker in rosette than in stem leaves. The cells of the

spongy parenchyma are decidedly more irregular in rosette leaves, and the tissue contains a maximum of air spaces. Palisade tissue is least developed in basal stem and rosette leaves, as well as in the basal region of the leaves themselves.

CHRYSANTHEMUM LEUCANTHEMUM.—The outer epidermal wall and cuticle of the upper and middle stem leaves are very much alike in thickness, but both are decidedly thicker than the corresponding epidermal wall and cuticle of the basal stem leaves, the latter being only about one-half as thick. Rosette leaves do not differ much from each other in the thickness of epidermal wall and cuticle, but the maximum thickness may probably be found in the middle leaf. Rosette leaves, as a whole, have a thinner epidermal wall and cuticle than stem leaves, being only one-half to two-thirds as thick. The palisade tissue is better developed, as a whole, in stem than in rosette leaves, and decidedly better developed in both stem and rosette leaves in apical and middle leaves than in basal leaves. The spongy parenchyma is slightly better developed in rosette leaves and in both kinds of basal leaves. Here is found also the maximum development of air spaces.

OENOTHERA BIENNIS.—The stem leaves are thickest in the apical region and gradually become thinner toward the base. There is also a gradual increase in thickness from the apical to the basal stem leaves. The upper rosette leaves are thickest in the apical region and become thin toward the base of the leaf. In the middle and lower rosette leaves the greatest thickness is found in the middle region. From this region they gradually become thinner toward both the apex and base of the leaf.

The spongy parenchyma in stem leaves gradually diminishes from the apical to the basal region of the leaf, but there is a gradual increase in amount from the apical to the basal leaves. In the upper rosette leaves the spongy parenchyma also gradually decreases from the apex of the leaf to the base. In the middle and lower rosette leaves, however, the greatest percentage of spongy tissue is found in the apical and basal regions. In stem leaves the palisade tissue is most developed in the apical region and least in the basal region. The maximum development is probably found

in the apical and middle regions of the basal leaves. The palisade cells of rosette leaves are decidedly broader or thicker than those in stem leaves, but are relatively slightly longer. The maximum development is found in the apical and middle regions of the leaf, or in those parts of the leaves having the greatest exposure to light and other desiccating influences.

The largest epidermal cells are found in the middle region of both stem and rosette leaves. It is also in the middle of leaves that both upper and lower epidermal cells have the greatest vertical diameter. The outer epidermal wall and cuticle of stem leaves are thickest in the apical leaves, and gradually become thinner toward the basal leaves. In the upper stem leaves the outer wall and cuticle diminish from the apical to the basal region. In the middle and basal stem leaves there is less difference, and in the lowest leaves there is practically no difference in thickness of the epidermal wall and cuticle in different regions. In the upper stem leaves there is not much difference in the thickness of the epidermal wall and cuticle of the upper and lower sides of the leaf; but in the lower stem leaves the thickness is decidedly greater in the upper than in the lower epidermis. The greatest difference in thickness is found in the lowest leaves.

In rosette leaves the situation in thickness of epidermal wall and cuticle is similar to that found in stem leaves. In the upper rosette leaves, however, there is a greater difference in thickness of wall and cuticle between the apical and basal regions of the leaf. The hairs on both stem and rosette leaves are longest and most abundant on the midrib and have larger veins than elsewhere. The hairs are most abundant on the upper stem leaves and gradually diminish in number and size to the basal leaves, where they are quite small (except on veins) and only half or even less than half, as abundant. On the upper leaves they are longer and more abundant on the lower than on the upper surface, and increase in length and abundance from the apex to the base. On the middle stem leaves they are similar in size and abundance on both sides of the leaf, but slightly decrease in number from apex to base. On the basal leaves the hairs are considerably reduced in size, but otherwise the situation is similar to that found in middle stem

leaves. On rosette leaves the hairs are slightly more abundant on the upper than on the lower surface, and gradually diminish in size and number toward the basal leaves. On the lower side of the basal leaves there are very few hairs except along the margin, where they are long and abundant. The chlorenchyma contains an abundance of needle-shaped crystals of calcium oxalate, arranged in bundles (raphides). These raphides are slightly more abundant in rosette than in stem leaves.

VERBASCUM BLATTARIA.—In this species the palisade tissue is best developed in the floral leaflets and in the upper stem leaves. Here the layers are well organized and compact, and the cells reach their maximum length. In the basal stem leaves the palisade cells vary considerably in length, some being quite long, while others are quite short. Moreover, the layers are poorly organized. In the middle and upper stem leaves 3 layers are well organized, a fourth layer being only partly organized. In the basal leaves there is no trace of a fourth layer. The thickness, or transverse diameter of the palisade cells, also increases appreciably from the upper to the lowest stem-leaves. In rosette leaves there is a gradual increase in the size of palisade cells from the upper to the basal leaves. In the latter the palisade tissue is poorly developed, the cells being very irregularly and loosely arranged, and scantily supplied with chloroplasts.

With the exception of a few hairs on the ribs of rosette leaves and lowest stem leaves, this plant is devoid of hairs. The basal stem leaves and rosette leaves have the largest epidermal cells, which also have the largest vertical diameter. The upper epidermal cells of both stem and rosette leaves always have a decidedly greater vertical diameter than the cells of the lower epidermis.

The floral leaflets and upper stem leaves have the thickest outer epidermal wall and cuticle. In these leaves there is very little difference between the upper and lower epidermis. In the upper rosette leaves we also find a thicker epidermal wall and cuticle, but the difference is less pronounced than in stem leaves.

A summary of the comparative study of the upper, middle, and lower stem leaves and the corresponding upper, middle, and lower rosette leaves, based upon the 5 species just considered,

and in addition *Leonurus Cardiaca* and *Verbascum Thapsus*, is as follows.

In general the lowest stem and rosette leaves, as well as the basal part of all leaves, are most protected and most shaded, and therefore have the most mesophytic structure. The leaves are thinnest; the outer epidermal wall and cuticle are thinnest; the palisade parenchyma is developed most poorly; and spongy parenchyma, containing a maximum of air spaces and a minimum of chloroplasts, is developed most highly.

The upper stem leaves are relatively xerophytic in structure. This is especially true of the apical region of these leaves. We frequently find the maximum thickness of leaf, maximum thickness of epidermal wall and cuticle, and a maximum development of palisade tissue, which in many instances develops almost equally on both sides of the leaf. The middle and lower stem leaves are almost invariably thinner than the corresponding rosette leaves. The spongy parenchyma is better developed in rosette than in stem leaves. This was true of 75 per cent of all sections studied.

The palisade parenchyma in stem leaves is better organized, more compact, and the cells relatively longer and narrower as compared with the thickness of the leaf. In rosette leaves the layers of palisade tissue are frequently less perfectly organized, less compact, and the cells larger. Palisade cells of rosette leaves are decidedly broader and usually longer than those of stem leaves; but the amount of palisade tissue and the length of the cells, when compared with the average thickness of the leaves, are less in rosette than in stem leaves. The absolute length of palisade cells in the first layer is greater in rosette leaves than in corresponding stem leaves in 70 per cent, in the second layer in 55 per cent, and in the third layer in 28 per cent of all sections studied. In 30 per cent of all stem sections studied the second palisade layer was not developed. The same was found to be the case in 29 per cent of rosette sections studied. Likewise, the third palisade layer was not developed in 81 per cent of all stem sections studied, or in 66 per cent of all rosette sections studied. The number of sections considered in each case was the same (75 stem and 75 rosette sections). With the exception of the upper stem leaves, where the

upper and lower epidermis frequently have an outer wall of approximately equal thickness. the upper epidermis has a thicker wall than the lower. In 93 per cent of the cases the outer epidermal wall and cuticle of stem leaves were found to be thicker in stem than in rosette leaves. The thickest epidermal walls are usually found in the outer two-thirds of upper stem leaves. On the other hand, rosette leaves have epidermal cells with the largest vertical diameter and contain a maximum of air spaces.

Lepidium virginicum and *Capsella Bursa-pastoris* produce both summer and winter rosettes. When these rosettes are compared, it is found that summer rosettes have slightly thicker leaves (thinner in *Capsella*), a thicker cuticle, and a thicker outer epidermal wall. The palisade parenchyma also is better developed. There are frequently more layers, and the cells are longer and narrower. These differences are most pronounced in *Lepidium*.

SUMMARY ON CHLORENCHYMA.—Typical xerophytic leaves have a relatively compact and well developed palisade tissue; also a relatively small amount of spongy parenchyma with small air spaces. The mechanical tissue is usually also better developed than in mesophytic and shade leaves. Since rosette leaves are usually broad, close to the ground, frequently more or less shaded, and therefore in most respects better protected than stem leaves, it should not be surprising if the former were found to be more mesophytic than the latter. That this seems to be true, at least of the forms studied, is shown by the following deductions.

1. Rosette leaves, as a rule, have a greater amount of chlorenchyma than stem leaves. This is true of at least 80 per cent of all plants studied.

2. Rosette leaves have a greater amount of spongy parenchyma than stem leaves, although the percentage of the chlorenchyma is slightly greater in the latter than in the former.

3. The percentage of air spaces in both palisade and spongy parenchyma is also greater in rosette than in stem leaves. This is true of about 86 per cent of all plants studied. In a considerable number of instances, however, the differences are slight.

4. The number of palisade layers is much the same in both kinds of leaves, but the average length of palisade cells, in at least

80 per cent of the types studied, is greater in rosette than in stem leaves. This is correlated perhaps with the greater thickness of the chlorenchyma in the former. The thickness of palisade cells, in at least 90 per cent of all cases, is also greater in rosette than in stem leaves.

5. The average size of spongy parenchyma cells is also greater in rosette than in stem leaves. This is true of about 90 per cent of all plants studied.

6. Sclerenchyma tissue seems to be about equally well developed in ribs and veins of both kinds of leaves. The conductive tubes in veins of approximately equal size have a slightly larger lumen and a wall slightly thicker in rosette than in stem leaves. The conductive system of rosette leaves is better developed in rosette than in stem leaves, although this rule is not without exceptions.

On the whole, therefore, it may be said that, so far as the structure of chlorenchyma is concerned, stem leaves are more xerophytic in structure than rosette leaves, although the latter appear to be more xerophytic so far as the greater thickness of epidermal wall and cuticle are concerned. In some instances the xerophytic character of stem leaves, as compared with the rosette leaves of the same plant, is so pronounced as to be easily detected with the naked eye.

Conclusions

1. Hairs are most abundant in the upper stem leaves and decrease to the basal leaves; they are also most abundant in the upper rosette leaves and decrease to the basal leaves. In general, however, the stem leaves are more hairy than the rosette leaves.

2. Stomata are usually smaller, more nearly round, and more abundant, per unit area, on stem than on rosette leaves.

3. As a rule, the epidermal cells of rosette leaves are larger than those of stem leaves and have more sinuous lateral walls. The shape of the cells is usually correlated with the shape of the leaf.

4. The blade of rosette leaves is thicker than that of stem leaves, chiefly owing to a greater development of spongy parenchyma. This is not true, however, of stem leaves that are long,

narrow, and of a decidedly xerophytic form and structure as compared with rosette leaves.

5. The outer epidermal wall of rosette leaves is thicker, as a rule, than in stem leaves. The maximum thickness occurs in middle stem and apical rosette leaves. The thickness of the cuticle varies with the thickness of the epidermal wall, the thickest walls having the thickest cuticle. Rosette leaves in the large majority of instances have the thickest cuticle. The preponderance of epidermal protection is in favor of rosette leaves. In stem leaves of xerophytic form the preponderance of epidermal protection is in favor of stem leaves.

6. In a comparison of the different stem and rosette leaves of the same plant it is obvious that the lowest stem and lowest rosette leaves, as well as the basal part of all leaves, have the thinnest epidermal wall, thinnest cuticle, the most poorly developed palisade tissue, the maximum development of spongy tissue and air spaces, and the minimum development of chloroplasts. The upper stem leaves are relatively xerophytic in structure, especially in the apical region of these leaves. The middle and lower stem leaves are usually thinner than the corresponding rosette leaves. The palisade parenchyma in stem leaves usually is better organized, more compact, and the cells relatively longer and narrower, as compared with the thickness of the leaf, than in rosette leaves. The thickness of palisade cells of rosette leaves is greater, in most cases, than in stem leaves. This is also true of the absolute length in the great majority of instances.

7. When the chlorenchyma in middle stem and middle rosette leaves is compared we may conclude: (1) that rosette leaves, in most cases, have a greater amount of chlorenchyma than stem leaves (this is especially true of spongy parenchyma); (2) that in most cases rosette leaves also have more air spaces than stem leaves; (3) that there is little difference in the number of palisade layers in the two kinds of leaves, but in most cases the absolute size of the palisade cells (length and thickness) is greater in rosette than in stem leaves; (4) that the average size of cells of the spongy parenchyma is also greater in rosette than in stem leaves; (5) that sclerenchyma tissue is about equally developed in both kinds of

leaves, but the conductive tissue is slightly better developed in rosette than in stem leaves.

On the whole, typical rosette leaves, where there is considerable shading and protection, are decidedly more mesophytic than stem leaves. In winter leaves on stolons or runners there is a tendency toward greater xerophytism than in stem leaves, but on the whole the rosette leaves are more mesophytic in structure than stem leaves.

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IMPERFECTION OF POLLEN AND MUTABILITY IN THE GENUS ROSA*

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(WITH PLATES IV–VI)

During the winter of 1915–1916 I made a study of all the species, of which specimens were obtainable, of the genus *Rosa*. This was done in connection with work on other genera of the family Rosaceae, notably on *Rubus* and on *Crataegus;* and it has been interesting to note that in all 3 genera there is indication of a large amount of hybridism, and that the multiplication of species is startlingly great.

From the Arnold Arboretum of Harvard University, I have been able to obtain flower buds of 32 different species of *Rosa*. Of 3 of these species I have several varieties; 5 varieties of *R. spinosissima*, 3 of *R. rugosa*, and 2 of *R. virginiana*. The buds were taken when on the point of opening, thus making sure of the maturity of the pollen. They were then preserved in alcohol until such time as it was possible to examine them.

I prepared sections of about half of the species gathered, with a view to determining how many showed sound pollen and how many showed a greater or smaller proportion of undeveloped or imperfect pollen. For this purpose the buds were first imbedded in celloidin to make sure that there would be no shrinkage of the parts. Sections were cut with the microtome, stained with Heidenhain's haematoxylin and safranin, and finally mounted in balsam.

For nearly a century it has been known that one of the most important and most easily recognized characteristics of a hybrid is imperfect pollen. DUTROCHET[1] in 1832 recognized the morphological sterility of the hybrid and pointed out that pollen abortion is a criterion for hybridism. GAERTNER,[2] in 1849, speaks of the

*Contribution from the Laboratories of Plant Morphology of Harvard University.

[1] DUTROCHET, HENRI, Sterility of hybrid plants. Gard. Mag. **8**:500. 1832.

[2] GAERTNER, C. F., Versuche und Beobachtungen über die Bastarderzeugung im Pflanzenreich. Stuttgart. 1849.

importance of the pollen conditions in determining the fertility of a hybrid as follows:

Die wichtigste Theil der Befruchtungstheile der Bastarde ist der Pollen. Es ist nun aber zu bemerken, dass ein vollkommen normal gebildeter Pollen sein Ovarium nicht absolut zu befruchten vermag, weil manche mit wirklich potentem Pollen bestäubte Blumen nicht selten doch abortiren und unbefruchtet abfallen: obgleich in der Regel von dem Vorhandensein eines vollkommenen Pollens bei den reinen Arten auf die Fruchtbarkeit einer Pflanze geschlossen werden darf.

Since, therefore, imperfect pollen is a well known characteristic of hybrids, and one of the easiest means of identifying them, it is from an examination of the pollen of the species of *Rosa* that their probable genetical status can be determined most easily. The thin sections through the anthers, when examined microscopically, give one a remarkably clear view of the pollen grains in all positions in anthers in all parts of the different buds.

GAERTNER is again in harmony with our modern ideas in his observations concerning the difference between perfect and imperfect pollen grains, or, as he calls them, fertile and infertile; for even a hybrid may have the means, however imperfectly developed, of reproducing its kind. GAERTNER states as follows:

Die Gestalt und Grösse der Körner des Pollens der Bastarde in der nämlichen Anthere ist weit mehr verschieden, als man es nach FRITZSCHE und H. V. MOHL in den der reinen Arten zuweilen antrifft.

In den Antheren aller fruchtbaren Bastarde, befinden sich kleinere und grössere Körner mit einander vermischt in verschiedenen Verhältnissen zum Theil äusserst kleine von verschiedenen Graden der Unförmigkeit länglichte, eingeschrumpfte, leere Bälge, ohne flüssigen Inhalt—am deutlichsten findet man dies bei solchen Individuen, welche eine geringe Fruchtbarkeit besitzen. . . . . Aus der Grösse und Qualität der Pollenkörner kann man daher in manchen Fällen mit ziemlicher Zuverlässigkeit auf die Fruchtbarkeit oder Unfruchtbarkeit eines Bastards schliessen.

Die reine Farbe bezeichnet in den meisten Fällen die Potenz des Pollens.

Die Verstäubung des Pollens der Hybriden ist wie schon bemerkt sehr mangelhaft; er vertrocknet häufig in den Antheren, wenn sich diese auch öffnen.

Der Inhalt des Pollens der Bastarde ist sehr verschieden und auch selbst bei den fruchtbaren gering, meistens fehlt er aber gänzlich und der Pollen ist dann trocken und ballt sich nicht. . . . . Wenn der Pollen seine regelmässige Gestalt und Grösse hat so enthält er gewöhnlich eine flüssige ölige Materie.

That is, normal pollen is perfect morphologically, fully formed, and having normal protoplasmic contents; while abnormal or imperfect pollen, such as often characterizes known hybrids, on the contrary, is usually shrivelled and has little or no protoplasmic contents, consequently making the grain quite impotent.

The pollen of *Rosa* is largely in the last named condition, imperfect, and therefore probably sterile to a considerable extent. Of the 32 species secured from the Arnold Arboretum, 2 show entirely perfect pollen, and in 3 others imperfectly developed grains were slightly intermingled; that is, showing only 1–10 per cent bad pollen. Seventeen show a very large percentage of imperfect grains (about 50–100); and the remaining 20 show 10–50 per cent.

This enormous degree of infertility of pollen in the genus probably accounts for much of the difficulty systematists seem to have encountered in establishing species. ENGLER and PRANTL[3] speak of the genus thus:

> Die allbekannte, von Dichtern aller Kulturvölker gepriesene Rose bildet eine scharf umgrenzte Gattung, die sich durch den Bau der Blütenachse den Sanginsorbeae und Pomoideae, durch den übrigen Blütenbau den Potentilleae durch die Tracht insbesondere der Gattung Rubus anschliesst. Sie ist fast über die ganze nördliche gemässigte Zone verbreitet, geht auch in die Gebirge der Tropen über, fehlt jedoch auf der Suedl. Halbkugel.
>
> Die Zahl der Arten kann man bei mittelweiter Fassung des Artbegriffes auf etwa 100 ausschlagen, doch sind schon allein aus Europa mehrere hundert Arten niederen Ranges beschrieben worden.

The last edition of GRAY'S *Manual* recognizes 15 species of wild roses of the eastern United States and Canada, and in the last edition of *Field, forest, and garden botany*, in which are included cultivated species and varieties, there are 24 species.[4] Nine of the wild species, so-called, of GRAY (M.), I have been able to obtain from the Arnold Arboretum, also 4 of the cultivated species. The 20 odd species remaining that I have analyzed are importations, hybrids, etc., grown specially in the Arboretum. Of the 3 divisions made of the species of *Rosa* according to the percentage of bad pollen present, I shall first take up the group in which the proportion was

[3] Die natürlichen Pflanzenfamilien (p. 46).

[4] In the following pages the *Manual* is indicated by (M.), and *Field, forest, and garden botany* by (F.F.G.).

less than 10. In this group are *R. rugosa*, *R. cinnamomea*, *R. Kelleri*, *R. pendulina*, and *R. Moyesii*.

R. rugosa (figs. 5, 13) has almost no imperfect grains as may be seen in the figures, practically all of the grains being perfectly formed and full of protoplasmic contents. Fig. 5 shows the pollen teazed out of the anthers on to a slide. Fig. 13 is a cross-section through an anther, showing the pollen grains in their normal positions in the loculus of the anther. It is worth while to note also the generous quantity of pollen in the single loculus. GRAY (F.F.G.) groups *R. rugosa* among the principal types of exotic garden roses which are "much mixed by crossing and changed by variation." The reason, doubtless, for the purity of *R. rugosa* as compared, for instance, with *R. rubiginosa* (fig. 6) is its geographical seclusion on the islands of Japan. The varieties of *R. rugosa* show evidence of contamination, as will be shown later.

R. cinnamomea and the 3 other species in this group (*R. Kelleri*, *R. pendulina*, and *R. Moyesii*) have not been figured. All may be found in the Arnold Arboretum. They are all practically without imperfect pollen grains. *R. pendulina* comes from the mountains of Europe, and *R. Moyesii* comes from China.

In the second and much larger group the percentage of imperfect pollen is 10–50. In this group are *R. spinosissima altaica*, *R. spinosissima* (garden variety hybrid), *R. spinosissima*, and *R. spinosissima hispida;* also *R. spinosissima paniculata* (garden variety). *R. spinosissima fulgens* (garden variety), because of its larger percentage of undeveloped grains, belongs to the third and last group. With these are *R. Harrisoni* (garden hybrid), *R. gymnocarpa*, *R. Manetti* (garden hybrid), *R. blanda*, *R. seraphini*, *R. wichuriana*, *R.* no. 306 Wilson, *R. pratincola*, *R. multiflora*, *R. davurica*, *R. acicularis*, *R. hemispherica*, and *R. ferruginea*. Of *R. rugosa alba* and *R. virginiana alba*, which properly belong in this group, I shall speak later in connection with other varieties of the same species in the third group.

R. spinosissima and its 5 varieties present some very interesting conditions. Fig. 12 is a cross-section of the typical anther of the so-called species. It is very clearly seen that about 40 per cent of the grains in the loculus are shrivelled and without protoplasmic

contents; and the contrast between these and the perfect grains is very marked.

In comparing the species *R. spinosissima* with the recognized garden hybrid, a variety of the species and called *R. spinosissima* garden variety hybrid (fig. 10), the latter shows less pollen in the loculus, but about the same percentage of shrivelled grains. *R. spinosissima paniculata*, another garden variety, has only about 10 per cent of its pollen grains undeveloped; while still a third garden variety, *R. spinosissima fulgens*, has a larger percentage than any of the group I have examined. This last, as may be seen in fig. 11, has an abundance of pollen grains in the loculus, but about 50 per cent of them appear as tiny, shrivelled cells.

The two remaining varieties of *R. spinosissima*, *R. spinosissima altaica* and *R. spinosissima hispida*, are apparently the least contaminated of the varieties. The first, a Siberian rose (fig. 8), has a considerable amount of pollen in the loculus, and only about 10 per cent of its grains are bad. The second (fig. 9) presents an almost identical situation; and this is a European variety of the same species.

The next species in the group is *R. Harrisoni*, a recognized garden hybrid. Fig. 18 shows the poorly developed pollen typical of this species, about 40 per cent of the grains being imperfect if not entirely shrivelled. GRAY (F.F.G.) described "*R. Eglanteria* L. a yellow Eglantine rose. Like a sweetbriar, but lower. Austrian briar, Persian yellow, and Harrison's yellow are forms of this." I have not been able to obtain specimens of *R. Eglanteria* L., the parent, but certainly the offspring is an excellent example of the condition of badly developed pollen usually accepted as indicating hybridism.

R. Manetti, another garden hybrid (fig. 17), presents a condition analogous to that found in *R. Harrisoni*. *R. gymnocarpa*, a northwestern North American rose, is much less contaminated than the last two, only about 20 per cent of its pollen grains being imperfect.

The remaining species of the group under discussion present conditions more or less similar to those already described. *R. blanda* has about 20 per cent bad pollen; *R. seraphini* has only about 10 per cent; *R. wichuriana* has numerous shrivelled grains;

R. no. 306 Wilson, a Chinese wild rose, shows about 15 per cent bad pollen; *R. pratincola* has about 25 per cent of its pollen grains undeveloped; *R. multiflora* has about 20 per cent bad pollen. The last is a rose native to Japan and China, and cultivated here. GRAY (F.F.G.) records it among the principal types of exotic garden roses: "*R. multiflora* Thunb. from Japan and China. Hardy in the Middle States, a double form of an old garden rose, the single form not common. Polyantha roses are offshoots of this chiefly through hybridization with *R. indica*."

R. davurica is a Siberian rose which shows more imperfection of pollen than *R. spinosissima altaica*, having 25–30 per cent of its pollen undeveloped, in contrast with 10 per cent in the other species. *R. acicularis*, another rose native to Siberia, is in a similar condition of probable contamination; this species is now wild in the Northern Hemisphere. *R. hemispherica* is a Persian yellow rose, probably like *R. Harrisoni*, an offspring of *R. Eglanteria*. I examined the variety *R. hemispherica plena*, and found the pollen in bad condition. The last of the species in the group is *R. ferruginea*, native to the mountains of central Europe, and here also the pollen was to a large extent abortive, a condition interesting when compared with that found in *R. pendulina*, likewise a native of the European mountains, but almost without bad pollen.

In the third group are those species with 50–100 per cent bad pollen. This group is not quite as large as the second group, but presents conditions even more interesting. It includes *R. kamchatica*, *R. cordifolia*, *R. rugosa plena*, *R. rugosa alba*, *R. rugosa arnoldiana*, *R. oxyodon*, *R. rubiginosa*, *R. setipoda*, *R. mollis*, *R. macrophylla*, *R. canina biserrata*, *R. arvensis*, *R. gallica*, *R. alba*, *R. damascena*, *R. virginiana plena*, and *R. virginiana alba*.

Since the conditions as they appear in the species *R. rugosa* have already been shown, I shall first take up its 3 varieties. *R. rugosa plena* has every appearance of a typical hybrid, as evidenced by a large degree of sterility in its pollen (fig. 14). It seems clear that about 90 per cent of the pollen is abnormal; and the contamination is still more marked when we compare it with *R. rugosa* (fig. 13). *R. rugosa alba* (fig. 15) is not in such bad condition as *R. rugosa plena*, for in this case only about 40 per cent of the grains are

Three species of this last group remain to be mentioned. *R. setigera* shows a large percentage of microsporic degeneracy. *R. virginiana plena* with about 90 per cent of its pollen bad, and *R. virginiana alba* with but 25 per cent of its pollen imperfect, are varieties of the *R. virginiana* Mill. of GRAY'S *Manual*. The latter, known sometimes as *R. lucida* Ehrh., is a dwarf wild rose found on the margins of swamps and rocky shores from Newfoundland and eastern Quebec to New York and eastern Pennsylvania.

The preceding statistics show clearly that the species of *Rosa* are in a very marked degree characterized by abnormal pollen. It is true likewise that abnormal pollen is largely sterile. Pollen sterility for nearly a hundred years has been recognized by plant breeders as a prominent characteristic of hybrids; and another well known characteristic of hybrids is their extreme variability. Since both extreme pollen sterility and variability are prominent features of hybrids, the conclusion seems inevitable that most of the so-called species of *Rosa* are in reality hybrids.

This conclusion is most interesting when viewed from an evolutionary standpoint. Are new species the result of gradual changes or sudden leaps? The answer to this depends largely upon the definition of the term species. In the lower vascular plants the conditions of spore abortion and hybridization appear to be very rare. The term species in these cases, therefore, is used to distinguish groups of plants wholly distinct from one another and probably genetically pure. JEFFREY[6] has shown by microscopical investigation that morphologically sterile pollen does not occur in plants that are monotypic, isolated geographically or through the time of flower maturity. He has likewise made a comparison of the "conditions of sporogeny found in the lower plants, the Bryophyta, Pteridophyta, and gymnosperms, which are not characterized by enormous multiplication of species, with the sporogenic features of the angiosperms in which multiplication of species has run riot." In this comparison he found that in the lower forms of Embryophyta, from the Bryophyta to the gymnosperms, "infertile spores and hybridism were conspicuous by their

[6] JEFFREY, E. C., Some fundamental morphological objections to the mutation theory of DEVRIES. Amer. Nat. **49**:5–21. *figs.* 7. 1915.

absence." Among the gymnosperms he examined Cycadales, Ginkgoales, Coniferales, and Gnetales, and found "a single species of *Abies* with evidence of abortive pollen grains of hybrid origin."

The photomicrographs of *Lycopodium complanatum* and *Pinus divaricata* (figs. 1, 2) show clearly the morphological condition typical in both genera, fertile spores uncontaminated by any abnormal, sterile grains. JEFFREY states that "the genus *Pinus* is very old and its species accordingly very distinct"; and he has not yet found "the slightest evidence of hybridization here or in other numerous and widely distributed species of conifers, other than the *Abies* mentioned above."

In the angiosperms, on the contrary, hybridism as a condition widespread in nature is commonly recognized. For example, in this country and in Europe systematic botanists agree that hybridism is extremely common as a natural condition in certain genera of the Rosaceae. BRAINERD has shown that a great many "natural hybrids" of *Rosa* and *Rubus*, occur; and JEFFREY[7] in a recent article says as follows:

> Not only are certain of the Rosaceae recognizable as hybrids on account of their transitional external features of organization, Mendelian phenomena, etc., but certain others which have not revealed themselves as hybrids in these ways are clearly such as a result of a study of their spores. . . . . Taking morphological features into account, as well as the data of the systematic botanists, there are three kinds of individuals; pure species, recognized species with pollen showing they are concealed hybrids or crypthybrids, and recognized hybrids or phenhybrids.

These 3 classes, typical of many angiospermous genera, make it difficult to determine what individuals shall form a species. In the higher vascular plants, therefore, the term species is obviously used in a very different sense from that in which it is applied to the lower vascular plants. Clearly crypthybrids should not be species in the same sense in which the name is used of *Pinus* and other morphologically normal genera. But they are generally admitted by the systematist as good species because of their relative constancy and the absence of observed intergrading types, though the morphological conditions are undoubtedly those of hybrids.

[7] JEFFREY, E. C., Spore conditions in hybrids and the mutation hypothesis of DEVRIES. BOT. GAZ. **58**:322–336. *pls. 22–25.* 1914.

Now if crypthybrids could *justly* be called true species, it might possibly be admitted that they to some extent support the mutation theory of DEVRIES. But unfortunately they are frequently, although not universally, very variable, and this variability would appear on morphological grounds to be the result of hybridization.

On the other hand, the natural hybrid or phenhybrid found in the angiosperms and resulting from a cross between distinct species, with no segregation as in Mendelian crosses, but a blending of the parent characters, may breed true to these respective characters, in which case a new and distinctive form is perpetuated, and to this the systematist may justly give a specific rank. Such forms, however, are usually characterized by a large amount of sterile pollen, unlike the true species in which the pollen is morphologically perfect. Hence the term species is used here in a sense somewhat different from that ordinarily implied.

In proportion to the extension of the term species, the number of species has grown astonishingly. This multiplication of species is probably largely due to hybridization, judging from the morphological data afforded by the Rosaceae; and generally the new "species" are crypthybrids. HOAR[8] of this laboratory has been investigating *Rubus* and has reached results corresponding to those recorded here with regard to *Rosa*. Hybridism appears to be even more rife in *Crataegus*, and the multiplication of species is likewise greater, as is shown by the result of work carried on by Miss LORA STANDISH.[9]

It is interesting to note how many of the species of the genus *Rosa* in cultivation at the Arnold Arboretum are really crypthybrids, and how many are true species and phenhybrids. Take the 3 groups as presented according to their pollen sterility. To the first group only can the term species be accurately applied, that is, in the strict sense of the word as used of species of *Pinus* and *Lycopodium;* for only in this group is the percentage of sterile pollen so negligible that the members may be considered, with some degree of probability, genetically pure.

[8] HOAR, C. S., Sterility as the result of hybridization and the condition of the pollen in *Rubus*. BOT. GAZ. **62:** 370–388. 1916.

[9] STANDISH, LORA, What is happening to the hawthorns? Jour. Heredity **7:** 266–279. 1916.

In the second group there are 3 phenhybrids and 3 garden varieties probably of hybrid origin though not so designated. The remaining 14 are crypthybrids, several of which are treated as species in standard systematic works; as for example, *R. blanda* with 20 per cent of its pollen grains abortive, and *R. rubiginosa* with pollen almost completely undeveloped.

In the third and last group I find 3 phenhybrids; one natural hybrid of known parentage, *R. alba;* two recognized hybrids, *R. rubiginosa* regarded as derived from *R. canina*, and *R. damascena* which is allied to *R. centifolia* and parent with *R. indica* of "hybrid perpetual roses"; and two garden varieties of *R. rugosa* which are also of recognized hybrid origin. The remaining 12 of this group are crypthybrids.

These crypthybrids of *Rosa* are particularly interesting in connection with the several theories of the origin of species. We know that they are common not only in the Rosaceae, but, as has been shown to be probable, also in the Onagraceae and other families of the angiosperms. Such forms, though recognized as species, obviously cannot rank with pure species in the sense in which that term is applied to gymnosperms, etc., in evolutionary discussions; for, as JEFFREY has recently stated (see footnote 6), "The conduct of such forms is conditioned more or less by their mixed blood."

In this connection it is interesting to note the conditions presented by *Oenothera Lamarckiana* and other species of the genus as described by DEVRIES and other authors. Here we have, as is the case in *Rosa*, a considerable degree of variability accompanied by a large amount of pollen sterility. Upon *Oenothera* and forms manifesting similar peculiarities DEVRIES has mainly based his mutation hypothesis.

To go back to the original question, are new species the result of gradual changes or sudden leaps? The Darwinian hypothesis, as has been pointed out, is in large measure supported by the species of *Pinus*. But, as I have shown, the term species when used of *Pinus* has an altogether different significance from that which it has when used of *Rosa;* and consequently, the problem of evolution as presented by the species of *Rosa* must be an entirely

different one. There must be careful distinctions made between the 3 classes of individuals; and the search for the true solution of the problem of the origin of species becomes thereby a matter of great complexity. As for the mutation hypothesis of DEVRIES, the morphological and systematic evidence set forth with regard to the conditions in *Rosa*, and the similar conditions brought out with regard to *Rubus*, *Crataegus*, and the Rosaceae as a whole, seem to lend it little support, since the mutability here is obviously the result of hybridization in nature.

Conclusions

1. The species of *Rosa* are characterized by a large amount of abortive pollen and also by great variability.

2. Both pollen sterility and variability have long been recognized as two main characteristics of hybrids.

3. The species of *Rosa*, therefore, are largely of hybrid origin.

4. On account of the great number of crypthybrids and phenhybrids in angiosperms, the term species has a very different meaning from that which it has when applied to the lower vascular plants and the gymnosperms.

5. The mutability of the species of *Rosa* cannot properly be used in support of the mutation hypothesis, since this phenomenon is obviously the result of hybrid contamination in nature.

In conclusion the writer wishes to express her most sincere thanks to the Director of the Arnold Arboretum for permission to collect material; and to Professor E. C. JEFFREY for advice and assistance.

HARVARD UNIVERSITY

EXPLANATION OF PLATES IV–VI

PLATE IV

FIGS. 1–6.—Pollen.

FIG. 1.—*Lycopodium complanatum;* ×125.

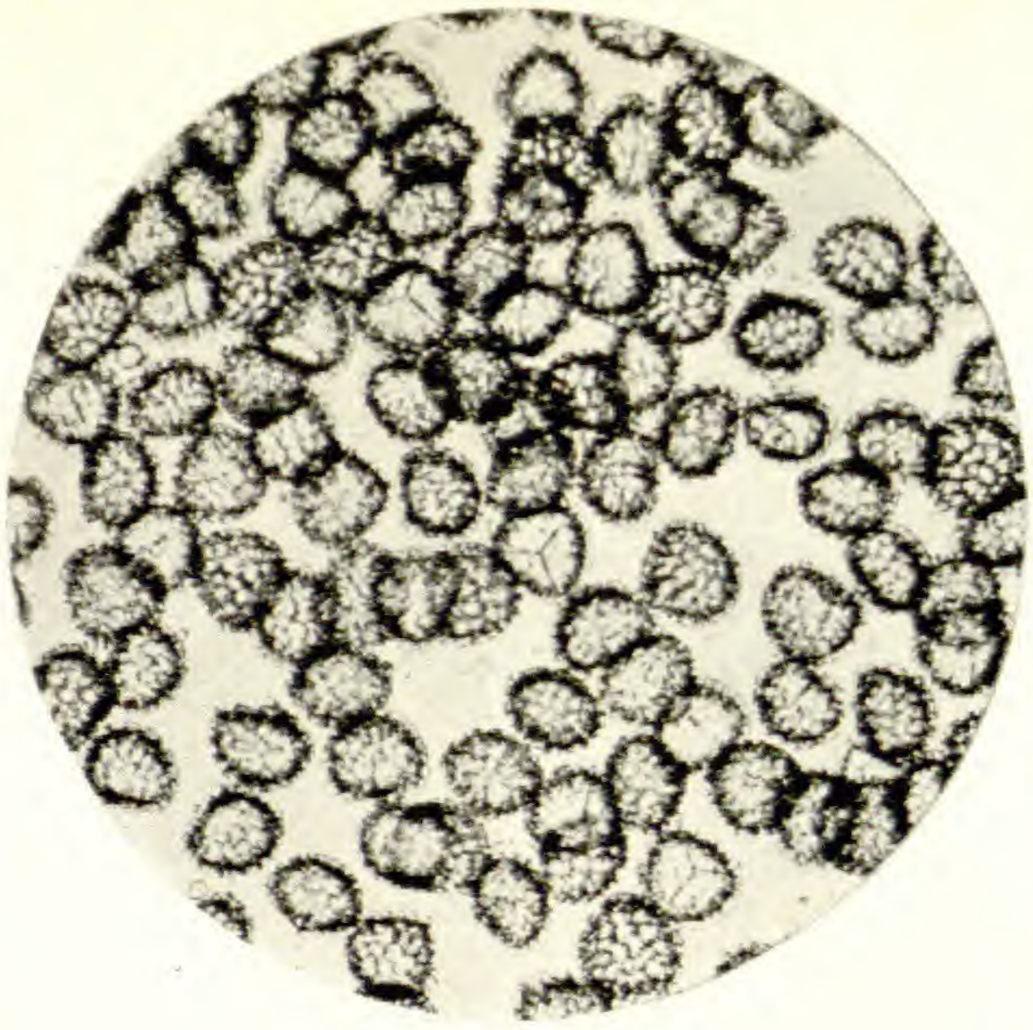
FIG. 2.—*Pinus divaricata;* ×125.

FIG. 3.—*Rosa alba;* ×250.

FIG. 4.—*Rosa alba;* ×375.

FIG. 5.—*Rosa rugosa;* ×250.

FIG. 6.—*Rosa rubiginosa;* ×375.



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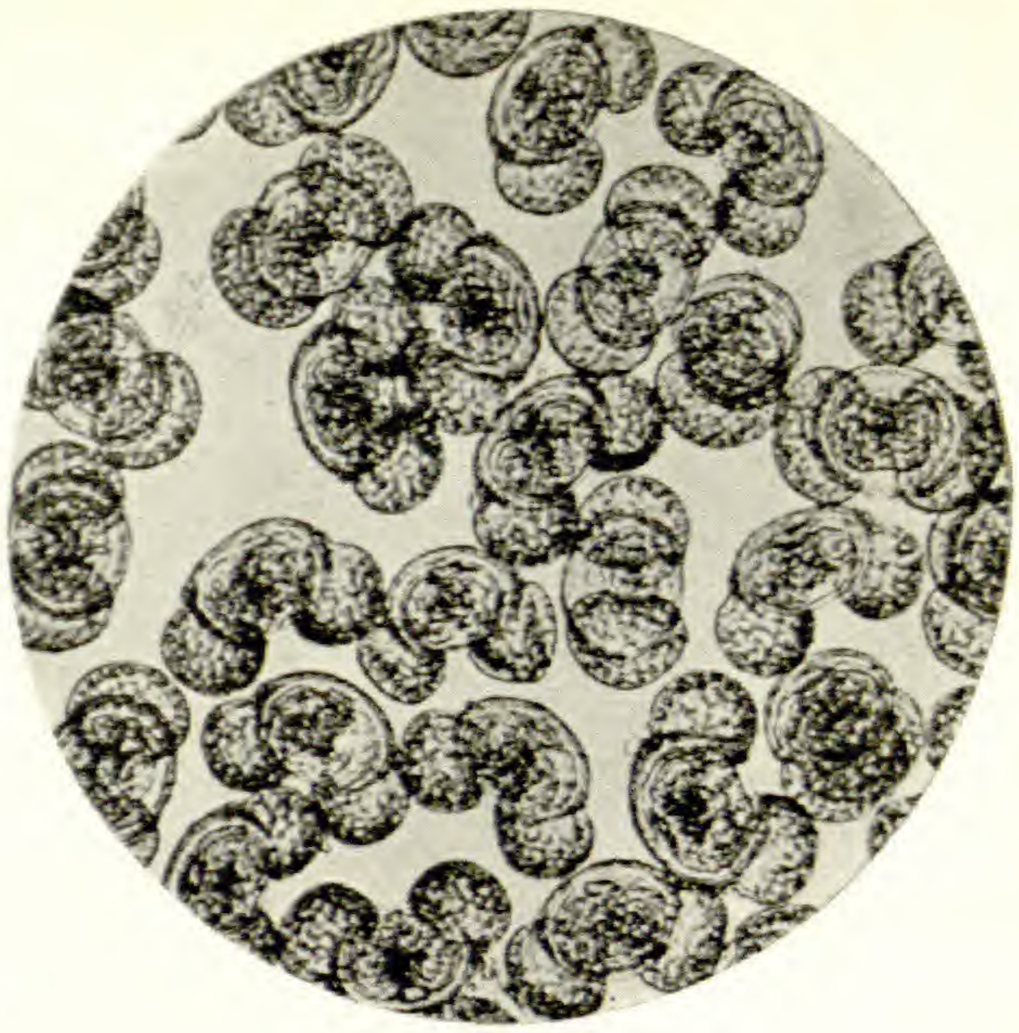

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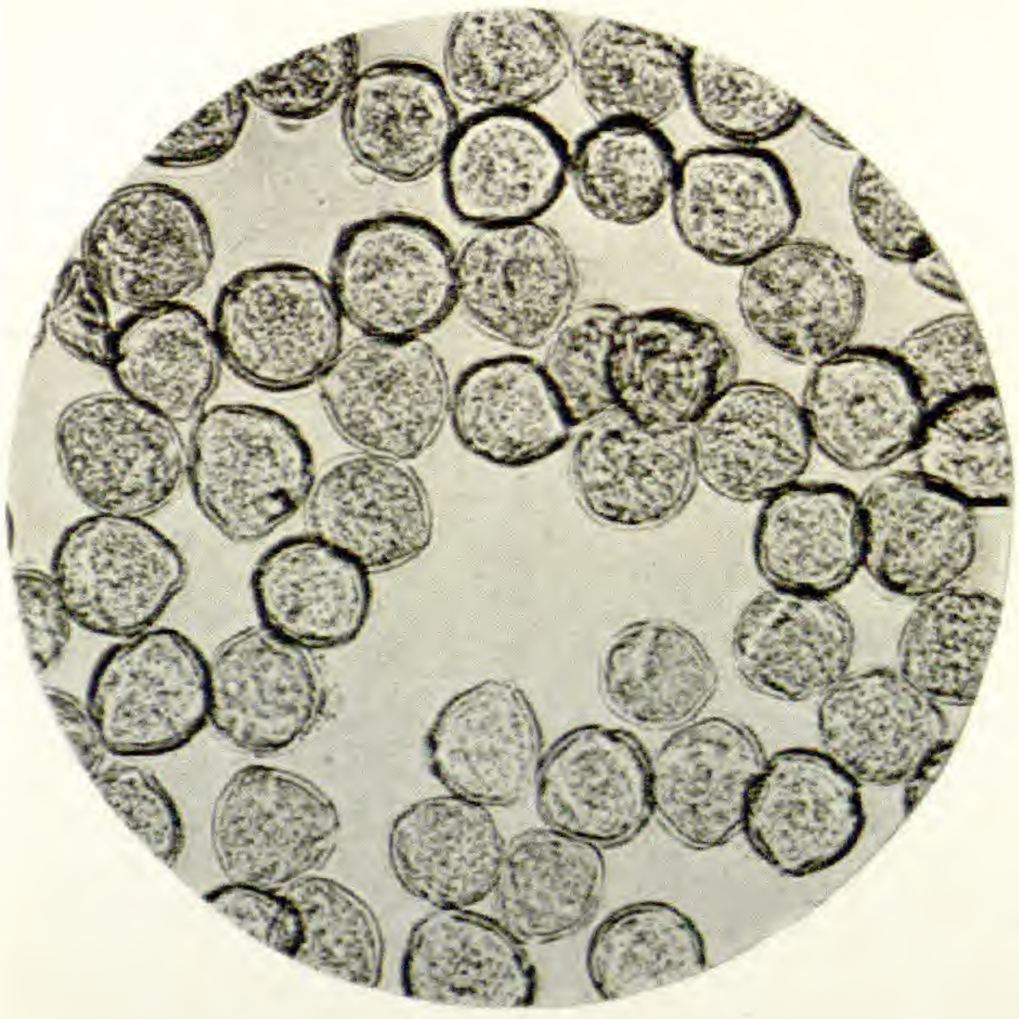

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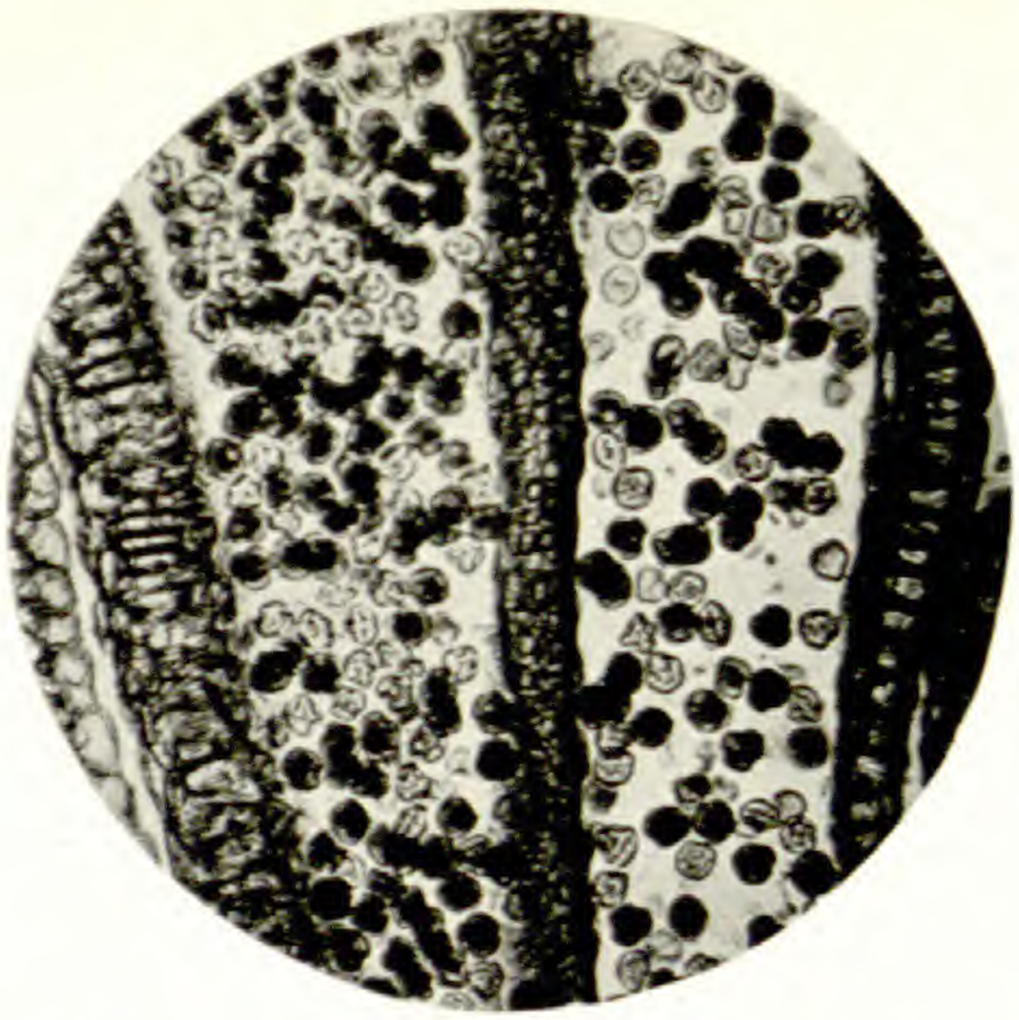

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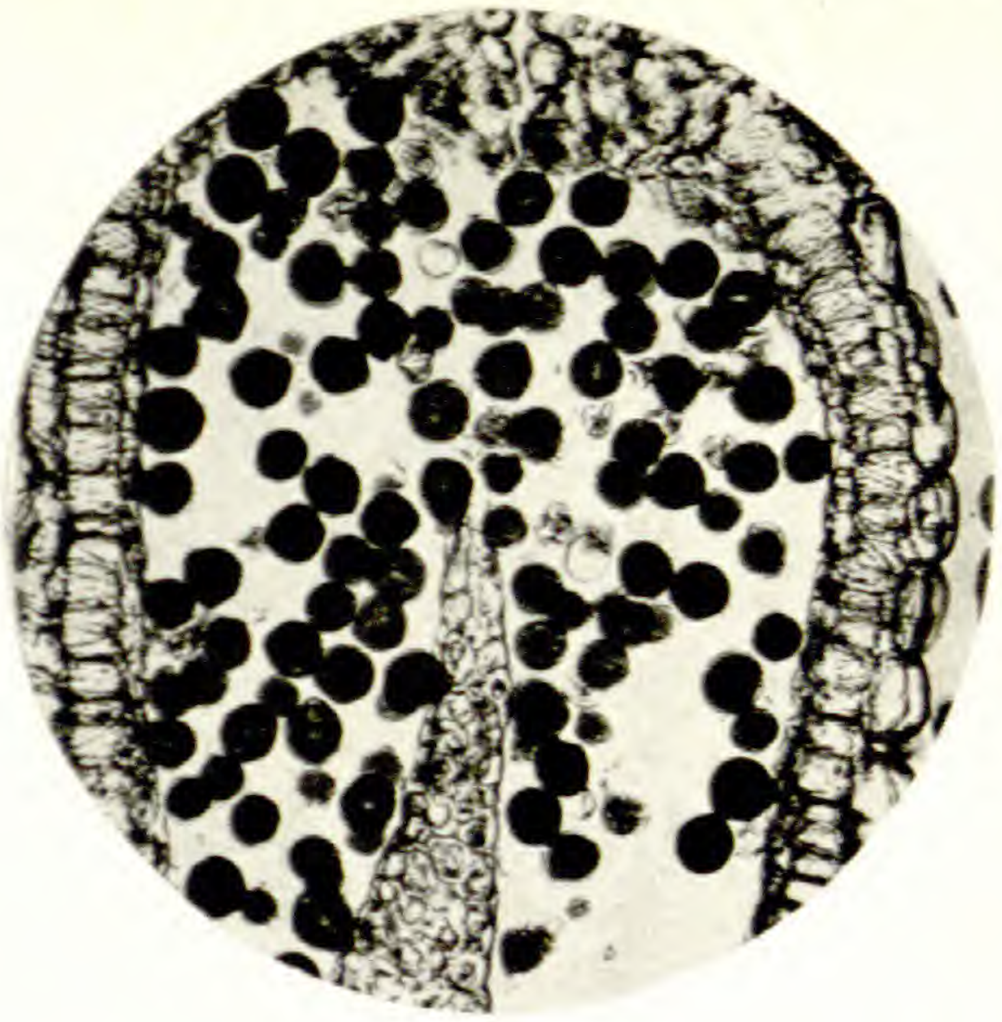

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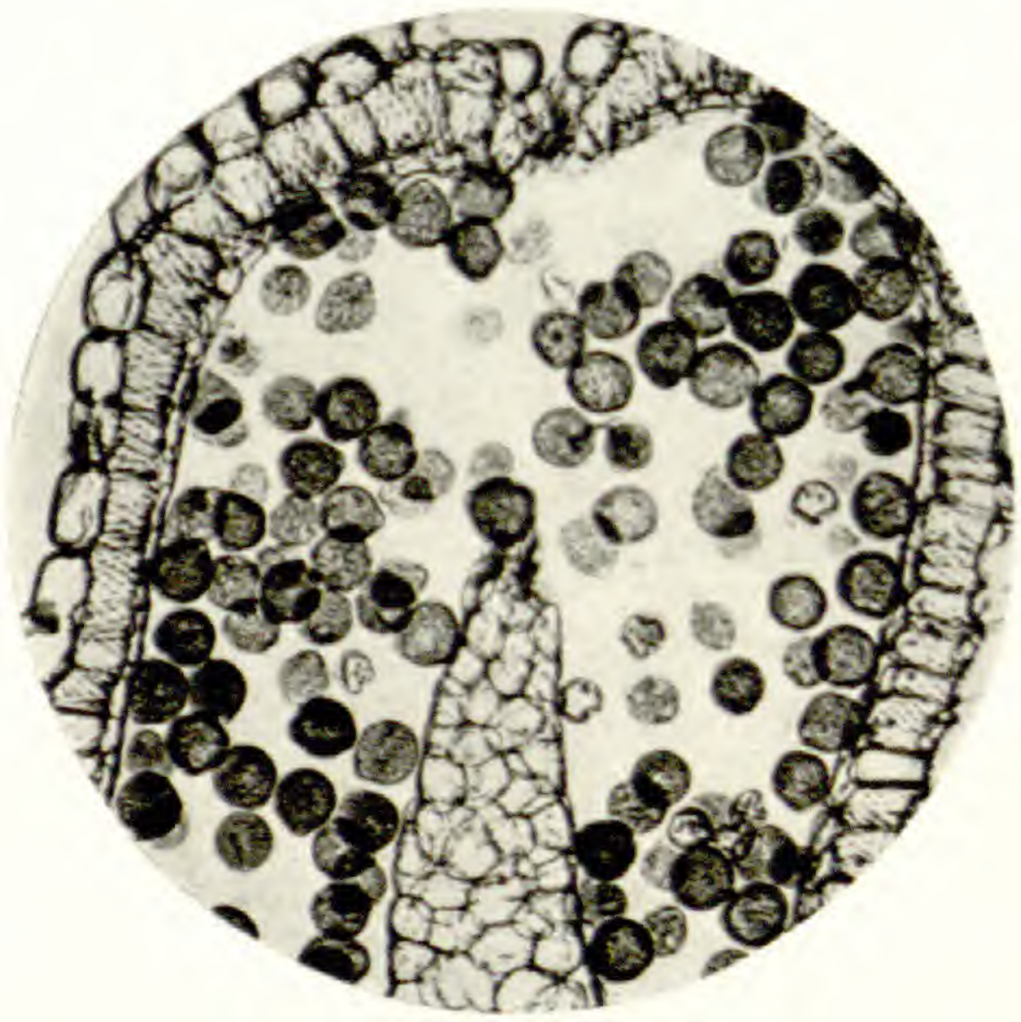

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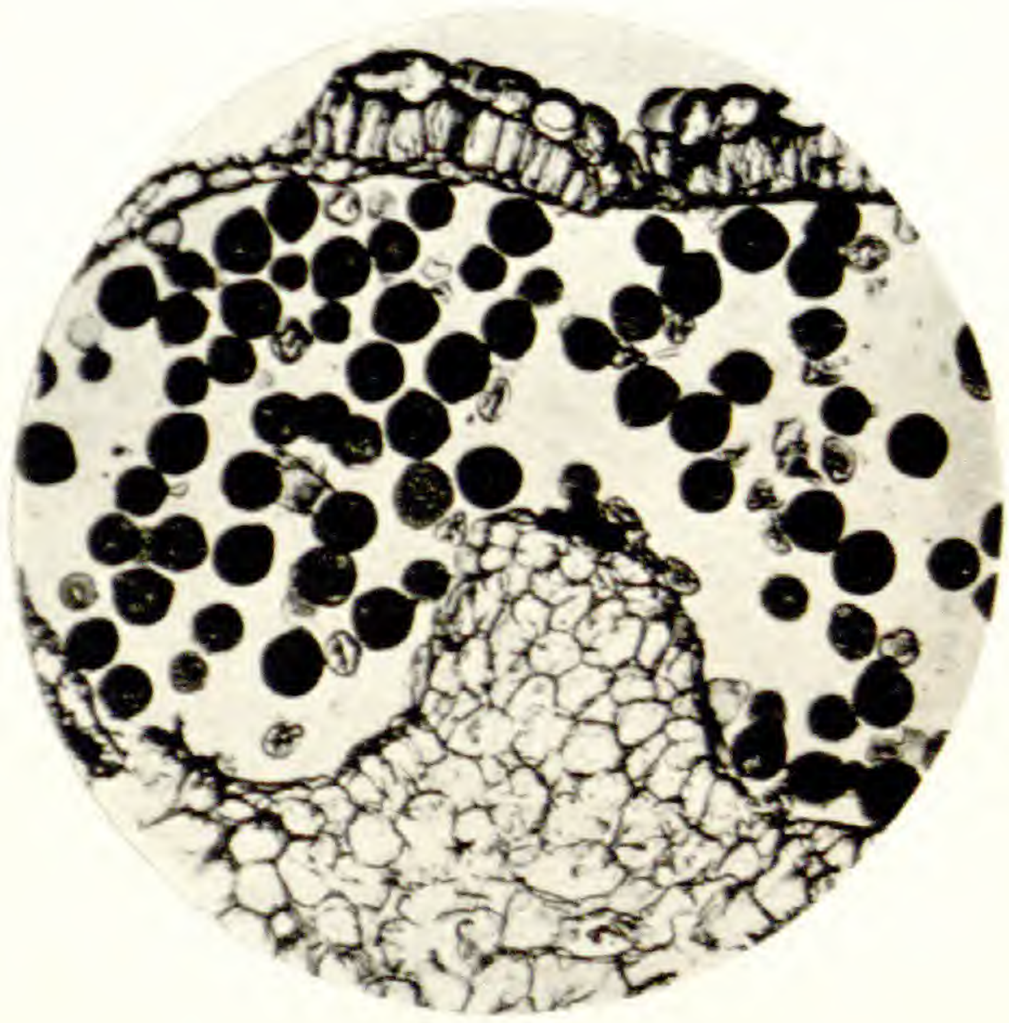

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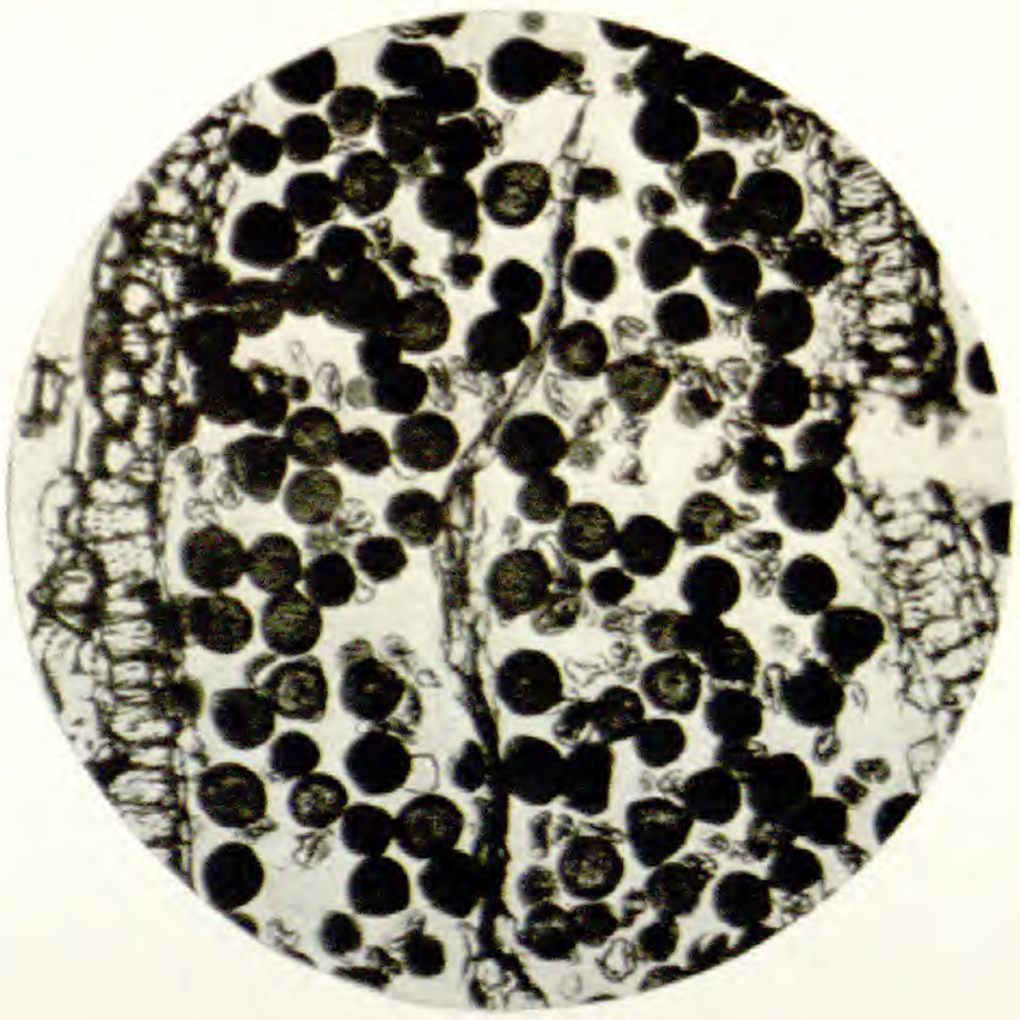

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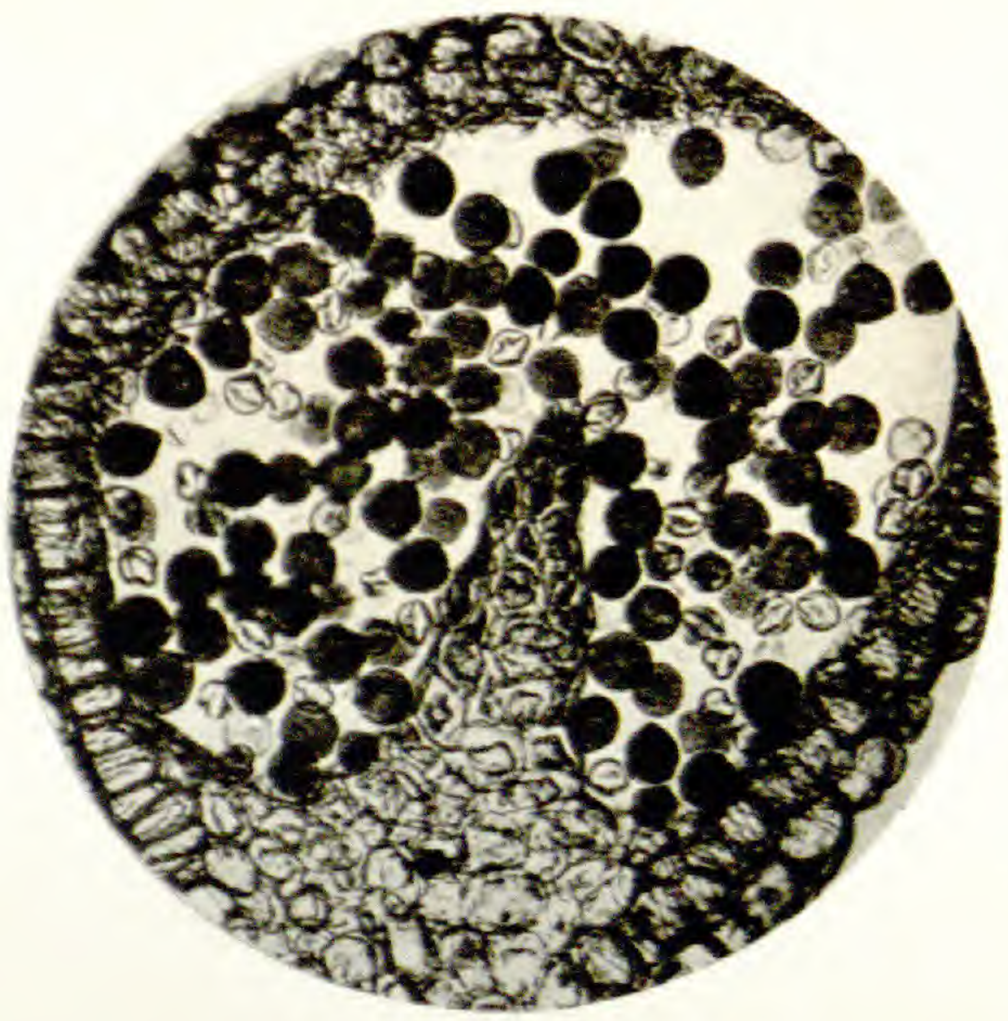

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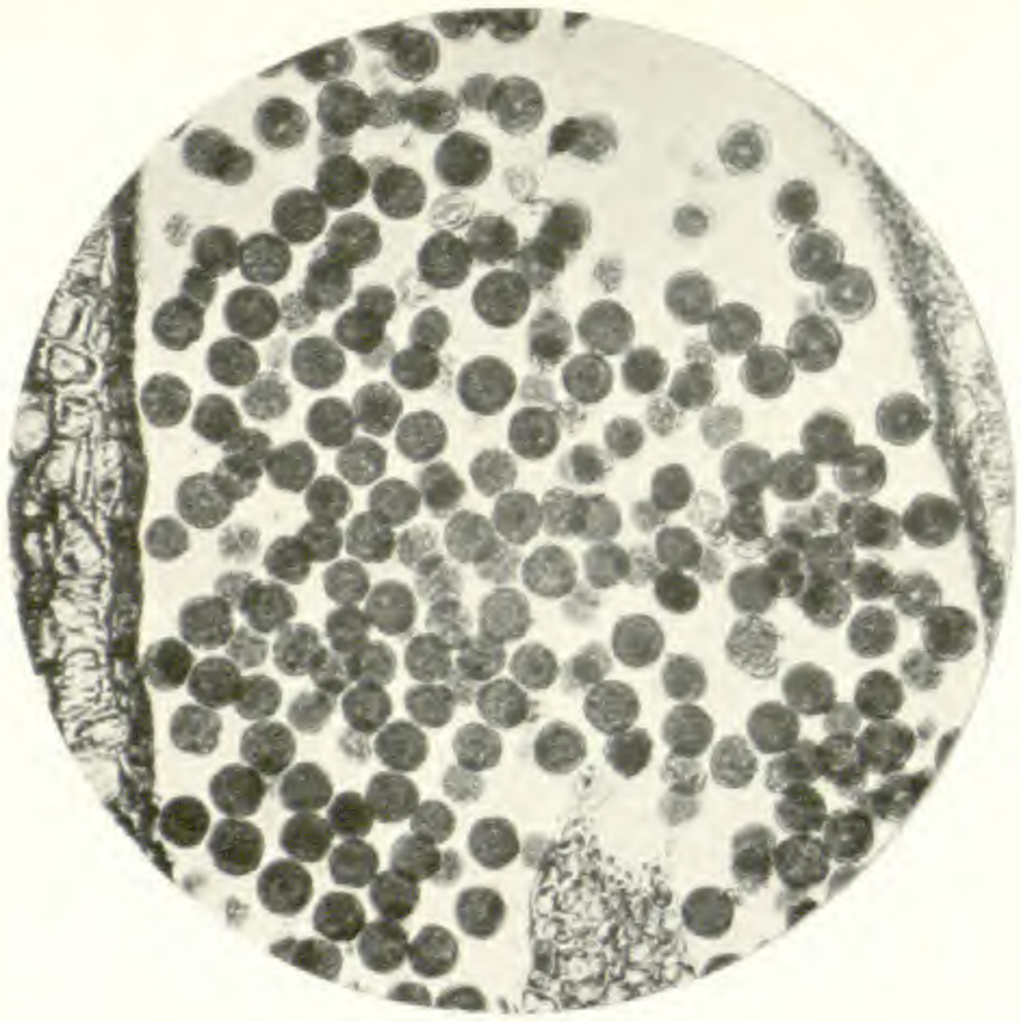

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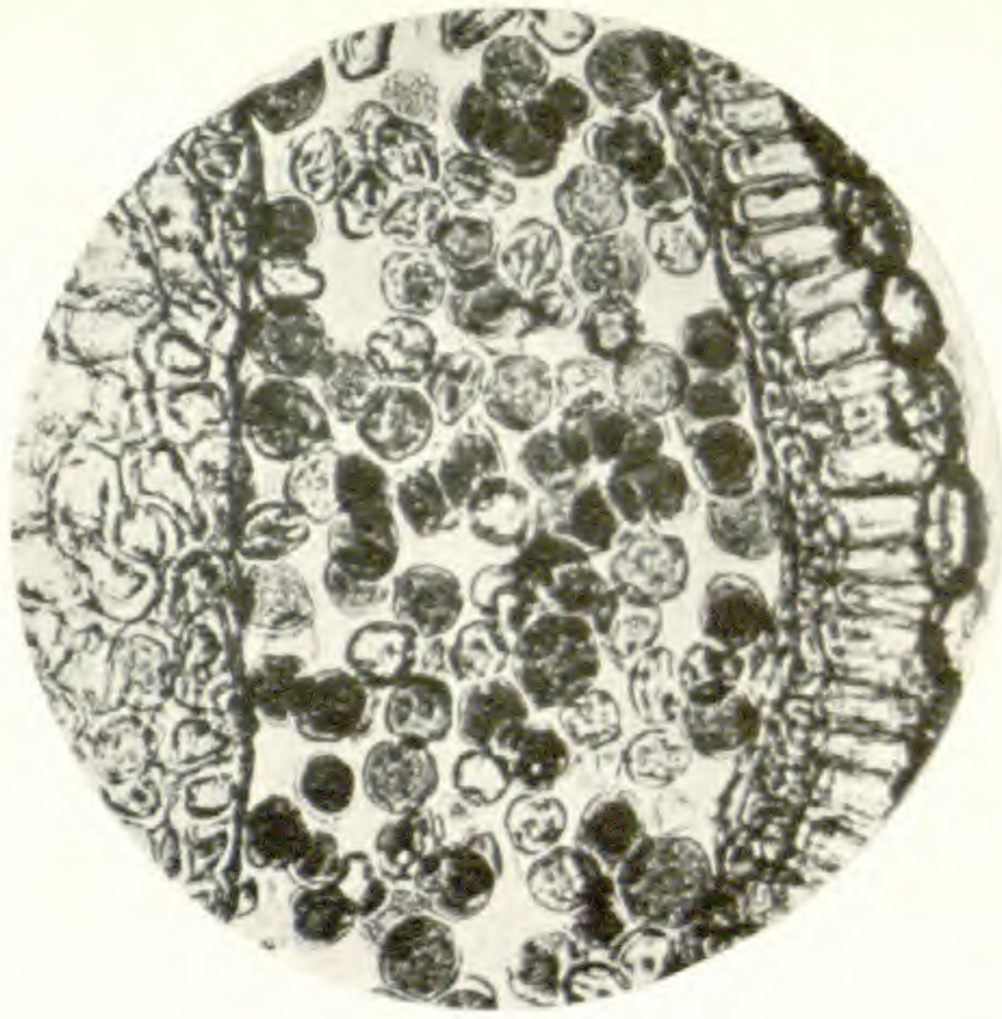

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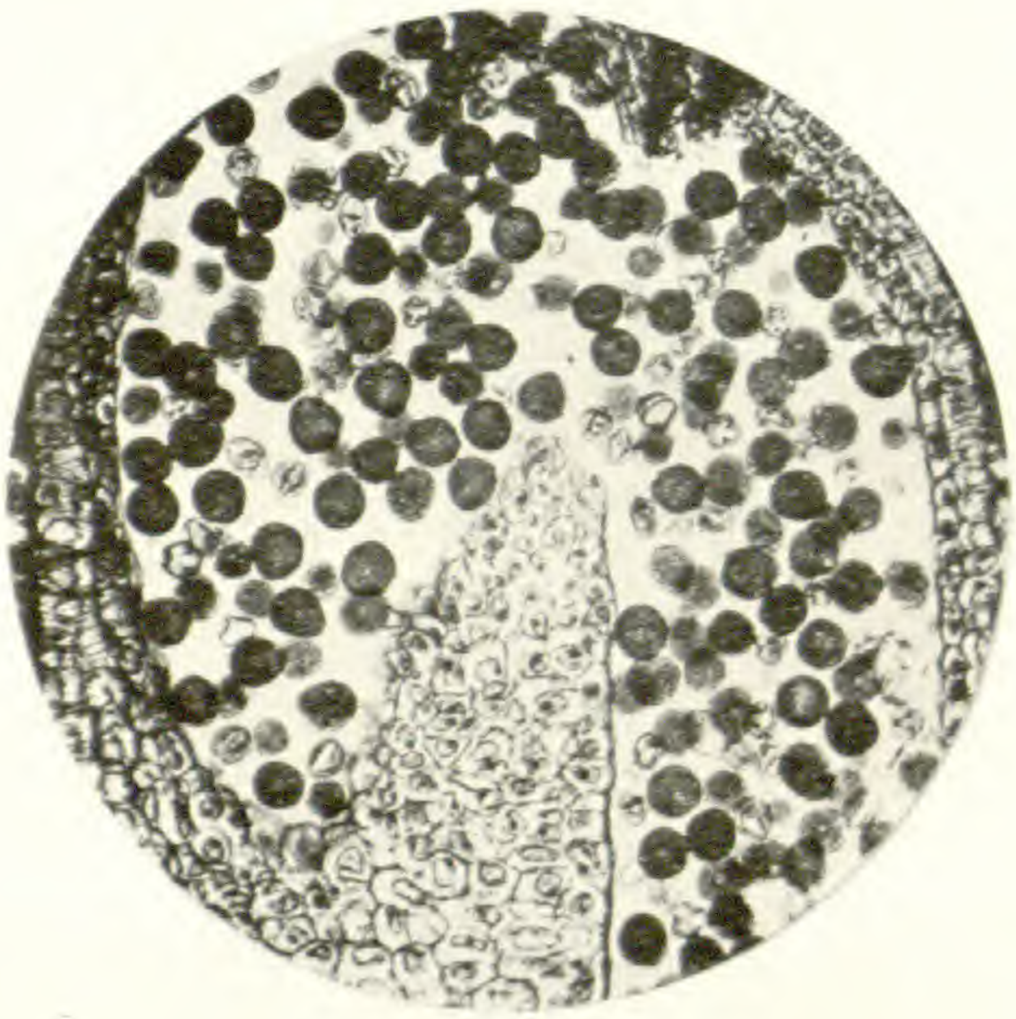

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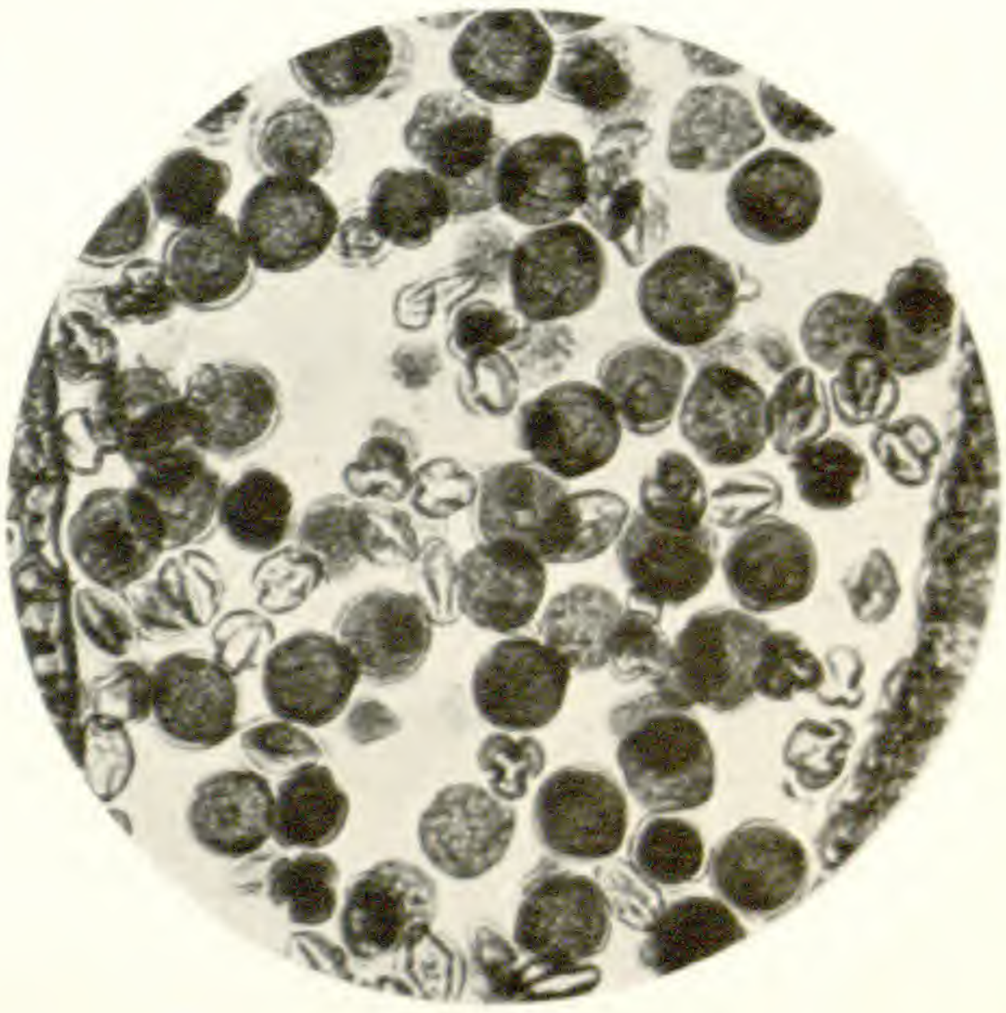

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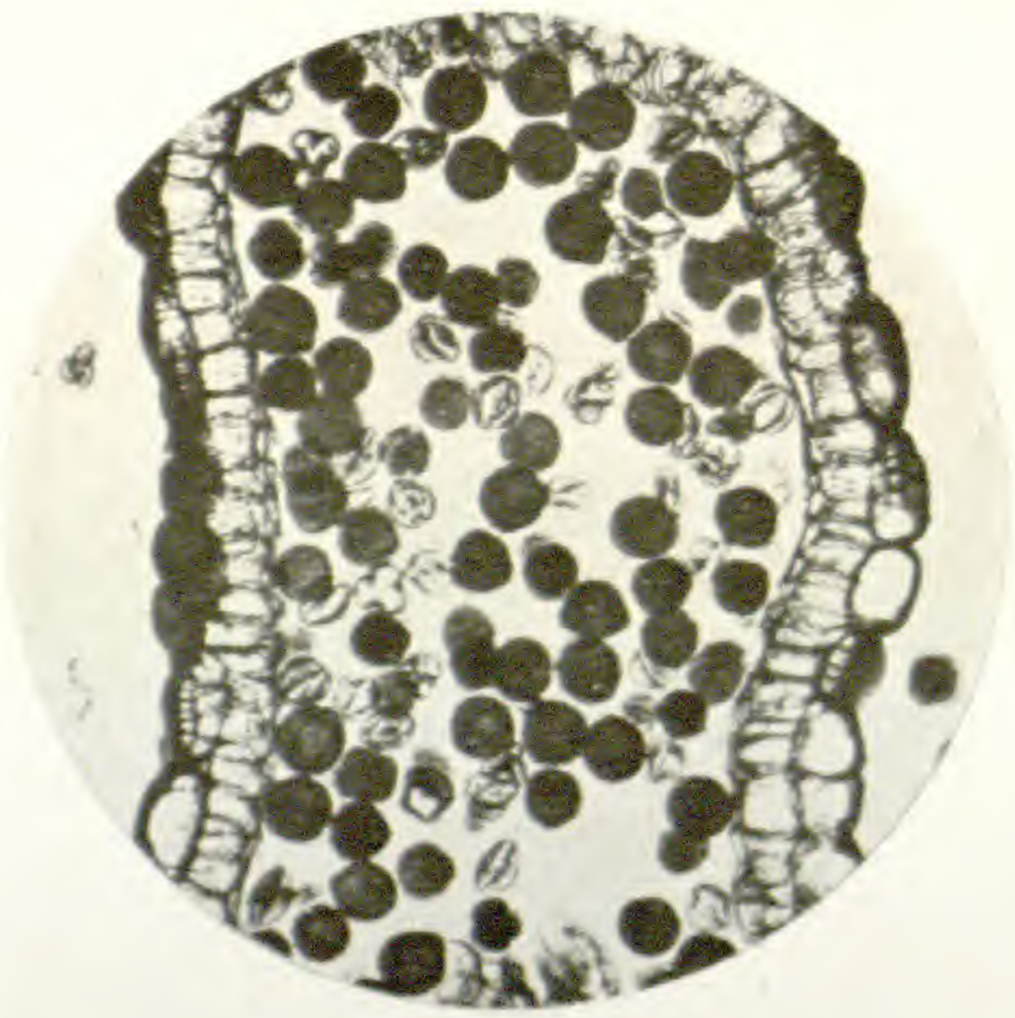

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18

COLE on ROSA

PLATE V

FIGS. 7–18.—Transverse sections of anther.
FIG. 7.—*Rosa oxyodon;* ×125.
FIG. 8.—*Rosa spinosissima altaica;* ×125.
FIG. 9.—*Rosa spinosissima hispida;* ×125.
FIG. 10.—*Rosa spinosissima* (gard. var. hyb.); ×125.
FIG. 11.—*Rosa spinosissima fulgens;* ×125.
FIG. 12.—*Rosa spinosissima;* ×125.

PLATE VI

FIG. 13.—*Rosa rugosa;* ×125.
FIG. 14.—*Rosa rugosa plena;* ×125.
FIG. 15.—*Rosa rugosa alba;* ×125.
FIG. 16.—*Rosa kamchatica;* ×250.
FIG. 17.—*Rosa Manetti;* ×125.
FIG. 18.—*Rosa Harrisoni;* ×125.

MORPHOLOGY OF KETELEERIA FORTUNEI

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 225

A. H. HUTCHINSON

(WITH PLATES VII AND VIII AND THREE FIGURES)

Since its discovery by FORTUNE, *Keteleeria* has aroused interest as an endemic Chinese conifer. It was found first near the temple of Foo Chow Foo, and reports of recent explorers locate it near ancient shrines. Whether *Keteleeria*, as seems most probable in the case of *Ginkgo*, was a sacred tree and has been preserved by a religious order is a matter of conjecture.

Because of limited knowledge, even of the gross structure and characteristics, it is not surprising that the form now known as *Keteleeria* has been variously placed by systematists. LINDLEY named this form *Abies jezoensis*, mistaking it for a Japanese species of that name (14). MURRAY, in 1862, showed that the form in question differed from *Abies jezoensis* and called it *Abies Fortunei* after the original discoverer. In 1868 CARRIÈRE made a new genus *Keteleeria*, naming it after KETELEER, a Belgian horticulturist. PARLATORE placed the same form under the genus *Pinus*, as *P. Fortunei;* by BENTHAM and HOOKER it was classified with *Tsuga;* BENTHAM and MASTERS again placed it in the genus *Abies;* while by ENGLER it is described under *Abies*.

CARRIÈRE'S reasons for making a new genus were that the form in question differs from *Picea*, since it has erect cones; it cannot be included with *Abies* because the cone scales are persistent; and at the same time, in habit and general aspect it resembles *Podocarpus*. Further, PIROTTA (14) states that a new genus is justified because of the arrangement of the staminate strobili ("fiori maschili"). The bud of the staminate strobili is borne either in the axils of the leaves of the preceding year or at the apex of a branch. PIROTTA regards the cone clusters as true "inflorescences." Each "inflorescence" consists of a short peduncle dilated at the apex into a receptacle-like body which is invested

by scales; the lower scales are short, those above becoming increasingly longer. The "flowers" are situated on the margin of the dilated peduncle in the form of a circle or false crown, one or two "flowers" being situated near the center of the receptacle. The number of staminate cones in a cluster ranges from 6 to 10. It is rather remarkable that the other genus of Abietineae (*Pseudolarix*) which shows such an arrangement of staminate cones is also an endemic of China. PIROTTA regards this character of sufficient importance to warrant the division of Abietineae into the Euabietineae, including those forms whose staminate strobili are single (*Abies, Picea, Pseudotsuga, Tsuga*), and the Pseudoabietineae, including those forms whose staminate strobili are in clusters (*Keteleeria* and *Pseudolarix*).

PIROTTA (**15**) has examined also the anatomical structure of the root, stem, and leaves. The root is characterized by a primary axial resin canal, by secondary canals arranged irregularly in the secondary wood, and by the presence of resin-bearing "idioblasts" in the secondary cortex. In the branches there are resin canals and mucilage-bearing "idioblasts" in the primary cortex only. The leaves are bilateral and contain 2 marginal resin canals and also mucilage "idioblasts" in the mesophyll.

The vascular anatomy has been studied also by HOLDEN (**11**), who says "*Keteleeria* has the wood structure of *Abies*. Ray tracheids are entirely absent even in such primitive structures as the first annular ring, cone-bearing branches, cone axis, and are not recalled after wounding, although there is an abundant formation of traumatic resin canals."

RADAIS (**17**) has classified conifers according to the distribution of the resin ducts ("caneau sécréteurs") in the megasporophylls. Upon this basis *Keteleeria* is placed with *Cedrus* and *Picea*, cross-sections of the sporophyll, about the middle of the seed, showing resin ducts in both inner and outer parenchyma; in *Tsuga, Larix, Pseudotsuga*, and *Abies* they are situated in the inner parenchyma; and in *Pinus*, in the outer parenchyma only. This classification, according to the admission of the author, is "surtout artificiel."

The anatomy of the staminate strobilus has been described by AASE (**1**). The general tendency in the evolution of conifers

to proceed from separate bract and scale to the fused bract and scale is noted; in the first group the bract and scale are separate almost to the base of the appendages, and both are about equally prominent. To this group belong *Keteleeria*, *Pseudotsuga*, species of *Abies*, and species of *Larix*.

MALE GAMETOPHYTE.—The multiplication of the cells in the male gametophyte follows the sequence characteristic of the Abietineae. There are 3 successive primary divisions; by the first and second the 2 polar ("prothallial") cells are cut off, the third resulting in the formation of the antheridial cell and the tube nucleus (figs. 1–5). This stage is the most advanced found in available material, and is believed to be the stage at which the pollen is shed. With respect to the development of the male gametophyte at the time of shedding, *Keteleeria* would resemble *Pinus* and might be contrasted with *Abies* and *Picea*. The appendage-like outgrowths of the exine and the inflation of the region between the exine and intine, caused by this growth, result in the production of wings, such as are characteristic of the Abietineae.

The mitoses involved in the development of the male gametophyte are similar to those described for *Abies* and *Picea* (**12,13**). In each of the first 3 mitoses the spindle fibers become oriented in such a way that they surround the polar nucleus; later they radiate from it, appearing in cross-section as tufts of fibers. This peculiarity of the mitotic figure doubtless is associated with the unequal apportionment of the cytoplasm to the resulting nuclei, an inequality which results in more favorable conditions for the more centrally placed nucleus.

In *Keteleeria* the development of the male gametophyte is not uniformly as described. Fig. 5 shows 4 nuclei medianly placed and almost equal in size. The association of such gametophytes with others whose nuclei and cells are unequal and differently placed indicates that the degree of development depends upon conditions, rather than being foreordained. When inclosed by a wall containing little cytoplasm the nucleus soon disintegrates. Fig. 4 shows 3 nuclei which are "prothallial" in nature; the third under ordinary conditions would be regarded as antheridial; in

this case the nucleus corresponding in origin to the tube nucleus has taken the central position. In the struggle the nucleus which is most centrally placed gains the ascendency, the others being crowded to the wall.

MORPHOLOGY OF THE OVULATE STROBILUS.—The anatomy of the megasporophyll has been studied by AASE (1). "In *Keteleeria Fortunei* one bundle originates near the base of the gap in the strobilus cylinder and supplies the bract. It remains undivided throughout its course. Two bundles, one from each side of the gap, supply the scale; the two bundles soon unite, forming one inverted bundle, that is, its xylem faces the xylem of bract." However, in the early stages the strands connected with the 2 ovules are separate. The evidence supports the theory that the scale with its megasporangia represents a fertile bud in the axil of the bract.

The material studied shows that at the time the pollen is shed the megasporangium has reached the mother-cell stage. There is only one megaspore mother cell and it is the fourth cell from the epidermis (fig. 9), characters which still further emphasize the relation of *Keteleeria* to the Abietineae.

SIEVE TUBES.—The sieve tubes of *Keteleeria* are large, 8–10× 200–400μ, and are well differentiated. Concerning the sieve tubes of gymnosperms, DEBARY (5) states that "the oblique terminal faces are directed toward the radial planes. Sieve plates are placed in one or two longitudinal rows over the terminal faces and the whole remainder of the radial lateral face. They form roundish spots separated by high intervening portions. These spots are coarsely latticed, while in the cavities of the coarse lattice the very delicate sieve structure is seen." The appearance as seen in a surface view only has been described, and this has led to an erroneous idea of the structure. The description given by HABERLANDT (6) and DEBARY might apply to the structures as represented in fig. 6. However, transverse and tangential sections show no thickenings or depressions in the walls of the sieve tubes. The appearance of "delicate sieve structures" described for radial sections is caused by the presence of granular inclusions in protoplasmic aggregations which are situated on either side of groups

of perforations in the sieve tube walls. The protoplasmic masses are connected by delicate strands which penetrate these perforations (figs. 7, 8).

The general tendency in the modification of sieve tubes from the lower to the higher vascular plants is toward an increasingly smaller number of sieve plates. First, there is a decrease in the number of walls upon which the plates occur. In some ferns each sieve tube wall contiguous with the wall of a similar cell bears sieve plates, while in most angiosperms they occur on the terminal walls only. Again, there is a tendency toward diminution in the number of plates on a given surface. In ferns (**5,** p. 180) there are several rows of plates, or they may be closely crowded together; in *Vitis* there are a number of elongated plates on the oblique septae; in cucurbits there is a single plate. In *Keteleeria* the occurrence is limited to the walls seen in radial sections and the oblique terminal walls. This is true of gymnosperms in so far as the records are available. The plates are arranged in a single interrupted series of groups. In this respect *Keteleeria* is much more advanced than *Encephalartos* (**5,** p. 181, *fig. 78*); the latter has plate groups closely distributed over the radial faces. Moreover, the plate groups are much less numerous on the radial faces in *Keteleeria* than on the oblique terminal faces. This is a further advance toward the condition in angiosperms. It seems probable that the investigation of other forms in this respect would give valuable evidence with reference to genetic relationships.

EMBRYO.—The embryo of *Keteleeria* is of considerable morphological interest; it throws light upon the polycotyledonous embryo of Coniferales, and also the meristem of the primary root shows characters heretofore unknown among gymnosperms (**2, 7, 8, 9, 10**). PIROTTA (**16**) has described the seedling.

In the embryo, as found in the mature seed, the following regions occur: cotyledons, leaf bud, and primary root. Beginning at the exterior, a cross-section of the root, taken near the central region (figs. 24, 25), shows the coleorhiza, the cortex, the region of meristematic cells and mucilage cells, and the central axis.

There is a cotyledonary tube which extends throughout approximately two-thirds of the length of the embryo. The cotyledons

proper are terminal upon the cotyledonary tube, and their length is about one-sixth of that of the tube. At the base the tube is in the form of a hollow cylinder (fig. 19) above; the inner surfaces become rectangular, then star-shaped in outline (figs. 16, 17); finally, the 4 cotyledonary tips become separate (fig. 16).

Occasional seedlings of *Abies* and *Pinus* have been described (7, 8, 9, 10) having limited cotyledonary tubes, but no such pronounced structure as occurs in *Keteleeria* has been recorded. Although the material did not show the earlier stages, it seems evident that the situation here is similar to that existing in angiosperms (3, 4); that cotyledons, whether several as in Coniferales, two as in dicotyledons, or one as in monocotyledons, are all similar in origin; that the cotyledonary growth is, primarily, that which results from a meristematic ring about the leaf bud, the number of cotyledons being dependent upon the number of the loci of increased growth. In *Keteleeria* the major part of cotyledonary elongation is uniform throughout the entire ring of the growth region.

With the exception of the central axis, the regions of the primary root are similar to those of other conifers; in *Keteleeria* this region is continuous throughout, while in other conifers described it is broken by the meristematic region. It is evident that such a modification in structure is due to the nature of the meristem. Since the meristem of certain conifers, including *Keteleeria*, is being described in another paper, details may be omitted here.

The differentiation of tissues as they occur in the embryo of the mature seed is advanced beyond the stage usual for conifers (7, 8, 9, 10). In the primary root the first cells to become differentiated are those which later become mucilage tubes (shown in black, figs. 15–27). The cells cease to divide and become vacuolate (fig. 11); the nucleus disintegrates; the cells are greatly elongated by division and growth of the surrounding cells and become mucilaginous in content. Similar cells, except that they are much shorter, are formed in the coleorhiza. The cells of the cortex become filled with food materials, generally in the form of starch (fig. 12). The cells forming a hollow meristematic cylinder about

the central axis divide in either of two planes (fig. 13). The first xylem elements, having the usual spiral thickenings of the protoxylem, become differentiated in the cotyledons; there are 4 groups of protoxylem, with 2–4 strands in each group, extending

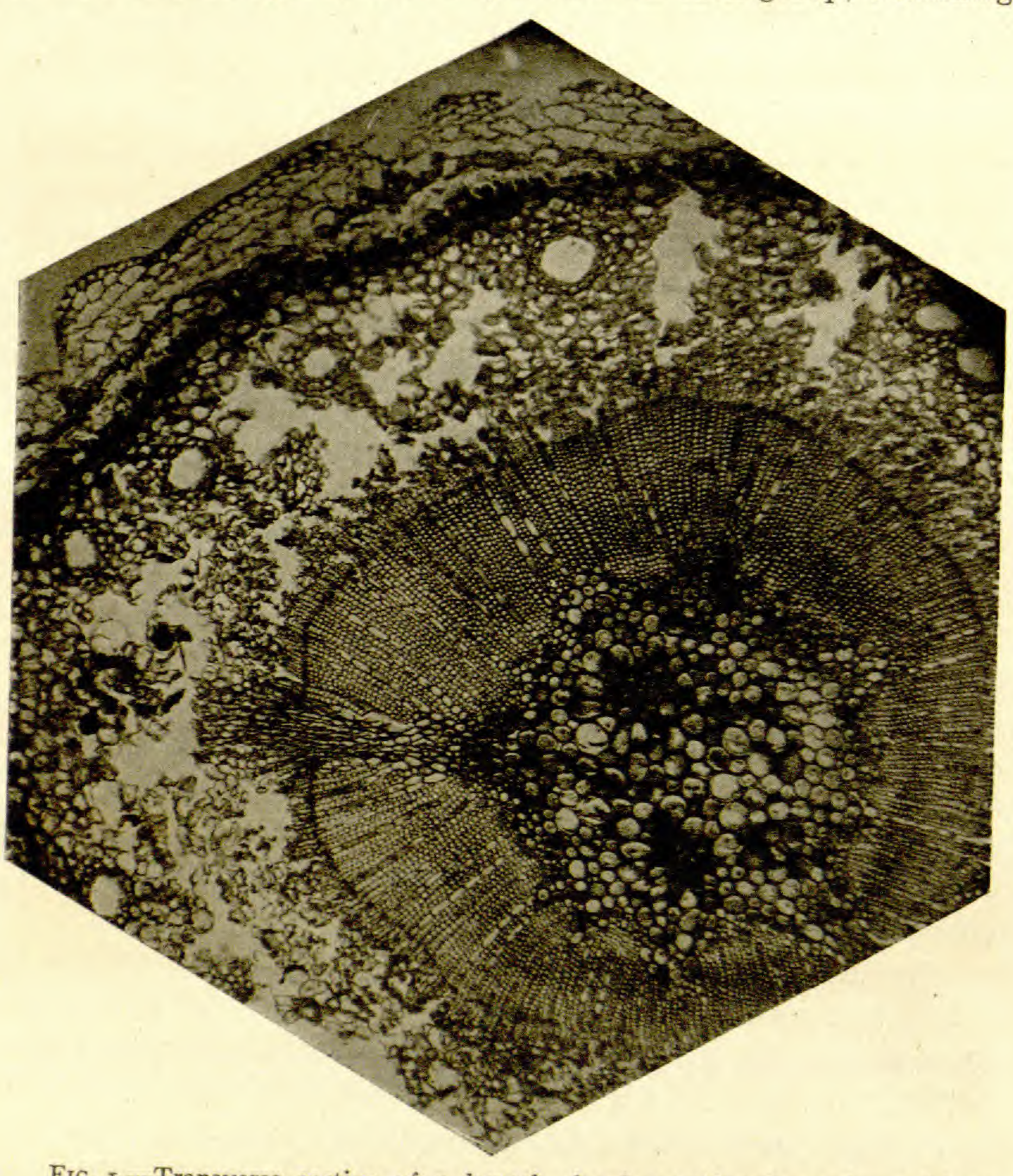


FIG. 1.—Transverse section of a branch showing epidermis, cork cambium, cortex with resin ducts, phloem, xylem, pith, and a leaf trace.

from near the tip of the cotyledon to the base of the cotyledonary tube. The protoxylem is endarch (figs. 16–20). It is significant that at this time there is no xylem present in the primary root or in the leaf bud (stem tip).

The 4 meristematic regions of the cotyledonary tube, with which the protoxylem is associated, and also the meristem of the leaf bud, connect with the hollow meristematic cylinder of the primary root. The central axis extends beyond this junction point, thereby modifying the structure generally known as the cotyledonary plate (fig. 15) (**7, 8, 9, 10**).

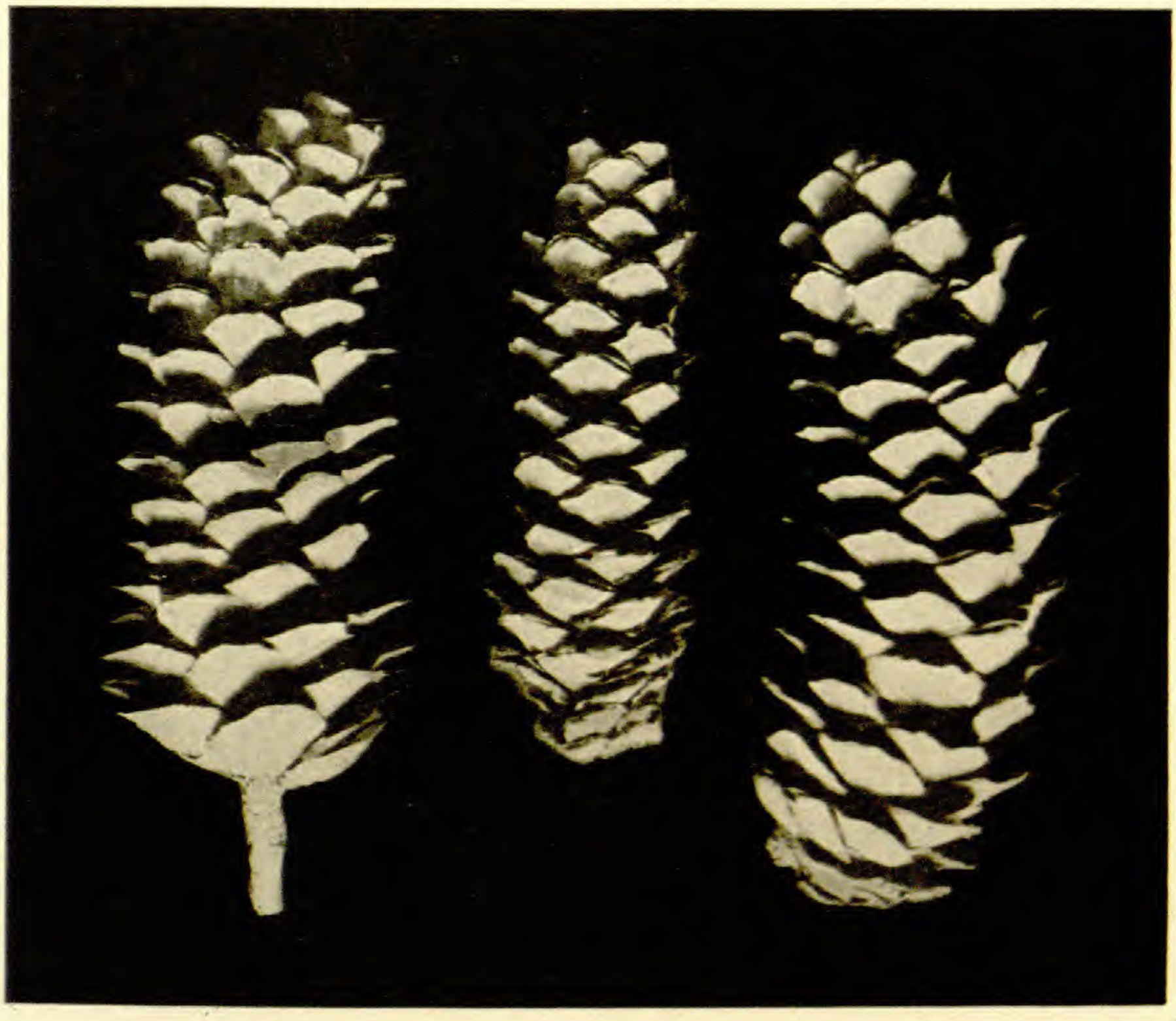


FIG. 2.—Mature ovulate cones

Summary

The following characters of the form in question warrant a genus *Keteleeria*, belonging to the Abietineae.

1. *Ovulate strobilus.*—(1) The cones are erect (text fig. 2); (2) the scales are persistent (text fig. 2); (3) the scale and bract are separate nearly to their bases (text fig. 3); (4) there is a single megaspore mother-cell (fig. 9).

2. *Staminate strobilus.*—(1) The staminate cones are borne in clusters on a fertile branch (text fig. 3); (2) there are 2 abaxial microsporangia on each sporophyll; (3) the pollen is winged (figs. 1–5).

3. *Male gametophyte.*—The pollen is shed in the 4-celled stage, consisting of 2 polar cells, the antheridial cell, and the tube nucleus (figs. 2–3).

FIG. 3.—Above, megasporophylls with scale and bract separate nearly to the base; below, fertile branches bearing groups of staminate strobili; between, 2 winged seeds.

4. *Vascular anatomy.*—(1) Resin ducts do not occur in the secondary wood except as traumatic responses (text fig. 1) (11); (2) there are no ray tracheids nor are they "recalled by wounding" (11).

5. *Embryo.*—(1) There is an extensive cotyledonary tube (figs. 15–20); (2) a central axis extends throughout the primary root (fig. 15).

6. *Leaves.*—(1) The leaves are spirally arranged on ordinary branches; (2) there are 2 very closely associated vascular strands and 2 marginal resin ducts (**15**).

7. *Sieve tubes.*—These present single interrupted rows of plate groups on the radial and oblique terminal faces. Paired protoplasmic accumulations, one on either side of each plate, are connected by strands which penetrate small perforations in the intervening walls (figs. 6–8). The sieve tubes are more numerous on the oblique terminal faces, an advance toward the angiosperm condition.

8. *Cotyledonary tube.*—This is significant in connection with the theories of the origin of polycotyledony.

The writer wishes to express his thanks to Professor J. M. COULTER and Professor C. J. CHAMBERLAIN for material provided and for advice and direction during the progress of the investigation.

UNIVERSITY OF BRITISH COLUMBIA

LITERATURE CITED

1. AASE, H. C., Vascular anatomy of the megasporophylls of conifers. BOT. GAZ. **60**:277–313. 1915.
2. COULTER, J. M., and CHAMBERLAIN, C. J., Morphology of gymnosperms. Chicago. 1910.
3. COULTER, J. M., and LAND, W. J. G., The origin of monocotyledony. BOT. GAZ. **57**:509–519. 1914
4. ———, *ibid.* Ann. Mo. Bot. Gard. **2**:175–183. 1914.
5. DEBARY, A. (BOWER, F. O., and SCOTT, D. H.), Comparative anatomy of the phanerogams and ferns. 1884.
6. HABERLANDT, G. (DRUMMOND, M.), Physiological plant anatomy. McMillian. 1914.
7. HILL, T. G., and DEFRAINE, E., On the seedling structure of gymnosperms. Ann. Botany **22**:689–712. *pl. 35.* 1908.
8. ———, *ibid.* Ann. Botany **23**:189–227. *pl. 15.* 1909.
9. ———, *ibid.* Ann. Botany **23**:433–458. *pl. 30.* 1909.
10. ———, *ibid.* Ann. Botany **24**:319–333. *pls. 22, 23.* 1910.
11. HOLDEN, R., Ray tracheids in the Coniferales. BOT. GAZ. **55**:97–115. *pls. 1, 2.* 1913.
12. HUTCHINSON, A. H., The male gametophyte of *Abies balsamea.* BOT. GAZ. **57**:148–152. *figs. 15.* 1914.
13. ———, The male gametophyte of *Picea canadensis.* BOT. GAZ. **59**:287–300. *pls. 15–19. fig. 1.* 1915.

14. PIROTTA, R., Sul genere *Keteleeria* di Carrière (*Abies Fortunei* Murr.). Bull. Soc. Toscana Orticultura **12**:1–8. 1887.

15. ———, Sulla struttura anatomica della *Keteleeria Fortunei*. Rend. R. Acad. Lincei **6**:561–565. 1890.

16. ———, Sulla germinazione e sulla struttura della piantina della *Keteleeria Fortunei*. Ann. R. Inst. Bot. Roma **6**:31–34.

17. RADAIS, MAXIME, Anatomie comparée du fruit des Conifères. Ann. Sci. Nat. Bot. **14**:165–368. 1894.

EXPLANATION OF PLATES VII AND VIII

FIGS. 1–5.—Male gametophyte; ×1630.

FIG. 1.—First polar cell and second primary mitosis.

FIGS. 2, 3.—Two polar cells, antheridial cell, and tube nucleus; in fig. 3 the wall which later cuts off the antheridial cell has not yet been formed; the radiating fibers may be noted.

FIG. 4.—Three nuclei have been crowded to the wall ("prothallial cells"); tube nucleus is central.

FIG. 5.—Four medianly placed nuclei, alike in size and structure.

FIGS. 6–8.—Sieve tubes with sieve plates; ×1630.

FIG. 6.—From a longitudinal radial section.

FIG. 7.—From a longitudinal tangential section.

FIG. 8.—From a transverse section, a cell from the pith ray being shown also.

FIG. 9.—Megasporangium showing megaspore mother-cell.

FIGS. 10–14.—Cells from different regions of the embryo.

FIG. 10.—Cells from meristem of cotyledon.

FIG. 11.—Cells becoming differentiated to form mucilage tubes.

FIG. 12.—A cortical cell.

FIG. 13.—Cells from meristem of primary root.

FIG. 14.—From protoxylem of cotyledonary tube.

FIGS. 15–27.—Semidiagrammatic drawings of the embryo; ×40.

FIG. 15.—Longitudinal median section; attached numbers indicate region from which accompanying transverse sections have been taken.

FIGS. 16–27.—Transverse sections.

FIG. 16.—Cotyledons.

FIGS. 17–20.—Cotyledonary tube, showing meristematic region and protoxylem (black).

FIG. 20.—Cotyledonary tube and leaf bud.

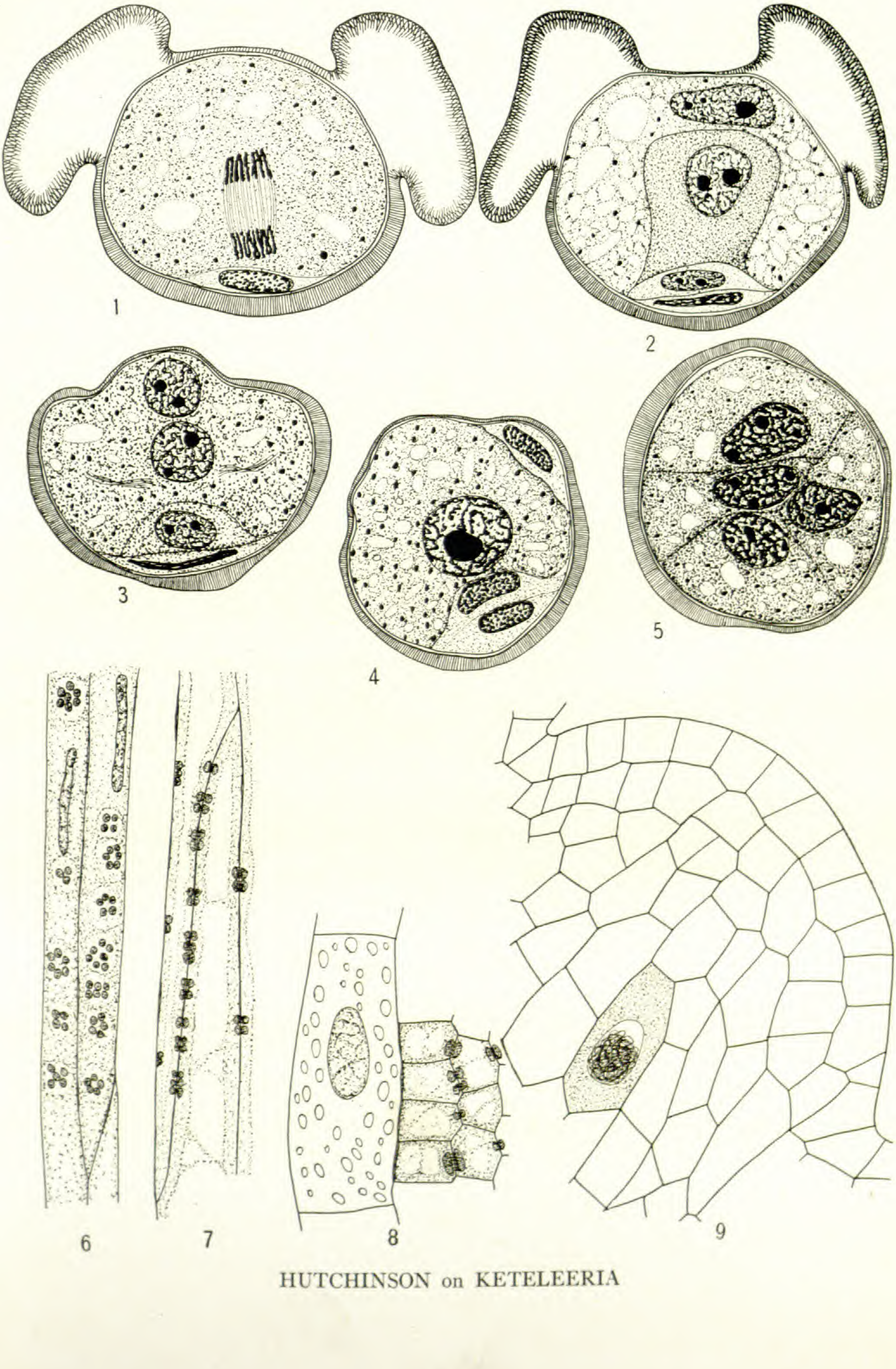
FIG. 21.—Junction of hollow meristematic cylinder of primary root and 4 meristematic regions of cotyledonary tube.

FIGS. 22, 23.—Cortex, region of meristem, and central axis.

FIGS. 24, 25.—Showing 4 regions; coleorhiza, cortex, region of meristem, and mucilage tubes; also central axis.

FIG. 26.—Section of coleorhiza, cortex, and central axis.

FIG. 27.—Section of coleorhiza and central axis.



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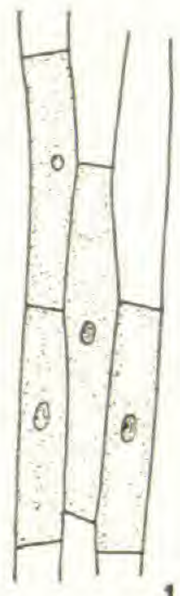
HUTCHINSON on KETELEERIA



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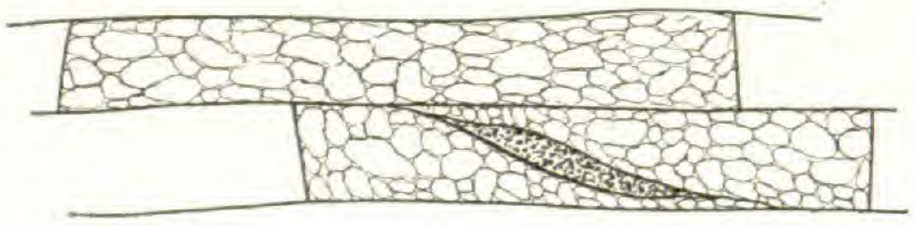

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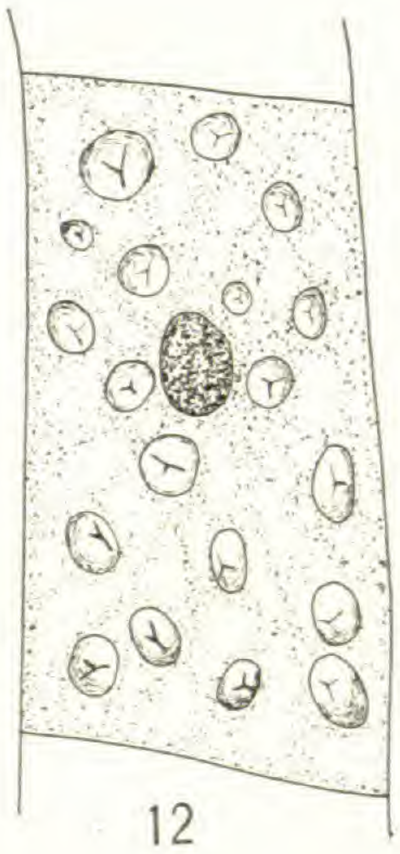

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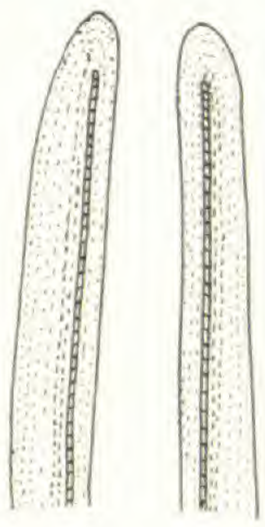

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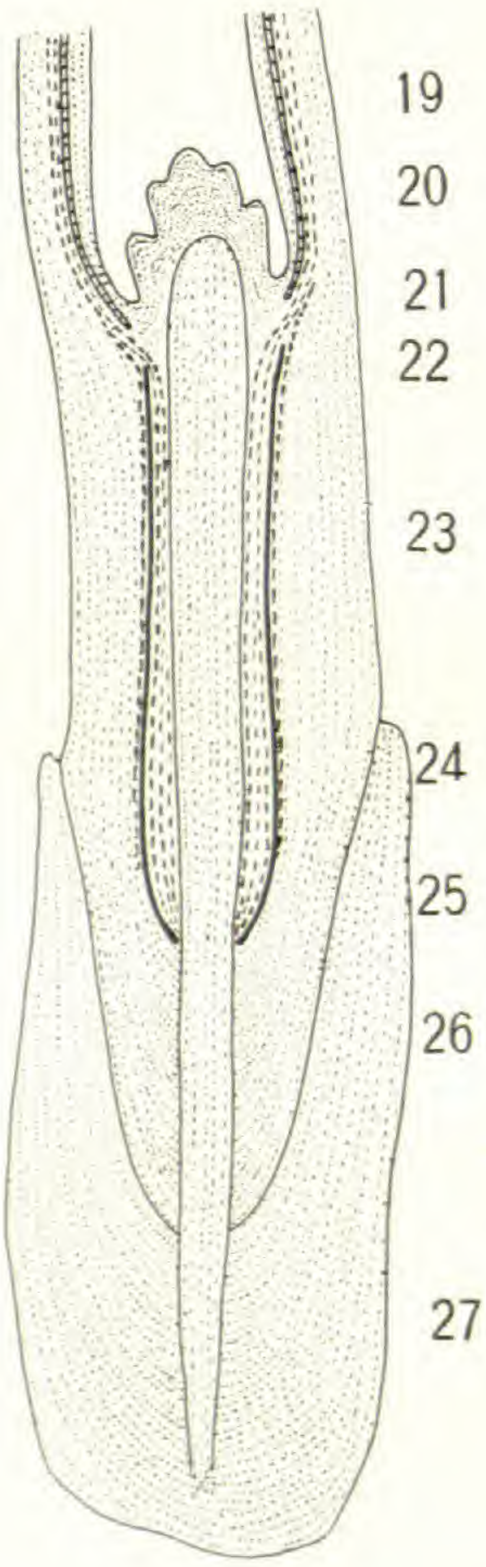

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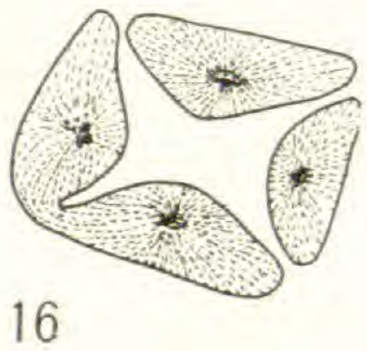

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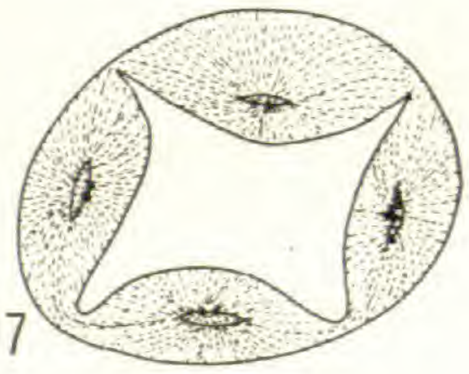

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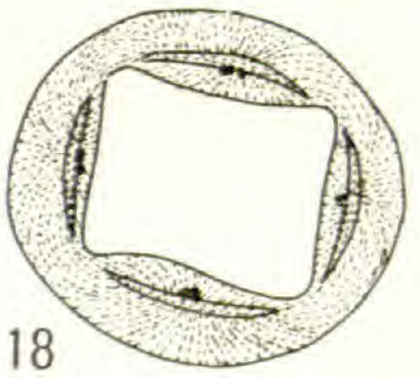

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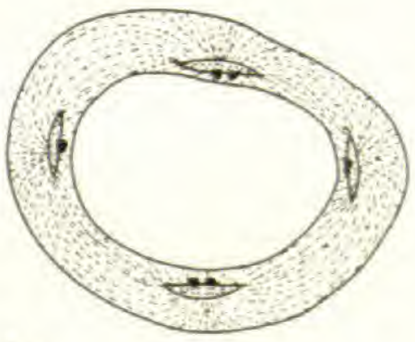

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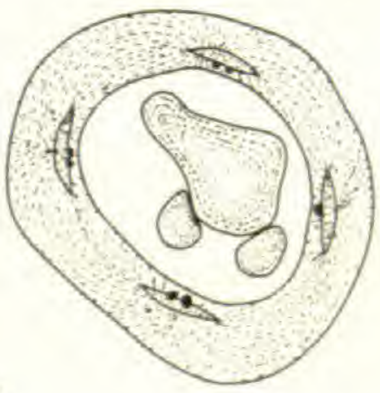

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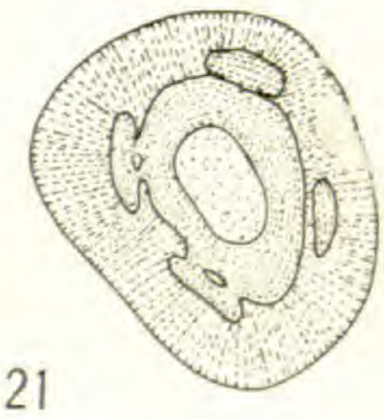

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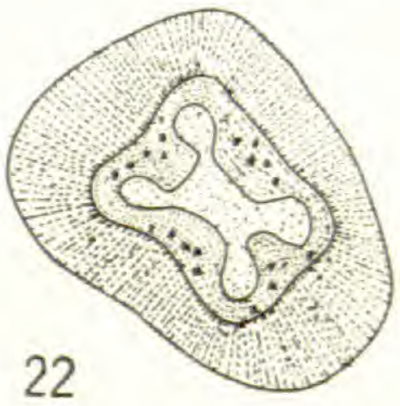

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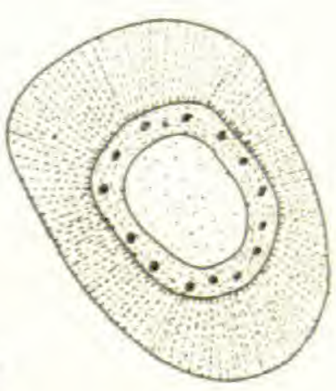

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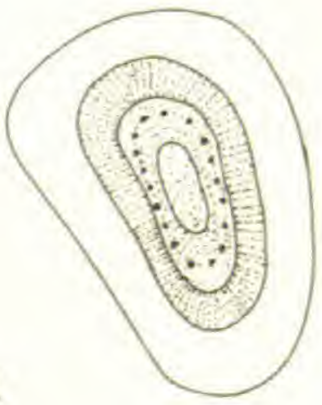

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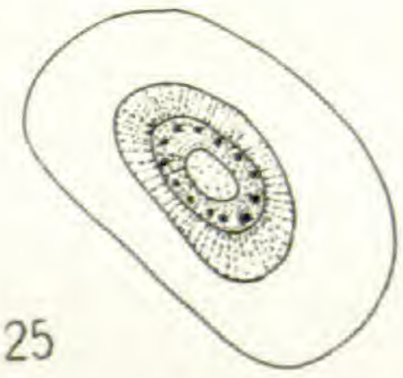

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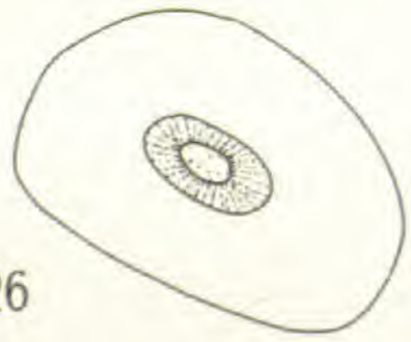

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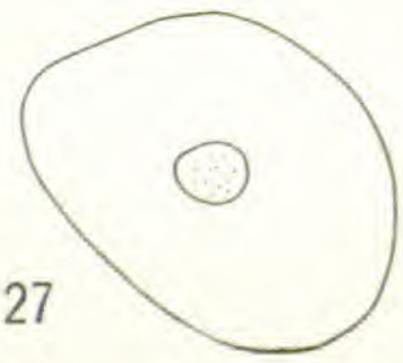

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HUTCHINSON on KETELEERIA

THE POLLINATION OF VALLISNERIA SPIRALIS

ROBERT B. WYLIE

(WITH PLATE IX AND SIX FIGURES)

Vallisneria has long been counted one of the classic examples of cross-pollination. Living vegetatively as a submersed aquatic, its dioecious flowers are brought together at the surface of the water in most ingenious fashion. These highly specialized flowers present the strongest contrasts, not only in size and structure, but in behavior as well, and give this plant its rank as one of the climax types with respect to floral differentiation. Specializations of such evident advantage for cross-pollination in a form so admirably situated for vegetative propagation seem to emphasize the importance of sexuality, or at least of seed production, in the higher plants.

While the general method of pollination in *Vallisneria* is well known, many interesting facts seem never to have been published, and the underlying principle has not been emphasized. The figures current in textbooks are highly generalized, and some of them are far from accurate. The story which they are intended to illustrate is likewise incomplete or in some cases highly distorted. In any event, neither figure nor story has done justice to the intimate history of pollen transfer in this remarkable plant.

It will be noted at once that the following account diverges radically from that suggested by KERNER'S (1) beautiful and widely copied figure. A comparison shows that these differences relate not only to the size and structure of the flowers, but are even more fundamental in character. KERNER emphasizes the fact that pollination is brought about through the contact of flowers floating on a level water surface; there follows an outline of a method of pollen transfer through the special agency of the surface film of water. The general drawing (pl. IX) is based on photographs of living flowers, measurements, and camera drawings of parts.

The epigynous seed-bearing flowers of *Vallisneria* are borne singly, each within its spathe at the end of a long scape, sometimes over a meter in length, which anchors the floating flower to the short upright stem at the bottom of the pond. Upon reaching the surface of the water through the elongation of this axis, the spathe opens at its outer end, but remains as a partial investment of the ovary until the seeds are nearly mature. The 3 spoonlike sepals soon separate, disclosing the 3 bifid stigmas which are coiled in the center of the flower (pl. IX). These fleshy stigmas are densely clothed with the stigmatic hairs, and their snowy whiteness constitutes the most conspicuous part of the flower. Rudimentary petals and slender staminodia are present, but as they seem functionless their discussion may be deferred to a subsequent paper.

The anchoring scape usually elongates sufficiently to permit the opening flower to assume an inclined position in the water as it is carried to one side by wind or current. The ovary, which is 20–25 mm. long before fertilization, is usually straight until the flower opens and has taken its position at the surface; later it often curves considerably in response to gravity, thus bringing the floral parts more nearly parallel with the surface of the water. This bending of the ovary at this stage is quite marked in plants growing in aquaria where the flowers are left undisturbed for some time.

The exposed floral parts are waxy and consequently are not wetted by the water, with the result that the flower comes to rest with a portion of its weight resting on the sepals and margins of stigmas supported by the surface film. This produces a slight depression of the water about the flower, perhaps 15 mm. in diameter, which is abruptly declined at its inner margin next to the pistillate flower. This sloping surface film plays an important part in capturing the floating staminate flowers, and later is intimately bound up with the actual transfer of pollen to the stigmas. Too much emphasis cannot be laid on the complete dependence of this plant upon the surface film of water for its pollination processes.

The staminate flowers are borne crowded numerously within the globose spathe which remains short-stalked at the bottom of

the pond. A count of several of these flower masses showed an average of over 2000 flowers packed within each spathe, the whole group the homologue of the single pistillate flower which is solitary within its spathe. The staminate inflorescence resembles a large fern sorus surrounded by an indusium. This similarity is carried further by the striking resemblance of the slender-stalked unopen staminate flowers to polypod fern sporangia. Massed within the spathe these flowers are joined to the axis by slender pedicels of varying length, so as to completely fill the space between the stem and the spathe.

The pollen-bearing flowers are very tiny, less than 1 mm. in diameter before opening, and are simple in structure. The floral parts consist of 3 sepals, 2 functional stamens, and rudiments of petals. The sepals are of unequal size and are not symmetrically disposed; 2 are similar and stand nearly opposite; while the third and smaller one is placed laterally between them. This reduced sepal is the first to open. Numerous tapering and curved hairs cover the region about the base of the stamens and are doubtless of some importance, although their functions are not clear. The 2 stamens stand close together and have their parallel filaments united up to a point near the anthers (pl. IX).

At maturity the tip of the spathe opens slightly and the staminate flowers begin detaching from their slender stalks. The uppermost are the first to be shed, and 2 or 3 days may be necessary to empty a single spathe. These detached flowers rise slowly through the water to the surface and there very slowly open. In this respect *Vallisneria* stands in sharp contrast to the writer's (2) observations on *Elodea canadensis*. In that form the staminate flowers upon release dart to the surface and there fairly explode, scattering their pollen on the surface of the water. In *Elodea*, however, it is the free floating pollen that functions, while in *Vallisneria* the pollen retained in the anthers has the better chance of reaching the stigmas. SVEDELIUS (3) reports that the sepals of the detached staminate flowers of *Enalus acoroides* snap back upon reaching the surface of the water, although the pollen is retained in the anthers. In *Elodea canadensis*, and perhaps in *Enalus*, the snap of the sepals seems to be due in part at least to the release of

gases imprisoned between the floral parts under water. The writer (4) has noted elsewhere that in *Elodea ioensis*, which has a long-stalked staminate flower, a bubble of gas is generally associated with the partly opened sepals, giving extra buoyancy to the submerged flowers, which tug at their anchorage like captive balloons.

No prolonged observations were made on the possible periodicity in the release of the staminate flowers of *Vallisneria*, although doubtless there is a relation between their detachment and the gases given off by the plant during times of brighter illumination. On one occasion it was observed that as the sun came up from behind a building and its direct rays fell on the spathe of the staminate inflorescence the rate of detachment was considerably increased for a time. In *Elodea canadensis* (2) there is a correlation between the coming of strong light in the morning and the rate of detachment of the staminate flowers. SVEDELIUS reports that the staminate flowers of *Enalus acoroides* are released mainly (if not exclusively) at periods of low tide. This habit is of peculiar significance from the fact that at high tide the pistillate flowers of that plant are wholly submerged and pollination would be impossible. No explanation of this relation was suggested in the paper.

The sepals of the staminate flower of *Vallisneria* completely invest the stamens until some time after the flower reaches the surface. They then slowly recurve, the smaller one being first to open (pl. IX), and as it touches the water it seems to function in orienting the flower so that when the pair of lateral sepals open there are formed 3 boatlike structures which engage the surface film and float the flower. This tiny flower, with its upraised stamens and pollen mass, is so snugly fitted to the surface film by its 3 broad areas of contact that it is kept in equilibrium under all ordinary circumstances. They are rarely overturned, even by rather vigorous agitation of the water, but maintain a strict right angle to the surface film. So slender an object as a needle if thrust into the water among these floating flowers and slowly withdrawn will be covered by the flowers that have been drawn up with the film of water about the needle and may be seen standing out radially from it on all sides. Once overthrown, however, they are not again righted, but lie partly under water.

This definite engagement with the surface film does not hinder the free movement of the staminate flowers on the water. In open areas they are caught by every passing breeze and are hurried along the surface of the water. On windy days they go scudding by the observer like tiny flecks of foam. Where the plants grow abundantly they often mass along the windward shores in broad zones of snowy white (fig. 1).

The anthers dehisce before the flowers open, and the sticky pollen from the pair of stamens of a given flower usually forms a single pollinium (pl. IX). Even if the products of the 2 anthers form

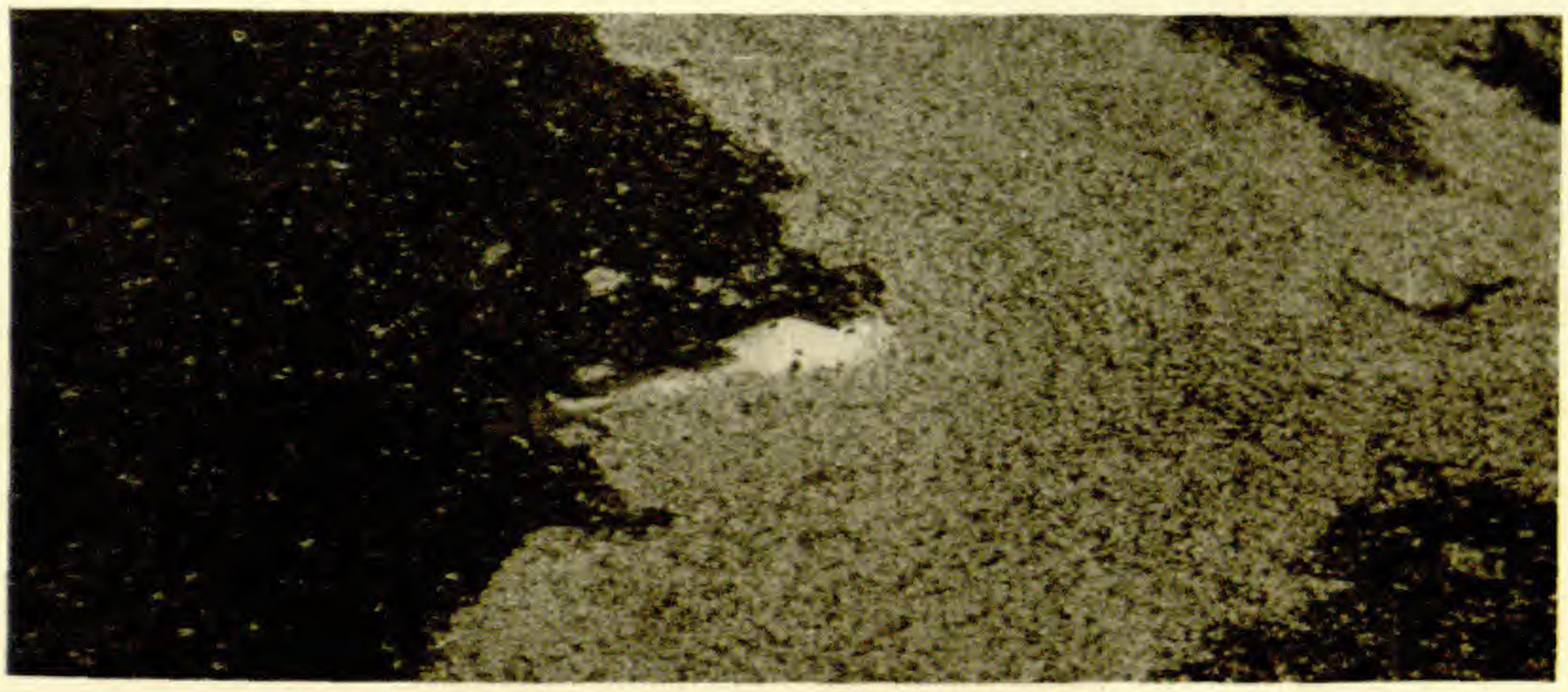


FIG. 1.—Floating staminate flowers along margin of East Okoboji Lake in northwestern Iowa; the dead fish shown near center of picture was about 8 inches long.

separate pollen masses, these lie so close together as to be practically tangential, and are never widely separated, as shown by KERNER (1). Seen under moderate magnification these masses of pollen gleam like clusters of pearls. The microspores, which are slightly oval in form, are about 65 μ in diameter and have a nearly smooth exine. Their adhesiveness is of great advantage in holding the pollen together until it is rubbed off against the stigmas, and doubtless assists after pollination in holding the spores to the stigmatic hairs. The pollen output is limited, averaging about 100 spores to the flower, but varying considerably.

The floating staminate flowers are carried along by the wind, and coming within the radius of the declined surface film about the pistillate flower slide into the little depression, where they are

retained. In this manner possibly as many as 50 staminate flowers may be caught in a single depression, thus forming conspicuous white patches on the surface of the water. It is interesting to note how successfully these areas of associated flowers hold together even when the water is quite rough. COWLES (5) mentions these film pockets, but gives no details other than to compare them with those of *Elodea canadensis*. In this latter form, as previously described (2), the floating pollen grains are caught in the depressions that are formed about the tiny pistillate flowers. SVEDELIUS speaks of a similar "capturing" of the pollen-bearing flowers in *Enalus acoroides*, but does not attribute this to the influence of the surface film, although obviously the case closely parallels that of *Vallisneria*.

FIG. 2.*—Flowers floating on surface of water in a small aquarium surrounded by black paper; pistillate flower in center.

The sepals of the innermost of the captured staminate flowers in *Vallisneria* are of course in contact with the margins of the pistillate flower (fig. 2), but later arrivals are held back as they form only a single layer in the depression. It should be noted at this time that contact between flowers on a level water surface, such as KERNER figures, could not lead to pollen transfer, as the pollinia are upraised over the center of the flower. But with any slight declination of the film about the pistillate flower, even in quiet water, there might be contact between the innermost pollinia and the stigmas (fig. 2). Obviously, however, any movement resulting in a further depression of the pistillate flower would cause the surface film to become more abruptly declined, thus tipping the staminate flowers more sharply inward (fig. 3) and thereby

* FIGS. 2–6 constitute a series illustrating changes in relations of associated flowers floating at surface of water when subjected to submergence by pulling on scape of pistillate flower.

making conditions more favorable for pollen transfer. The upward movement of the water due to a passing wave might serve to temporarily depress the floating pistillate flower weighted by its long stalk. Should the movement be sufficient to make taut the anchoring scape, even for an instant, the depression would become cuplike, with the inner staminate flowers standing at right angles to its nearly vertical walls (fig. 4). Many of the pollen masses would thus be forced directly into the stigmas, although the outer ones would still be held back at some distance. At a certain stage of depression, however, the lateral pressure of the water breaks the surface film above the flower; the sides of the cup snap together, roofing it over; and a considerable number of staminate flowers, with the pistillate flower, are thus shut tightly together in a common bubble beneath the surface of the water (fig. 5). It should be noted that this process has completely overturned the staminate flowers, and that these are now inverted upon the pistillate flower, with their pollen masses pressed sharply into the stigmatic surfaces. The photograph for fig. 5 was taken through perhaps a centimeter of water, and shows the out-turned bases of the staminate flowers lining the bubble as seen from above. To the right may be seen a number of free floating staminate flowers that were released from the depression as the flowers disappeared beneath the surface.

FIG. 3.—Positions assumed with slight tension on scape of pistillate flower.

Release of the tension on the scape permits the flowers to come again to the surface; the bubble breaks, and most of the flowers resume their original relations at the surface of the water (fig. 6). Examination, however, shows numerous pollen grains or even entire staminate flowers scattered over the surface of the stigmas. Fig. 6 shows that the group of free floating staminate flowers,

released when the group was submerged, has again entered the film pocket. This resulted from being blown into the radius of the declined surface.

Each passing wave thus brings a shift in the position of the flowers and furthers the wearing away of the pollinia upon the stigmas. During the time that the pistillate flower is at the surface the events outlined may be repeated hundreds of times with varying degrees of submergence. At all times, of course, there may be additions to the group from the free floating staminate flowers. Attention should be directed to the fact that any degree of depression is helpful, and that complete submergence, although this probably occurs frequently, is not necessary to adequate pollination.

FIG. 4.—Pistillate flower has been pulled farther down in water; depression is cup-shaped, and staminate flowers stand at right angles to the verticle walls.

When the pistillate flower is finally withdrawn into the water by the coiling of the scape, numerous pollen-bearing flowers may be trapped in the bubble of air that forms about the retreating floral parts as they disappear beneath the surface. As the volume of imprisoned air is gradually dissolved by the water, the pollinia would be pressed more and more strongly into the stigmas. Observations on isolated patches of pistillate plants show that the scapes will coil somewhat without pollination, so the retreat of the seed-bearing flower is the final step in pollination if the pollen-bearing flowers are present. At the first favorable opportunity the writer plans to make a study of the flowering habits of marked plants in the field to determine the length of time the flowers remain at the surface, and the influence of pollination upon the time of their retreat.

Despite the dioecism and complete separation of the flowers in *Vallisneria*, pollination seems to take place with remarkable

certainty, provided both kinds of flowers are near together in the same body of water. No doubt the pollen-bearing flowers often ride the surface of the water for considerable distances. They will float for days, and the pollen seems to withstand desiccation for a long time. In our laboratory aquaria the decline of microspores seems to be due to the attacks of fungi, and collections from the field often show hyphae among the spores.

From 200 to 450 ovules line the walls of the ovary, so that the entire pollen output of several staminate flowers would be necessary for fertilization, even if all the spores germinated. Fertilization seems to take place with certainty, for few ovules fail to develop into seeds. Scores of supernumerary pollen tubes are frequently seen lining the walls or wandering through the ovarian chamber among the ovules. Many of these meandering pollen tubes form enlargements at their distal ends similar to those previously reported for *Elodea canadensis* (2).

FIG. 5.—With further depression the water has closed over the flowers, now shut together in a common bubble; out-turned bases of staminate flowers may be seen on all sides, while pollen masses are being pressed directly into stigmas; near by are some pollen-bearing flowers that escaped when others were caught in the bubble (these moved during time of exposure and so are blurred in picture).

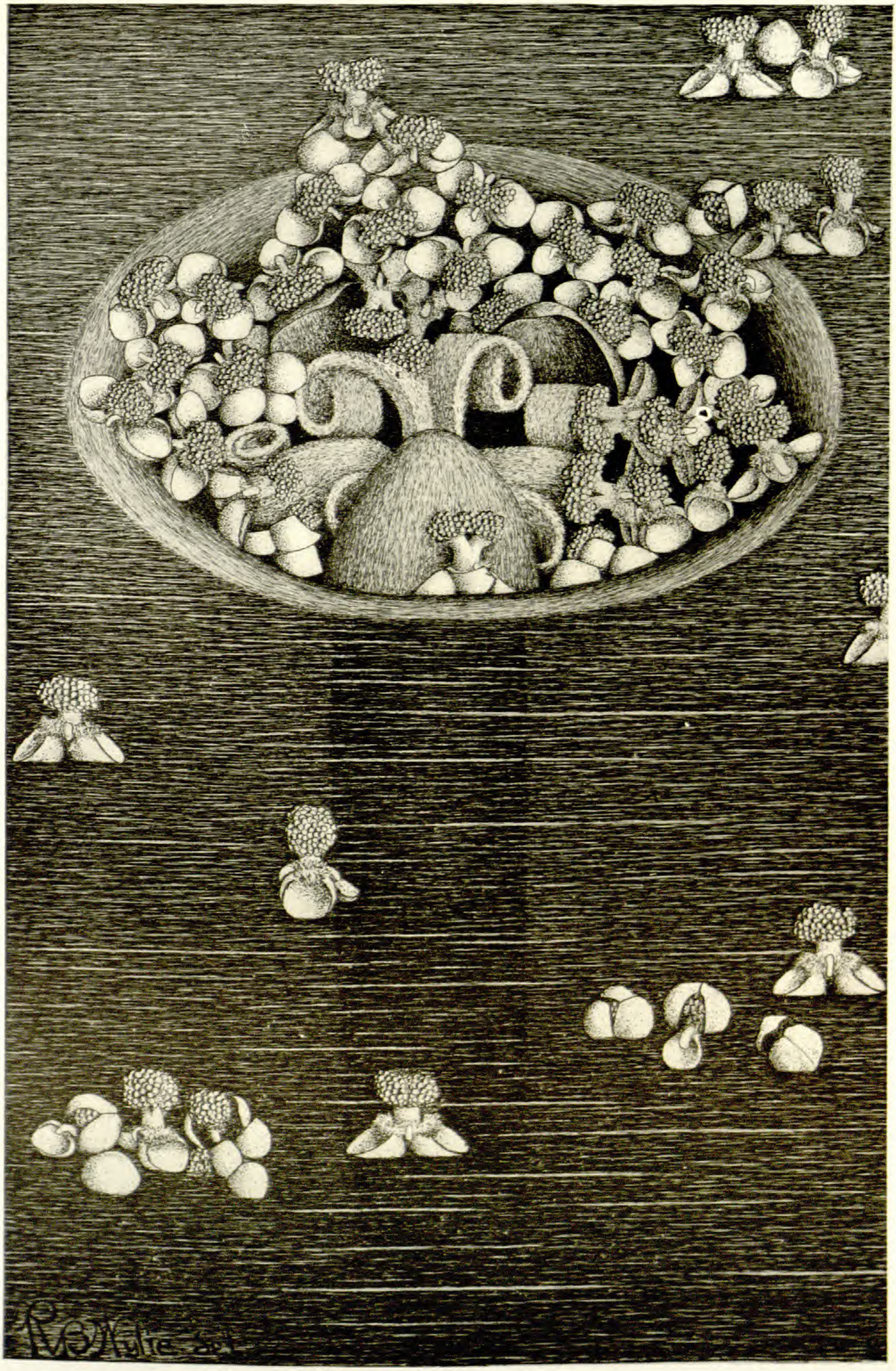
Turning now to KERNER'S widely copied figure illustrating his description of the pollination of *Vallisneria spiralis*, one is struck by the many points of contrast with the foregoing account. His illustration shows a pistillate flower with long slender ovary which, relative to the spread of the floral parts, is only about one-third of the diameter of that in our form. The spathe invests only the base of the ovary, whereas in ours it extends up almost to the sepals. The wide-spreading sepals are shown as straight, while the broad stigmas are flattened, outstanding, and raised entirely above the surface of the water. The stigmas, as shown in the

figure, have margins fringed with long hairs and spread much more widely than in our plant. Finally, the whole pistillate flower in KERNER's figure is placed in such relation to the surface of the water that it could be sustained there only on the supposition that it is supported by a stiff stem.

Similarly, KERNER has figured the staminate flower as markedly different from ours. It is represented as having 3 equal and symmetrically arranged sepals; the stamens are figured with long filaments at right angles and protruding beyond the margins of the sepals. Tiny clusters of pollen grains crown these wide-spreading filaments, carried after the fashion of the spar torpedo well over the margins of the flower. Compared with its companion blossom, the staminate flower is figured as many times larger than in our plant.

FIG. 6.—Release of tension on the scape has permitted submerged flowers to come again to surface; pollen and some entire staminate flowers may be seen on stigmas.

These and many other minor points of difference suggest that KERNER's account is highly generalized, and perhaps intended to convey only a general account of the pollination in this plant. It may only be accidental that most of the departures from the conditions found in our plant are necessary to make possible his proposed plan of pollen transfer. On the other hand, it may be that the European plant is essentially different from ours of the same name; if so, ours should be described as another species. That there may be considerable difference between the forms on the two continents is further supported by TURPIN's (**6**) figures, which show slender stamens somewhat divergent, and stigmas very different from those found in our form. In so far as one can depend upon published figures, it would seem that rather widely divergent plants are included in the species *Vallisneria spiralis*.



WYLIE on VALLISNERIA

The writer would welcome photographs, drawings, or specimens from distant regions for purposes of comparison.

In conclusion, it seems clear that *Vallisneria* offers a remarkable series of specializations mainly related to pollination at the surface of the water. A few aquatic plants have solved the problems of pollen transfer under water, and so may carry out their entire life history as submersed plants. A good example is seen in *Ceratophyllum demersum* L., which is pollinated below the surface and so may flourish at considerable depths in clear water. Neither *Vallisneria* nor *Elodea* shows any evidence of transition to subsurface modes of pollination, although this would seem to be a desirable goal for all aquatic flowering plants. On the contrary, they are perhaps carried further and further from this possible habit by their devices favoring pollination in air. Their specializations not only bespeak long association with water, but also constitute a remarkable series of adaptations to pollination at its upper limit through the agency of the surface film.

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LITERATURE CITED

1. KERNER, ANTON, Pflanzenleben. Leipzig. **2**:129–131. 1891.
2. WYLIE, ROBERT B., The morphology of *Elodea canadensis*. BOT. GAZ. **37**:1–20. 1904.
3. SVEDELIUS, NILS, On the life history of *Enalus acoroides*. Ann. Roy. Bot. Gard. **2**:267–297. 1904.
4. WYLIE, ROBERT B., A long-stalked *Elodea* flower. Bull. Lab. Nat. Hist. State Univ. Iowa **6**:43–52. 1913.
5. COWLES, HENRY C., Textbook of botany. New York. **2**:838. 1911.
6. LOTSY, J. P., Vorträge über botanische Stammesgeschichte. Jena. **3**:642. *fig.* 427. 1911.

EXPLANATION OF PLATE IX

A general drawing representing a group of associated flowers as they appear when slightly depressed; free floating staminate flowers outside the depression.

TOLERANCE OF FRESH WATER BY MARINE PLANTS AND ITS RELATION TO ADAPTATION

W. J. V. OSTERHOUT

Some effects of distilled water on protoplasm have been described by the writer.[1] Further investigations on this subject have shown remarkable differences between marine plants, and even between different cells of the same plant, with respect to their tolerance of fresh water. These differences are interesting from a physico-chemical standpoint, and significant because of their bearing on the theory of adaptation.

It is commonly supposed that most marine plants are killed by exposure to fresh water.[2] Some instances have recently come under the writer's observation in which death occurs with great rapidity. A good example of this is furnished by *Polysiphonia violacea*. Upon placing it in pure distilled water, many of the cells are killed within a minute. This is clearly shown by the fact that if they are replaced in sea water at the end of a minute they become disorganized and never recover. This effect of distilled water is not due to the presence of toxic substances acquired during distillation, for the water was prepared with especial precautions and was not toxic to sensitive species of *Spirogyra* and to sensitive root hairs. Moreover, the same effect is produced by water taken directly from ponds, rivers, and springs.

On the other hand, there are species which are quite tolerant of fresh water. Some years ago the writer[3] found marine algae growing along the sides of a steamboat. These were exposed alternately for some hours each day to salt water and to fresh water. They were also exposed daily to concentrated sea water and to strong sunlight, under which they reached a relatively high temperature. They included representatives of the red, brown, green, and blue-green algae, and were associated with a somewhat varied

[1] BOT. GAZ. **55**:446. 1913.

[2] Cf. PFEFFER, Pflanzenphysiologie **1**:415.

[3] Univ. Calif. Publ. Botany **2**:227. 1906.

fauna. One who is inclined to attribute this remarkable tolerance of fluctuations in salinity to a process of gradual adaptation will meet with many difficulties.

The writer recently had an opportunity, on the island of Mount Desert, Maine, to observe plants which are subjected to both fresh and salt water. At the mouths of brooks, in situations between tide marks which are exposed alternately to 6 hours of fresh water and to 6 hours of salt water,[4] there flourishes a surprisingly large flora, including representatives of the red, brown, green, and blue-green algae, and a flowering plant, the eel grass (*Zostera marina*).[5]

In some places tide pools are found in the beds of brooks. When the tide is out these pools are filled with salt water except for a layer of fresh water which rests on the top of the salt water and flows in a gentle current over it. The depth of the fresh water may be as much as 7 inches, and that of the salt water 2 or 3 feet. The line between the two layers is sharply marked.[6] In such places one portion of a plant may be exposed for several hours a day to fresh water, while the remaining portion is always in salt water. There seemed to be no differences between these portions of the plant.

What enables these plants to survive under such unusual circumstances? The current explanation is that they have gradually adapted themselves to these conditions. The eel grass might be

[4] As soon as the plants are covered with salt water by the rising tide, the fresh water no longer affects them, since it flows over the surface of the salt water without mingling much with it.

[5] Among the species which endure 6 hours of fresh water alternating with 6 hours of salt water may be mentioned the following, which were kindly identified by Dr. W. G. FARLOW: *Gomontia* sp., *Enteromorpha intestinalis*, *Monostroma Blyti*, *Fucus vesiculosus*. Some of these species, for example *E. intestinalis* and *M. Blyti*, endure much greater exposure to fresh water. Mr. F. S. COLLINS has noted that *Ilea fulvescens* (Rhodora **5**:175 and **6**:20; also Green algae of N.A., p. 206), *Enteromorpha micrococca* (Torr. Bull. **18**:336; also Green algae of N.A., p. 204), and *Pilinia minor* (Green algae of N.A., p. 292) stand exposure to fresh water. See also PFEFFER, Pflanzen-physiologie **1**:415 and OLTMANNS, Morph. u. Biol. der Algen **2**:173–183. 1905.

[6] Small animals in these pools (*Gammarus*, young eels, etc.) swim back and forth from one layer to the other without any sign of inconvenience. The boundary between the two layers is easily made visible by stirring; water-logged vegetable matter taken from the bottom of a fresh-water pool sinks to the boundary and remains there.

cited as an especially good example; its leaves are exposed alternately to fresh and salt water, but its roots, being covered by mud, are exposed to comparatively little change in salinity. The theory of adaptation might lead us to expect that the leaves of such plants would be much more tolerant of fresh water than the roots. This expectation is most strikingly confirmed by experiments, which show that the root cells of these plants are killed by fresh water in a few minutes, while the leaf cells can stand exposure to fresh water for several hours. But the argument must be reversed as soon as we make experiments with specimens of eel grass taken from salt water in places remote from the mouths of streams, where no opportunity for adaptation to fresh water occurs. In these plants we find the same differences between root and leaf with respect to their ability to withstand fresh water that we find in plants growing at the mouths of streams.

We must suppose, therefore, that characters which seem to be the result of adaptation were in this case present from the beginning and must be ascribed to entirely different causes. Doubtless this is also true of many cases which at present serve as typical instances of adaptation.[7]

There is much significance in the fact that leaf cells may withstand a much longer exposure to fresh water than the root cells of the same plant. One might be inclined to explain this by differences in the cell wall rather than by differences in the protoplasm, particularly as the cell wall in the root is usually more permeable to water than the cell wall in the leaf. It is clear that this is not the case here, however, for when leaf cells and root cells are placed side by side in hypertonic sea water, they are plasmolyzed with equal rapidity, and when replaced in ordinary sea water they recover at the same rate; this shows that their permeability to water and to the salts in the sea water is about the same in both cases.

Another consideration shows that the difference in the behavior of the cells cannot be due to differences in their permeability to water. This is the fact that death is not primarily due to absorp-

[7] Experiments with other species growing at the mouths of brooks showed that individuals which have had no opportunity for adaptation to fresh water show a great tolerance of it.

tion of water. In the process of dying the majority of cells exhibit little or no increase in size, showing that they absorb little or no water. Certain exceptional cells may swell and even burst, but this is not the rule.[8] Moreover, the cells die in isotonic cane-sugar solutions, although not as rapidly as in distilled water.[9]

We must look, therefore, for another explanation of these effects. It has been pointed out by LOEB that when death occurs in distilled water it must be due to diffusion from the protoplasm of substances which are necessary to its normal activity, and that doubtless the most important of these are inorganic salts. The reason why some protoplasm is more tolerant of distilled water may be that it parts less readily with certain salts which are combined (chemically or mechanically) with it.

It may also be true that the less tolerant protoplasm consists more largely of substances (globulins or other colloids) which undergo a change of state as soon as the concentration of salts falls below a certain limit. In order that the cell should be intolerant of distilled water the globulin (or other substance) need not constitute a large part of the protoplasm, for it might, even in small quantity, play an extremely important rôle, such as that of a protective colloid or of a constituent of the plasma members. These effects would be very simply explained by such an assumption.

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[8] In some cases failure to swell may be due to the rigidity of the cell wall, but certain cells which have no rigid cell wall fail to swell under these conditions.

[9] This has been shown for certain animal cells by LOEB, Pfluger's Archiv **97**:406. 1903.

BRIEFER ARTICLES

HENRY HAROLD WELCH PEARSON

(WITH PORTRAIT)

Professor H. H. W. PEARSON, professor of botany in the South African College at Cape Town, died November 3, 1916. He was born at Long Sutton, England, in 1870, and was educated at Cambridge, receiving the M.A. degree in 1900 and the Sc.D. in 1907. He was assistant curator of the Cambridge herbarium from 1898 to 1899, and then went to the Kew Gardens where he remained until 1903. From Kew he went to the South African College, where his principal work was done. His thorough training in taxonomy enabled him to utilize at once the opportunity of a rich and little studied flora, and he made numerous contributions to the *Flora of South Africa*, *Flora Capensis*, *Philosophical Transactions*, *Geographic Journal*, *Annals of Botany*, and other periodicals. His researches were not confined to taxonomy. There were ecological papers upon South African cycads and morphological studies of *Welwitschia* and *Gnetum*, two genera in which he was so deeply interested that he not only spent a great deal of time and money, but eagerly endured the hardship of collecting material in distant and almost inaccessible places.

In a letter dated September 8, 1916, he states that he has in press two papers on *Gnetum*, and that he was to make another trip to Damara-

land in January to study *Welwitschia* and get material for further investigation. In the same letter he writes: "I don't think there is the least chance for peace before September, 1917. I wish I could get away from present occupations and take a part, but I am not encouraged by the authorities."

Five years ago, while studying cycads in South Africa, it was my good fortune to become acquainted with Professor PEARSON, and to be entertained in his home. He was a vigorous, kindly man, intensely interested in his work and enthusiastic over the botanical possibilities of South Africa. During a stroll on Table Mountain he pointed out to me a magnificent site for a botanical garden, at that time only a dream or a vision; but with him a vision was followed by meditation, and then by determined effort to make his dream come true. The visions which he saw during such strolls have materialized in the National Botanic Gardens at Cape Town, with an unsurpassed location, and with such a rapidly growing collection of plants that the place is already one of the great gardens of the world.

By his untimely death—he was only 46 years old—science has lost a great botanist, but it is to be hoped that his high ideals of scientific work will still remain to guide the botanical policies of South Africa.—CHARLES J. CHAMBERLAIN, *University of Chicago.*

SOIL MOISTURE INDEX

Outdoor botanists in thinking of any plant are likely to associate with it some measure of its soil moisture relation. The terms xerophyte, mesophyte, and hydrophyte naturally come to mind, or at least the conceptions that these names connote. With college students, however, no such association of ideas is likely to occur unless special attention is directed to the subject. This may be done by requiring the student to examine the habitat, note the associated species, and estimate the water requirements. Such a plan was tried the past summer at the University of Colorado Mountain Laboratory at Tolland, Colorado. For this purpose a scale, which may be called a "soil moisture index," proved useful. A rule of the laboratory required that every plant studied should be recorded with an index number, this to be criticized and perhaps altered by the instructor.

The index numbers form a scale from 1 to 10 with the following significance: (1) lithophytes; (2) plants of driest, sterile soil; (3) hyper-xerophytes; (4) xerophytes; (5) xero-mesophytes; (6) mesophytes;

(7) plants of wet meadows or moist forest; (8) marsh plants; (9) plants partly submersed; (10) plants growing in water.

In using the index most plants will be grouped around 4, 6, 8, and 10. This will be the case especially with students who have had little experience in field work. Later, when a finer discrimination is developed, the intermediate numbers will be employed more often. If extreme nicety is desired, decimals may be used. They will be satisfactory in the study of some particular community, as a meadow or a prairie, in which the student becomes acquainted with relative moisture requirements of a number of species. If less accuracy is required, plus and minus signs furnish a useful means of distinction. Thus, the elms may be called no. 6, the red maple 6+, and the red oak 6−. Students in desert and arid regions will need to work out the meanings of the index figures 2 and 3. Others will probably be content with calling all xerophytes no. 4. A plant that occurs in a number of different habitats may be indicated by two or more numbers, as, for example, *Achillaea lanulosa* 6, 7, 8.

There is no deep philosophical conception underlying the use of the scale and no instrumentation is intended in connection with it. It merely emphasizes the importance of water in the substratum and furnishes a suitable terminology for description and a vocabulary for thinking about soil moisture relations. It is possible that in research work on floristics the use of this or some other suitable scale may be found desirable. Perhaps workers in different localities might agree on typical plants to represent the different index numbers. The scale may well be used for a better understanding of transition areas, such as occur between meadow and marsh or between meadow and dry grassland. Any piece of vegetation may be given an index number if the indexes of its component species (previously determined in other situations) be averaged. Thus one might recognize a no. 6 meadow, a no. 6.5 meadow, or a no. 7 meadow.—FRANCIS RAMALEY, *University of Colorado, Boulder, Colorado.*

CURRENT LITERATURE

BOOK REVIEWS

Evolution

A book by LOTSY[1] is the most recent contribution to the literature of evolution. The author is evidently handicapped by the intricacies of English spelling and punctuation, for there are scores of instances of misspelled words, incorrect punctuation, and faulty idiom. It is to be regretted that these types of elementary error were not eliminated by the publisher, for the book-making is otherwise excellent. Perhaps the commonest error is that of ignoring the accepted canons of word division at the end of a line. With great frequency monosyllabic words are divided and words are broken in the middle of a syllable. Unfamiliarity with English idiom also leads frequently to strikingly awkward expressions. For example, on page 29, the following meaningless sentence occurs: "This species undoubtedly are in an uninheritable way." On page 120 this paragraph occurs just as quoted: "While hare and rabbit don't pair in nature, a male hare doubtless would do so if sufficiently long isolated with female rabbits, in the absence of male rabbits on an island, as ressorts from the experiments of Mr. Houwink, showing that the hare looses its inborn aversion of a tame rabbit, if it is taken soon after birth from its mother, and sucked by a tame rabbit foster-mother." Similar passages are frequent.

Another peculiar type of error is in the use of "e.g." and "i.e."; for the author reverses our usage constantly. He has also invented another abbreviation which serves him well, using "f.i.," apparently for "for instance." Not infrequently Dutch and German words are substituted for English, as "bij" for "by," "unter" for "under," and "alle" for "all." Any English writer, in an evening's work, could have edited the book into acceptable form and it is to be regretted that some such editing was not done.

Apart from these most obvious mechanical imperfections the book is of considerable interest, in that it serves to emphasize the importance of hybridization as a factor in evolution. The author's position, however, is an extreme one, in that he holds hybridization to be the sole cause of variation. The principal ideas that form the backbone of his argument are as follows.

1. Linnean species, or *Linneons* as he calls them, are not species at all, but artificial groups of intercrossing types, that are constantly giving Mendelian ratios. All so-called mutations are merely extracted recessives, which if isolated produce new pure types.

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[1] LOTSY, J. P., Evolution by means of hybridization. 8vo. pp. viii+166. The Hague: Martinus Nyhoff. 1916.

2. A real species is "a group of individuals of identical constitution, unable to form more than one kind of gametes; all monogametic individuals of identical constitution consequently belong to one species."

3. A "Linneon," says Lotsy, "is a vestigial group of a once much larger group of differently constituted types, born of a cross, which is apt to simulate a species by the overwhelming majority of the dominant types it contains, as a result of free-intercrossing, combined with a favoring of the dominants by a process of selection, weeding out the weaker or more conspicuous recessives; this uniformity being more apparent then real, because pure dominants are indistinctible, in most cases, from dominant-hybrids."

4. In another place the author states briefly his idea of the causal factors of evolution. "The vera causa of the production of new types consequently is: crossing; the vera causa of their extinction: the struggle for life; the selection resulting from the latter is by no means a revival, but is the sign of the struggle of the doomed." Just what is meant one can only conjecture by the context.

In taking the position that no variations or mutations arise except as the result of crossing and subsequent segregation, the author throws out of court the mutants that have arisen in such carefully controlled experiments as those of MORGAN and his pupils on *Drosophila*. He challenges the reader to produce a single case of mutation in a true species, which, according to him, is a type that produces only one kind of gamete and shows no variability in F_1 and F_2 generations. In other words, if a mutation does occur it may be taken as prima facie evidence of impurity in the stock. Such an argument leads nowhere.

The author follows his theory to its logical conclusion and attempts to show that even classes and orders must have been the result of crossing. We fail to see the necessity of forcing a theory, that seems fairly reasonable when applied within limits, to such an absurd length. If we object, we are told that "a formation of new classes is not in action at the present moment, so that it is illegitimate to claim that one who wants to explain evolution must demonstrate how such a formation of new classes goes on."

In conclusion, the reviewer would like to recommend the reader to the second edition of this book, which, if it ever appears, will doubtless be a considerable improvement on the first.—H. H. NEWMAN.

MINOR NOTICES

Forestry for boys.—In a volume dedicated to the youth of America and to the leader of the Boy Scouts, MOON[2] has described in a readable manner the problems and processes of tree growth, forest development, and forest utilization. The extent and economic value of our forests are well emphasized

[2] MOON, F. F., The book of forestry. 8vo. pp. xvii+315. *figs. 64.* New York: Appleton. 1916. $1.75.

and the importance of their conservation made clear. The harvesting and utilization of the timber crop is described in an interesting manner, as well as the training and duties of the forester. Some attention is given to such forest industries as maple sugar making, nut growing, resin production, and wood distillation. A word is said about the value and care of shade trees, and a glance is taken at the future possibilities of forestry, everything being treated in a non-technical way likely to interest the "Boy Scout" and many of his elders. The latter part of the book is devoted to very brief descriptions of some 50 trees, each being illustrated by a small drawing of leaves and flowers or fruit.

While neither a textbook nor a scientific treatise, it is interesting and seems well suited to the purpose of interesting the public and more particularly the boys, in the forest and the forester as they concern the happiness and prosperity of our land.—GEO. D. FULLER.

Soil bacteriology.—A laboratory manual of soil bacteriology by FRED[3] is intended as a guide to teachers and students in courses given in soil bacteriology. The subject is logically developed and directions are given in clear, concise form. There is perhaps no branch of bacteriology so intimately associated with chemistry as soil bacteriology, and therefore considerable attention is given to this phase of the subject. There are a number of excellent illustrations in the book, and one of the most valuable features is the fairly complete assortment of recipes for preparing culture media suitable for the study of soil bacteria. Special sections deal with methods of quantitative and qualitative chemical methods of analysis. Provision is made at the conclusion of exercises for the student to record results in tabular form, a feature which adds materially to the value of the book.

It is being realized in agricultural schools that the study of soil bacteriology is of eminent importance, and this manual will undoubtedly be appreciated by those interested in such courses.—P. G. HEINEMANN.

North American flora.—The first part of Vol. 21 begins the Chenopodiales by presenting the Chenopodiaceae monographed by STANDLEY.[4] There are 195 species recognized, distributed among 27 genera. A new genus (*Meiomeria*) is based upon *Chenopodium stellatum* S. Wats. The large genera are *Atriplex* (96 species, 20 of which are new), *Chenopodium* (52 species, 13 of which are new), and *Dondia* (20 species, 7 of which are new). New species are also described in *Salicornia* (2) and *Endolepis*. One of the remarkable features of the family is the number of small genera, 13 being represented by a single species, and 4 by 2 species. In fact, 177 of the 195 species are included in 4 of the 27 genera.

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[3] FRED, EDWIN B., A laboratory manual of soil bacteriology. 12mo. pp. 170. Philadelphia: Saunders Co. 1916. $1.25.

[4] North American Flora **21**: part 1. pp. 1–93. Chenopodiales: Chenopodiaceae, by P. C. STANDLEY. New York Botanical Garden. 1916.

The sixth part of Vol. 9 concludes the presentation of the *Agaricaceae* by MURRILL,[5] 5 genera being presented, which include 165 species, 16 of which are described as new. The largest genus is *Clitocybe*, with 88 species and including 13 of the new species. The part closes with a list of corrections and a bibliography for the volume.—J. M. C.

Jackson's glossary.—A third edition of this well known volume has appeared.[6] The development of subjects in botany and the consequent growth in terminology has made a new edition imperative. Especially is this true in reference to the numerous "recently coined terms" in ecology. No glossary can be perfect, and it would be easy to pick flaws in this one, but it must be regarded as complete and trustworthy as such a book can be. It contains approximately 21,000 terms, so that it must be fairly representative of botanical terminology. With the increasing number of special fields of botany, even a trained botanist needs a convenient glossary on his shelves.—J. M. C.

Correspondence of Linnaeus.—Under the editorship of HULTH, the correspondence of LINNAEUS is to be published in a series of volumes, the first one of which has just appeared.[7] The collection includes letters "from and to" LINNAEUS. The extent of the correspondence is indicated by the fact that this first volume, of over 400 pages, includes 49 correspondents listed under the first two letters of the alphabet. Most of the letters are in Latin, and give an intimate and interesting picture of the biology and biologists of the Linnaean period.—J. M. C.

NOTES FOR STUDENTS

Cecidiology.—Three interesting American papers on the histology of galls have been published recently and demonstrate the increasing interest in the study of pathogenic structures.

STEWART[8] presents a very interesting paper on the anatomy of *Peridermium* galls. The studies were made from *Peridermium cerebrum* Pk. on *Pinus Banksiana* Lamb. Galls of various ages were used, but all of them from young branches. It appears that the infection usually takes place during the first year's growth of the shoot. The woody portion of the gall was very distinct from the normal tissue. The author summarizes his results as follows:

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[5] *Op. cit.* Agaricaceae, by W. A. MURRILL. **9**:375–426. 1916.

[6] JACKSON, BENJAMIN DAYDON, A glossary of botanic terms, with their derivation and accent. 8vo. pp. xii+428. Philadelphia: Lippincott. 1916. $3.00.

[7] HULTH, J. M., Bref och Skrifvelser af och till CARL VON LINNÉ. Vol. I. ADANSON-BRUNNICH. 8vo. pp. viii+429. Upsala: Akademiska Boktryckeriet. 1916.

[8] STEWART, ALBAN, Notes on the anatomy of *Peridermium* galls. Amer. Jour. Bot. **3**:12–22. 1916.

"(1) both an alternate and an opposite arrangement of bordered pits in the radial walls of the tracheids; (2) an unequal thickening of the walls and lumina of the tracheids; (3) very short tracheids with blunt end walls, which resemble parenchyma cells except in the pitting; (4) cells which are transitional between tracheids and parenchyma cells in the pitting; (5) the presence of true wood parenchyma cells; (6) a small production of thin-walled summer tracheids; (7) a probable absence of bars of Sanio from many of the tracheids; (8) an increase in the number of rays in the gall wood; (9) a tendency toward the production of multiseriate rays; (10) ray tracheids which are transitional between those of hard and soft pines; (11) the presence of a balled or whorled arrangement of tracheids in the tangential sections; (12) a great increase in the number of resin canals in the gall wood, but no such increase in the uninfected wood close by. The examination of this gall has revealed so many points of anatomical interest that a further study of this subject seems to be worth while. On this account the author expects from time to time to issue other papers on the anatomy of *Peridermium* galls on pines and other conifers." It remains for someone to make a study of the very early stages of this and many other abnormal plant growths.

ROSEN[9] has made a study of the histology of the grape leaf gall. The author has made a study of this gall from its very earliest stages to its maturity. He summarizes his results as follows: "(1) the *Phylloxera vastatrix* leaf gall starts to develop on embryonic bud leaves; in 24 hours the insect produces a depression at the periphery of which hairs are formed on the upper surface of the leaf; the depression is due to a lessened growth of the attacked mesophyll; (2) after 3–4 days of insect attack the lower half of the leaf tissue which surrounds the portion in which the proboscis is inserted has proliferated enormously; the whole thickness of the leaf in the region immediately around the proboscis shows no proliferation; that portion of the leaf which is beneath the insect does not proliferate, but the upper half at the sides of the insect grows and forms the walls of a large insect cavity; upper epidermal cells and several layers of mesophyll cells in the portion of the gall below the insect show peculiar thickening and dissolution of their cell walls; (3) gall development depends upon leaf development; when the leaf reaches its maximum size, after 12–15 days of development, the gall becomes mature; (4) a mature gall shows but slight cuticular development and very few stomata; the mesophyll is a huge mass of compact, thin-walled, partly empty cells, some of which are undersized, and others enormously elongated; the vascular elements are scattered by wedges of parenchyma cells; many unicellular and multicellular hairs grow out from the gall; (5) chemical work on this gall shows it to be a structure in which anabolic processes are lacking, and in which large amounts of simple sugars and simple proteins are present; (6) the development of this gall does

[9] ROSEN, HARRY R., The development of the *Phylloxera vastatrix* leaf gall. Amer. Jour. Bot. 7:337–360. 1916.

not seem to support the theory that the insect injects some chemical into the leaf which causes gall formation; (7) intumescences produced by chemical sprays result from entirely different kinds of hyperplastic responses than hyperplastic gall growth; (8) the investigation establishes the fact that the proboscis may pass through the entire thickness of the leaf; (9) the insect remains fixed, and that portion of the leaf in which the proboscis is fixed is marked by lack of growth as compared with the huge outgrowths which surround it; (10) the continuous sucking action by the insect at one fixed point for fifteen days is believed to be the initial stimulus for gall development."

Another very interesting paper is by WELLS,[10] who has made a study of the galls of our common American hackberry. The purpose of the paper as stated by the author is as follows: "(1) to present a survey of the known insect and mite galls of *Celtis occidentalis* L.; (2) to elucidate the history of the normal gall-bearing parts of the hackberry and that of the galls; (3) to study comparatively the structures treated, pointing out any significant conclusions and generalizations that may be attained in such a study." The author gives excellent descriptions of the external and histological character of the galls and concludes the paper with the following summary: "(1) there are 17 known species of zoocecidia occurring on *Celtis occidentalis*, belonging to 4 orders of arthropods (Acarinae 1, Lepidoptera 1, Hemiptera 5, Diptera 10); all are heteroplasias, that is, those forms of hyperplasias (abnormal increase in size through cell proliferation) whose cells and tissues differ from the normal; all, be it noted, are built up on the basis of the same germ plasm, namely, that of the single species of the plant mentioned; (2) the acarinous and lepidopterous galls are kataplasmas of those forms of heteroplasias whose cells and tissues do not vary widely from the normal; each shows specific and characteristic inhibition of differentiation; (3) the hemipterous and dipterous galls are prosoplasmas of those forms of heteroplasias whose cells and particularly whose tissue forms differ fundamentally from those of the normal parts; (4) in the prosoplasmas the types of cells found are closely comparable to those of the normal plant parts, but the tissue forms discovered are fundamentally new; no analogous structure forms are to be found in the tissues of the normal plant or its allies; (5) in the dipterous prosoplasmas, since the gall's specific tissue form characters are related to the species of insect, we have the unique case of the 'overlapping' of the hereditary constitution of an animal on that of the plant in the sense that factors associated with the insect determine the form character locally, rather than those normally associated with the plant's germ plasm; these latter plant factors suffer suppression; (6) it is suggested that in the field of zoocecidiology we probably have a unique place, heretofore unrecognized, to attack the problem pertaining to the mechanism used in the expression of hereditary characters." The paper is well illustrated.—MEL T. COOK.

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[10] WELLS, BERTRAM W., The comparative morphology of the zoocecidia of *Celtis occidentalis*. Ohio Jour. Sci. **16**:249–290. 1916.

Transpiration studies.—Among the more recent devices for the investigation of the conditions affecting transpiration is the porometer devised by DARWIN,[11] which has attracted the attention of several workers, leading to improvements by BALLS[12] and by JONES[13] resulting in self-recording instruments. KNIGHT[14] then somewhat simplified the device, and later, assisted by LAIDLAW,[15] produced an automatic instrument that is probably better than its predecessors for most forms of stomatal investigations. All agree in measuring the stomatal opening by the rate at which air passes through the stomata with a given pressure. A rather careful study by KNIGHT[16] of the methods to be employed in avoiding errors in the use of the porometer is suggestive to future investigators in this field. Among the interesting results obtained by this method there may be mentioned those of DARWIN, and of LAIDLAW and KNIGHT, who found indications that upon severing a leaf from a stem and allowing it to wilt, a temporary opening of the stomata immediately preceded the closure accompanying wilting.

In a recent investigation TRELEASE and LIVINGSTON[17] have made a comparison between the porometer and the standardized cobalt chloride paper methods, and have obtained results showing a general agreement of data from the two. In the daily march of transpiring power the two are in close accord during the morning hours up to about 8:00 A.M., but from that hour until 11:00 A.M. the porometer index continues to increase, while the cobalt paper index tends to decrease. After 11:00 A.M. the influence tending to decrease becomes evident in the porometer index also. This is taken to indicate that the porometer measures the diffusive capacity of the stomata, but fails to take into account other influences affecting foliar transpiring power. The divergence in the two records, therefore, may be an index of non-stomatal influences upon transpiration. On account of the limited data, these workers are not inclined to press this conclusion, but it appears to be an extremely probable suggestion.

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[11] DARWIN, F., and PERTZ, D. F. M., A new method of establishing the aperture of stomata. Proc. Roy. Soc. London B **84**:136–154. 1911.

[12] BALLS, W. L., The stomatograph. Proc. Roy. Soc. London B **85**:33–44. 1912.

[13] JONES, W. N., A self-recording porometer and potometer. New Phytol. **13**: 353–364. 1914.

[14] KNIGHT, R. C., A convenient modification of the porometer. New Phytol. **14**:212–216. 1915.

[15] LAIDLAW, C. G. P., and KNIGHT, R. C., A description of a recording porometer and a note on stomatal behavior during wilting. Ann. Botany **30**:47–56. *figs.* 3. 1916.

[16] KNIGHT, R. C., On the use of the porometer in stomatal investigation. Ann. Botany **30**:57–76. 1916.

[17] TRELEASE, S. F., and LIVINGSTON, B. E., The daily march of transpiring power as indicated by the porometer and by standardized hygrometric paper. Jour. Ecology **4**:1–14. *figs.* 2. 1916.

An investigation upon a much larger scale, resulting in an abundance of data, is reported by BRIGGS and SHANTZ.[18] It was carried on at Akron, Colorado, and the transpiration was determined by weighing plants potted in sealed cans upon the automatic scales recently described by the same authors.[19] Solar radiation, wet-bulb depression, evaporation, air temperature, and wind velocity were also measured, and the relationship between these physical factors and transpiration was shown. The plants employed were wheat, oats, sorghum, rye, alfalfa, and *Amaranthus retroflexus*, the hourly rate throughout the entire day being determined, the number of determinations ranging from 6 for *Amaranthus* to over 40 for alfalfa. The resulting data are expressed in tables and graphs which also serve to express their relationship with the physical factors. Correlation coefficients and method of least squares are also used to analyze these relationships and give some interesting results. Space permits the citing of their final conclusion only, to the effect that their results agree with those of other investigators that plants under conditions of high transpiration do not respond wholly as free evaporating systems, even if bountifully supplied with water. It is interesting to note that none of the plants here studied show the mid-day drop reported by TRELEASE and LIVINGSTON, by SHREVE, and by other observers at the Desert Laboratory.

MUENSCHER[20] has used the method of determining water loss by weighing and then making counts and measurements of the number and size of the stomata of *Phaseolus*, *Ricinus*, *Zea*, *Primula*, *Impatiens*, *Pelargonium*, *Triticum*, and *Helianthus*. He found no constant relation between the number and size of stomata in relation to unit area of leaf surface and the amount of transpiration. He also concludes that the amount of transpiration is not governed entirely by stomatal regulation. His work, however, does not show any explanation for any other control.

In one of the most recent publications upon this subject, by BAKKE and LIVINGSTON,[21] data are given upon the daily march of foliar transpiring power of different leaves of plants of *Xanthium* and *Helianthus*. These serve to emphasize the fact that the control of foliar transpiration by the plant is a complex one, especially as there is a great range in transpiring power among the different leaves of the same plant with considerable variation in time of the diurnal maxima. No very definite relation is established between age of leaves and their behavior, except that the oldest ones always show a low daily range of

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[18] BRIGGS, L. J., and SHANTZ, H. L., Hourly transpiration rate on clear days as determined by cyclic environmental factors. Jour. Agric. Research **5**:583–649. 1916.

[19] ———, An automatic transpiration scale of large capacity for use with freely exposed plants. Jour. Agric. Research **5**:117–132. 1915.

[20] MUENSCHER, W. L. C., A study of the relation of transpiration to the size and number of stomata. Amer. Jour. Bot. **2**:449–467. 1915.

[21] BAKKE, A. L., and LIVINGSTON, B. E., Further studies on foliar transpiring power in plants. Physiol. Researches **2**:51–71. 1916.

transpiring power and usually low maximum index values. In addition to the transpiration data, this paper contains a description of an improved apparatus for providing a standard evaporating surface.—GEO. D. FULLER.

Fossil cycads.—The second volume of WIELAND'S[22] memoir on American fossil cycads represents a large amount of additional work on material with structure preserved, and in particular of a new monocarpic trunk from the Black Hills discovered by Dr. N. H. DARTON. It is replete with admirable line drawings and half-tones representing both external form and internal structure. The material is in addition illustrated by 58 superb plates in heliogravure. The whole constitutes an achievement of which American paleobotany may well be proud.

Although the volume is described as systematic in its contents, it contains much that is of interest to the anatomist and the evolutionist. Considerable space is devoted to the anatomy of trunks of cycadeoidean forms, and the fact that the fibrovascular tissues are much more woody than in the living representatives of the cycads is emphasized. This is the consequence of the narrow rays and the sparse parenchyma, both features of contrast to the living cycadean cylinders. The author apparently has not found in American Mesozoic material the interesting reduplication of the central cylinder recently described by STOPES in a publication of the British Museum. This situation is interesting as it tends to discredit the hypothesis of WORSDELL that the reduplication of the cylinder in cycads is a vestige of the complex system of fibrovascular bundles found in certain species of *Medullosa*, etc. The situation in fact is comparable rather with that found in vines, and it is interesting to note in this connection that it is not improbable that the cycadeoidean genus *Anomozamites* was a climbing plant. The author emphasizes the statement that the mucilage cavities of the cycadeoidean forms were isolated cysts and did not constitute a system of canals as in living cycads.

Certain interesting statements are recorded in regard to the leaves, although most of these represent only elaborations of facts already known. The leaf trace departs from the cylinder as a large horseshoe-shaped strand which passes directly toward the leaf base, breaking up into numerous bundles in transit. This situation is in marked contrast to conditions in the living genera where numerous strands take their exit from the cylinder for each leaf and pursue a circuitous course through the cortex toward the leaf base. It is obvious that the cycadeoidean forms, so far as their anatomy is known, were unilacunar, that is, there was a single gap in the cylinder of the stem for each foliar supply; while in the living Cycadales the vascular system of the leaf is multilacunar. The cycadeoidean condition is obviously more primitive, as it is found in the reproductive axis and occasionally in the seedling of living forms. The ana-

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[22] WIELAND, G. R., American fossil cycads, Vol. II, Taxonomy. Carnegie Inst. Washington, Publication 34. 1916.

tomical conditions in the Cycadophyta as regards the number of foliar gaps for the vascular supply of the leaves illustrate the danger of using the number of leaf gaps as a phylogenetic criterion, as has recently been attempted in the case of the dicotyledons. The leaf supply of the cycadeoidean forms was distinguished from that in the petiole of existing cycads by the strong development of secondary growth in the bundles on the upper side.

The reproductive structures are considered in some detail in the light of additional facts. The reviewer, however, expresses a regret, which will doubtless be common to those interested in the evolutionary history of the seed plants, that so little further information has been secured in regard to the crucial apical region of the seed. The author still maintains his earlier position in regard to the cycadeoidean origin of the angiosperms. Although he is supported in this view by a number of eminent European paleobotanists, perhaps it is open to question whether a hypothesis which finds little valid support in the anatomical organization of either the reproductive or vegetative structures will in the long run prove acceptable. The Goebelian character of the author's morphology is everywhere apparent, but most strikingly perhaps when he ventures to compare the seed of the Pteridosperm with an angiospermous flower. Surely this is carrying the Goebelian definition of an organ as the tool of a function to its logical *reductio ad absurdum*.

The memoir under discussion contains a wealth of facts to which it is impossible to do justice in a review. It will rank with those of BERRY as a most notable recent contribution to American mesozoic paleobotany.—E. C. JEFFREY.

The cohesion theory.—The cohesion theory of sap ascent has received much attention in recent years, and much supporting evidence has been brought out; but JOST[23] does not agree with RENNER that it is practically substantiated, and that all we need to do now is to find out where the cohering water columns are located. He believes much experimental work is still necessary to determine whether this theory is correct. Proceeding from early experiments of SACHS and others, and using mainly such plants as *Sanchezia*, *Cobaea*, *Biota*, and *Chamaecyparis*, he has made a quantitative study of water delivery by the basal portion of decapitated plants, as compared with the transpiration need of the top before and after cutting. The principal experiments attempt to determine the influence of suction upon the rate of water delivery by the root, and to determine whether continuous negative pressures can be maintained by a transpiring plant. As suction and pressures of only one or two atmospheres were used, the experiments seem to the reviewer little adapted really to test the cohesion theory; and considerable space is occupied in giving single examples of unsuccessful experiments of various sorts, which

[23] JOST, LUDWIG, Versuche über die Wasserleitung in der Pflanze. Zeitschr. Bot. 8:1–55. 1916.

will have value no doubt in preventing useless attempts along the same lines. However, they provide JOST the opportunity to discuss the cohesion theory and to present his own views of it.

The actual results add little that is new. He found that suction on the root causes an increase in water delivery, especially in plants which normally exude sap on being cut, and that greater suction causes a greater root excretion of water than low suction. But there seems to be no proportion between amount of suction and rate of water delivery, as should be the case if the root acts merely as a filter in water intake. The maximum suction possible with an air pump could never cause a delivery sufficient to cover even a moderately estimated transpiration need. Completely surrounding the root with water, or replacing the atmosphere about the roots with hydrogen, or excessive cooling of the roots, leads to noticeable decrease of water delivery, even with strong suction.

Tensions existing in the water columns of the intact plant were demonstrated by the rapid intake of water by freshly cut tops, although the plants previously had been kept in condition of low transpiration. Suction on the cut end of the top, or pressure exerted upon it, produces only a temporary decrease or increase respectively in water intake by the top. It was found that plants could continue transpiration essentially undiminished at real negative pressures of 15–25 cm.

JOST comes to the conclusion that, even in low plants like *Cobaea*, *Chamaecyparis*, etc., just as in tall trees, very considerable negative pressures must exist, if we assume that the osmotic pull of transpiring leaves must pull in sufficient water through a passive filter-like root.

In the concluding section he questions whether it is possible for high negative pressures to exist continuously in the stems of plants, and proceeds to answer the question by subjecting stem tissues to high gas pressures. In *Ficus Carica* he found all the vessels easily penetrated by air, this experience running counter to the recent work of RENNER and HOLLE, which shows that there may be two kinds of vessels present, storage and conducting, the latter being more or less impermeable to air, and providing the cohering water columns. In such a case as *Ficus*, JOST thinks that those who hold to the cohesion theory must suppose either that there are no cohesion phenomena in plants with nothing but tracheae, or that certain vessels remain with cohering water columns by pure accident, or that in closed vessels entirely different conditions obtain from those which have been cut. The question of unbroken columns is vital to the cohesion theory, and those who adhere to it must expect to be called upon to prove the existence of cohering columns of water which can remain unbroken under the reduced or negative pressures involved in normal transpiration. The conservative view of so prominent an authority on plant physiology will help us to maintain a balanced perspective with reference to this important problem.—CHARLES A. SHULL.

Genetical investigations of maize endosperm.—FUJII and KUWADA[24] have noted chemical differences between the red and the purple pigments of the aleurone cells of maize, as indicated by differences in their solubility in alcohol and in 1 per cent aqueous solution of sodium carbonate, and in their color reactions with acids and alkalies. They state that the two pigments occur either separately or together in the same seed. The latter fact, if substantiated, should prove of interest to geneticists working with maize. The authors suggest that variations in intensity of aleurone color may be accounted for in part by the triploid nature of the endosperm, whereby reciprocal crosses may differ in having either 1 or 2 doses of the dominant factor concerned and F_2 seeds differ in having either 1, 2, 3, or no doses. A cumulative effect of dominant factors is assumed. They are apparently in error in attempting to apply the same assumption to red color of wheat, where, so far as known to the reviewer, the color is in the pericarp (diploid) rather than in the endosperm.

The genetical significance of the triploid nature of maize endosperm was earlier pointed out by HAYES and EAST[25] in reporting results of crosses between races with corneous and with floury endosperm. In reciprocal crosses between flinty and floury varieties xenia did not occur. In F_2 a 1:1 ratio was obtained whether the F_1 was self-pollinated or cross-pollinated by either the flinty or the floury parent. A half of each class of seeds resulting from self-pollination bred true and the other half again segregated. When F_1 was cross-pollinated by the flinty parent, all the corneous seeds bred true and all the floury ones proved to be hybrid, while the reverse was true when F_1 was crossed back to the floury parent. Two hypotheses are offered to account for the results: (1) the endosperm develops from the fused polar nuclei without double fertilization, or (2) the two polar nuclei dominate the single second male nucleus. A plant, pure for white endosperm but hybrid for endosperm texture, pollinated by a pure yellow corneous seeded race, gave a 1:1 ratio of corneous and floury seeds, all of which were yellow, thus demonstrating double fertilization and indicating the second hypothesis, that two doses of a factor for floury endosperm dominate one dose of the corneous factor and vice versa. Crosses of popcorn with both floury and dent types gave results more difficult of analysis, owing in part, the authors suggest, to differences in seed size, but two independent factor-pairs are indicated in at least some of the cases. It is also thought that two factor-pairs are concerned in the inheritance of sharp points characteristic of rice pop-corn.—R. A. EMERSON.

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[24] FUJII, KENJIRO, and KUWADA, YOSHINARI, On the composition of factorial formula for zygotes in the study of inheritance of seed characters of *Zea mays* L., with notes on seed pigments. Bot. Mag. (Tokyo) **30**:83–88. 1916.

[25] HAYES, H. K., and EAST, E. M., Further experiments on inheritance in maize. Conn. Agric. Exp. Sta. Bull. no. 188. pp. 31. *pls.* 8. 1915.

Genetics of flax.—Miss TAMMES[26] has made a genetical study of the flower characters of 6 varieties of the common flax, *Linum usitatissimum*. These varieties consisted of 3 dark blue, 1 light blue, and 2 white varieties. Besides the color of the flower, with which she worked chiefly, she studied the color of the anthers, the color of the seeds, the shape of the petals, the color of the veins in the petals, and the number and viability of the seeds produced. These latter characters she finds correlated with the color of the flower and dependent upon the same factors. The several varieties are described and their genetic formulae given, after which the author presents in tabular form the expected ratios and the observed results in the second and third generations. She concludes that the blue color is the result of two complementary factors, *B* and *C*. The presence of these two factors alone produces the light blue flowers, and the dark blue is brought about by the action of an intensifying factor *A* co-operating with *B* and *C*. Unless both *B* and *C* are present the flower will be white. The factor *A* acts as an intensifier only on the light blue of the petals and has no effect on the color of the anthers, on the color of the seed, or on the color of the veins in the petals. The factor *B* is not only one of the necessary factors for the production of the blue flower color, but even without the cooperation of *C* brings about the blue color of the anthers and the brown color of the seeds, prevents the crinkling of the petals which, were it not present, would be caused by the presence of *C*, and overcomes the tendency of *C* to lessen the number and viability of the seeds. The factor *C*, besides producing, with *B*, the blue color of the flower, brings about, when in a homozygous condition, a deeper pigmentation of the veins in the petals; and causes, when *B* is absent, a crinkling of the petals and a lessening in the number and viability of the seeds. In respect to the color of the anthers, which results from the presence of *B*, it is pointed out that although the 6 varieties studied are in agreement with the interpretation given, there is a variety, which has not yet been studied, that has blue flowers and *yellow* anthers. As this is contrary to the conclusions arrived at from the 6 varieties investigated, the author suggests that the factor *B* may be, not a single unit, but a complex, with some essential part or factor lacking in the variety with blue petals and yellow stamens. On the other hand, *B* may be a unit and the blue anthers may be lacking because some other necessary factor besides *B* is lacking in that variety. An investigation of this problem is promised.—BEN C. HELMICK.

Physiological temperature and moisture indices.—In extending his studies of the derivation and use of indices of temperature in relation to plant growth, LIVINGSTON[27] distinguishes 3 classes of such indices. The first is the sum-

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[26] TAMMES, TINE, Die genotypische Zusammensetzung einiger Varietäten derselben Art und ihr genetischer Zusammenhang. Extrait Recueil Trav. Bot. Néerland. **12**:217–278. 1915.

[27] LIVINGSTON, B. E., Physiological temperature indices for the study of plant growth in relation to climatic conditions. Physiol. Researches **1**:399–420. *figs.* 4. 1916.

mation of the daily mean temperature, above a certain fixed minimum, throughout the growing season. Such indices of temperature efficiencies for plant growth have been used largely by phenological students, notably in MERRIAM'S "law of temperature control." An advance upon this method was suggested by LIVINGSTON,[28] based upon the supposition that growth rate may follow the chemical principle of van't Hoff, doubling with each increase of temperature of 10° C., and the present publication proposes to give the indices a value based upon physiological experiment. LEHENBAUER'S[29] recent experiments upon the growth rate of maize seedlings at different temperatures affords data for the derivation of these indices which surpass those formerly proposed in taking account of the recognized principle of temperature minima, optima, and maxima; and also in showing a much greater rate of increase of index value with rising temperature between 2° and 32° C. Charts showing the climatic zonation of the United States according to each of the 3 classes of indices are suggestive and interesting for study and comparison. The third method clearly surpasses the others in correctness of principles involved, and its indices are used by the same author[30] in deriving a single index for both temperature and moisture. As a measure of the moisture conditions, the ratio of annual rainfall to annual evaporation as suggested by TRANSEAU is used, and this ratio is multiplied by the summation index of temperature efficiency for the same period, and the product is the proposed moisture-temperature index. The general scheme is a good one, and the resulting zonation of the United States is interesting in spite of the utter inadequacy of the evaporation data. It may be doubted also whether this rainfall evaporation ratio expresses the moisture conditions which determine plant growth as well as the soil moisture-evaporation ratio suggested by the reviewer. It is true that here again the lack of data will prevent the effective use of this ratio for years to come.—GEO. D. FULLER.

Taxonomic notes.—COLLINS and HOWE,[31] in studying specimens of red algae from Bermuda, southern Florida, and North Carolina, have recognized 4 new species of *Halymenia*.

SAFFORD[32] has published *Desmopsis* as a new genus of Annonaceae, to include 5 species from Mexico, Panama, and Costa Rica, that differ in several important characters from the Old World *Desmos* (*Unona* Vahl).

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[28] LIVINGSTON, B. E., BOT. GAZ. **56**:349–375. 1913.

[29] LEHENBAUER, P. A., Growth of maize seedlings in relation to temperature. Physiol. Researches **1**:247–288. 1914.

[30] LIVINGSTON, B. E., A single index to represent both moisture and temperature conditions as related to plants. Physiol. Researches **1**:421–440. *fig. 1*. 1916.

[31] COLLINS, F. S., and HOWE, M. A., Notes on species of *Halymenia*. Bull. Torr. Bot. Club **43**:169–182. 1916.

[32] SAFFORD, W. E., *Desmopsis*, a new genus of Annonaceae. Bull. Torr. Bot. Club **43**:183–193. *pls. 7–9. fig. 1*.

In the last number of HOOKER'S *Icones Plantarum* (V. 1:*pls. 3051–3075*, June 1916), the following new genera are described and figured: *Pappobolus* Blake (Compositae), *Mischopleura* Wernham (Ericaceae), *Neowollastonia* Wernham (Apocynaceae), *Dalzielia* Turrill (Asclepiadaceae), *Eriolopha* Ridley (Zingiberaceae), *Chloachne* Stapf, *Uranthoecium* Stapf, and *Danthoniopsis* Stapf (Gramineae). In addition to these 8 new genera, 7 new species are described.

KOIDZUMI,[33] in continuing his studies of Castanaceae, has recognized *Synaedrys* Lindl as a genus, extending it considerably, and has included under it 150 species transferred from *Quercus*. He also lists 234 species as remaining under *Quercus*.

RIDDLE[34] has presented a complete list of the known lichens of Bermuda, including 86 species representing 36 genera. New species are described in *Thelidium*, *Anthracothecium*, *Opegrapha*, *Bilimbia*, *Psorotichia*, and *Collema*.

WEST,[35] in continuation of his studies of algae, has described a new marine genus of the Volvocales (sub-family Carterieae), naming it *Platymonas*. He also describes new species in *Chlamydomonas* (2) and *Pteromonas*.—J. M. C.

Eocene floras.—The geographic area covered in the recent monograph by BERRY[36] is the mainland south of latitude 41 and east of longitude 100. The Antillean and Mexican regions are not included even for comparison on account of meager information in regard to them. The memoir is monumental in its character, consisting of nearly 500 quarto pages and 117 plates. A few ferns and monocotyledons are described, and no conifers. Most of the illustrations represent impressions of dicotyledonous leaves. The only anatomical illustrations are of a *Cupressinoxylon* and a *Laurinoxylon*, which are of the conventional and vague nature that too often characterizes such illustrations in publications of the U.S. Geological Survey. Apparently the Survey either should not publish anatomical data at all or intrust their preparation to some one equipped with a modern anatomical training. An important and valuable feature of the work is the attempt to correlate the presence of fossil forms with the principles of phytogeography. This appears to be very well done and is not open apparently to the grave objections which present themselves in the case of the conifers of the Mesozoic, which for the most part have been wrongly identified from their impressions and consequently cannot be used safely in

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[33] KOIDZUMI, GENICHI, On the classification of Castanaceae. II. Bot. Magazine (Tokyo) **30**:185–215. 1916.

[34] RIDDLE, LINCOLN W., The lichens of Bermuda. Bull. Torr. Bot. Club **43**:145–160. 1916.

[35] WEST, G. S., Algological notes. XVIII–XXIII. Jour. Botany **54**:1–10. *figs. 7.* 1916.

[36] BERRY, E. W., The Lower Eocene floras of southeastern North America. U.S. Geol. Survey. Professional paper 91. Washington. 1916.

discussing geographical distribution. The author sets a high example for American systematic paleobotanists. It is to be hoped, however, that the paleobotanical activities of the U.S. Geological Survey will not in the long run be confined to the systematic side, but that they will be extended, as has already been done in the case of European countries, to the crucially important although less abundant structural remains.—E. C. JEFFREY.

Lower Eocene plants.—BERRY[37] has published an extensive paper on the plants of the Lower Eocene of southeastern North America, being the result of several years of work on the fossil plants of the southern coastal plain. Naturally, much of the report deals with the stratigraphic relations illustrated by the plants, but the systematic descriptions are of great botanical interest. The orders represented, 34 in number, range from Pyrenomycetes to Rubiales, but 29 of the orders are angiosperms. *Caenomyces* is a new genus of Pyrenomycetes, including 6 species. The pteridophytes are represented by 5 new species, and *Meniphylloides* is proposed as a new genus of ferns. The gymnosperms are represented by 2 new species, one in *Zamia* and the other in *Anthrotaxis*, while 4 new species, representing as many genera, belong to the monocotyledons.

The bulk of the report, however, deals with the dicotyledons, 228 new species being described, distributed among 96 genera, among which are 7 new genera as follows: *Paraengelhardtia* (Juglandaceae), *Knightiophyllum* (Proteaceae), *Dalbergites* (Leguminosae), *Sterculiocarpus* (Sterculiaceae), *Bombacites* (Bombaceae), *Dillenites* (Dilleniaceae), and *Ternstroemites* (Ternstroemiaceae). One of the marked features in the composition of this dicotyledonous flora is the abundance of leguminous plants, of which 53 new species are described, 12 of which, for example, belong to *Cassia*. In addition to the new species assigned definitely to recognized families, 14 new species are described under form genera of uncertain relationship.—J. M. C.

Conjugate nuclei in Ascomycetes.—In a brief article, Miss WELSFORD[38] notes the fact that conjugate nuclei are common in the hyphae of well nourished mycelia of *Botrytis cinerea* and *Sclerotinia Libertiana*. In poorly nourished mycelia the paired nuclei are absent, as the nuclei under such conditions have time to move considerable distances apart before successive divisions occur. Miss WELSFORD observes that if conjugate nuclei occur generally in the mycelium of Ascomycetes, their presence in the ascogenous hyphae does not have the sexual significance usually attributed to it.—H. HASSELBRING.

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[37] BERRY, E. W., The Lower Eocene floras of southeastern North America. U.S. Geol. Survey. Professional paper 91. pp. 481. *pls. 117. figs. 16.* 1916.

[38] WELSFORD, E. J., Conjugate nuclei in the Ascomycetes. Ann. Botany **30**:415–417. *figs. 4.* 1916.

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THE

BOTANICAL GAZETTE

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TEMPERATURE AND LIFE DURATION OF SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 226

JAMES FREDERICK GROVES

(WITH FIVE FIGURES)

Introduction

In this investigation I have sought to determine the extent to which a study of the laws of the life duration of seeds at high temperatures (50–100° C.) will explain the process of degeneration of air-dried seeds at ordinary storage temperatures. In this connection it seemed especially desirable to determine: (*a*) the temperature coefficient (Q_{10}) for the death rate or life duration of seeds; (*b*) to what extent the formula which LEPESCHKIN (**22**) applied as a time-temperature formula for the coagulation of proteins and as a life duration-temperature formula for active living plant cells can be applied as a life duration-temperature formula to dry seeds; and (*c*) how far the temperature coefficient (Q_{10}) on the one hand, and the LEPESCHKIN formula on the other, when applied to actual measurements at high temperatures serve as a means of approximating the life duration of air-dried seeds at ordinary storage temperatures. With these questions in mind a number of determinations have been made on wheat of the Turkey-red variety.

Historical

The effects of high temperatures on dry seeds early engaged the attention of investigators. In 1875 JUST (**19**) showed that dried seeds of *Trifolium pratense* were killed at 120° C., and that

at lower temperatures (time of exposure not given) the speed of germination fell with heating. HÖHNEL (**17**) in 1877 found that most seeds with a moisture content below 3 per cent would endure a temperature of 110–125° C. for 15 minutes. In 1902 DIXON (**13**) summarized the earlier work on high temperatures of seeds as follows: "(*a*) imbibed protoplasm resists 30–40° C. more than the optimum temperature; (*b*) dry protoplasm resists 100° C. more than the optimum temperature of the protoplasm of imbibed seeds." DIXON found that the time required for germination was lengthened as the temperature increased, and that each seed had a heat point at which the time of germination began to increase. In 1899 JODIN (**18**) found that 30–60 per cent of desiccated seeds of peas and cress may be exposed to a temperature of 98° C. without losing their ability to germinate, if first dried at 60° C. for 24 hours and then heated to 98° for 10 hours.

The cause of the loss of viability in old seeds has been a matter of considerable discussion and investigation. DUVEL (**14**) states that seeds retain their viability longest in conditions which permit least respiration, implying that the food materials are exhausted. ACTON (**1**), by a careful analysis of old and new seeds, found that there was but a slight difference in their food content. In the course of these investigations he discovered that there was considerable diastatic and proteolytic enzyme action in new seeds, while in old seeds there was none. He assumed, therefore, that the loss of viability is related to the loss of enzyme activity. The investigations of THOMPSON (**31**), WAUGH (**32**), and others furnish some evidence for this conclusion. They found that old seeds with a low percentage of germination, when soaked in enzyme solution, showed an increase in viability. BROCQ-ROUSSEAU and GAIN (**8, 9, 10, 11**) in their earlier work found that enzymes gradually disappear with age. They tested 300 species of seeds and found no peroxidases in any of the samples secured before the eighteenth century. In the case of *Triticum* they found enzymes in samples as old as 200 years. In some cases the retention of enzymes was attributed to the hard coats of the seeds, and the loss of viability was stated to be due to some cause other than degeneration of enzymes. ASPIT and GAIN (**3**) found enzyme activity in seeds

long dead and in seeds killed by anesthetics. Miss WHITE (33) found no increase in the germination of seeds soaked in enzyme solutions, but rather a decrease due to an increased fungal action. She found the life duration of *Triticum* to be 17 years, with no loss of enzyme activity. According to her work the enzyme theory of the loss of viability is not tenable.

Some very significant work has been done on the time-temperature relation of coagulation of protein both in vitro and in the living cell. BUGLIA (7) found that the time required for the coagulation of blood serum varies with the temperature used. The time of coagulation was found to be a logarithmic function of the temperature. CHICK and MARTIN (12) found that the time required to precipitate egg albumen and haemoglobin from solution varies with the temperature and with the concentration of the solution. LEPESCHKIN (22) showed that the death of active plant cells by supramaximal temperatures is due to the coagulation of the cell protoplasm. He applied a logarithmic formula to express the relation of temperature to the time of coagulation of proteins in vitro as well as in the living cell. By the application of this formula to the determined time for coagulation at any two temperatures, one can calculate the time necessary for coagulation at any other temperature. On this basis LEPESCHKIN calculated the life duration of active *Tradescantia* cells at 20° C. to be 33 days, and at zero to be 3 years. He believes that the life duration of plant cells is very much longer than indicated because of a redispersal process, carried on by the active living cells, which counteracts the coagulation process.

The results of many workers in this field have been well summarized in a recent monograph by KANITZ (20), who has brought together the literature from several related subjects. He shows that in general the effect of temperature upon the rate of chemical processes is governed by the Van't Hoff law, that is, the coefficient for a rise in temperature of 10° C. (Q_{10}) is 2 to 3. From the experimental results at any two temperatures the value of Q_{10} may be calculated from the following equation (referred to as formula 1 and formula 2):

$$Q_{10}=\left(\frac{k_2}{k_1}\right)^{\frac{10}{t_2-t_1}} \text{ or } Q_{10}=10^{\frac{10\ (\log k_2-\log k_1)}{t_2-t_1}}$$

in which k_2 is the rate of reaction obtained at temperature t_2, and k_1 the rate of reaction obtained at temperature t_1.

Many processes in living organisms show a temperature coefficient approximately that of the Van't Hoff law within certain temperature limits. Some of these show high values of Q_{10} at lower or at critical temperatures. High values of Q_{10} are found also for life duration and for coagulation or denaturing of proteins.

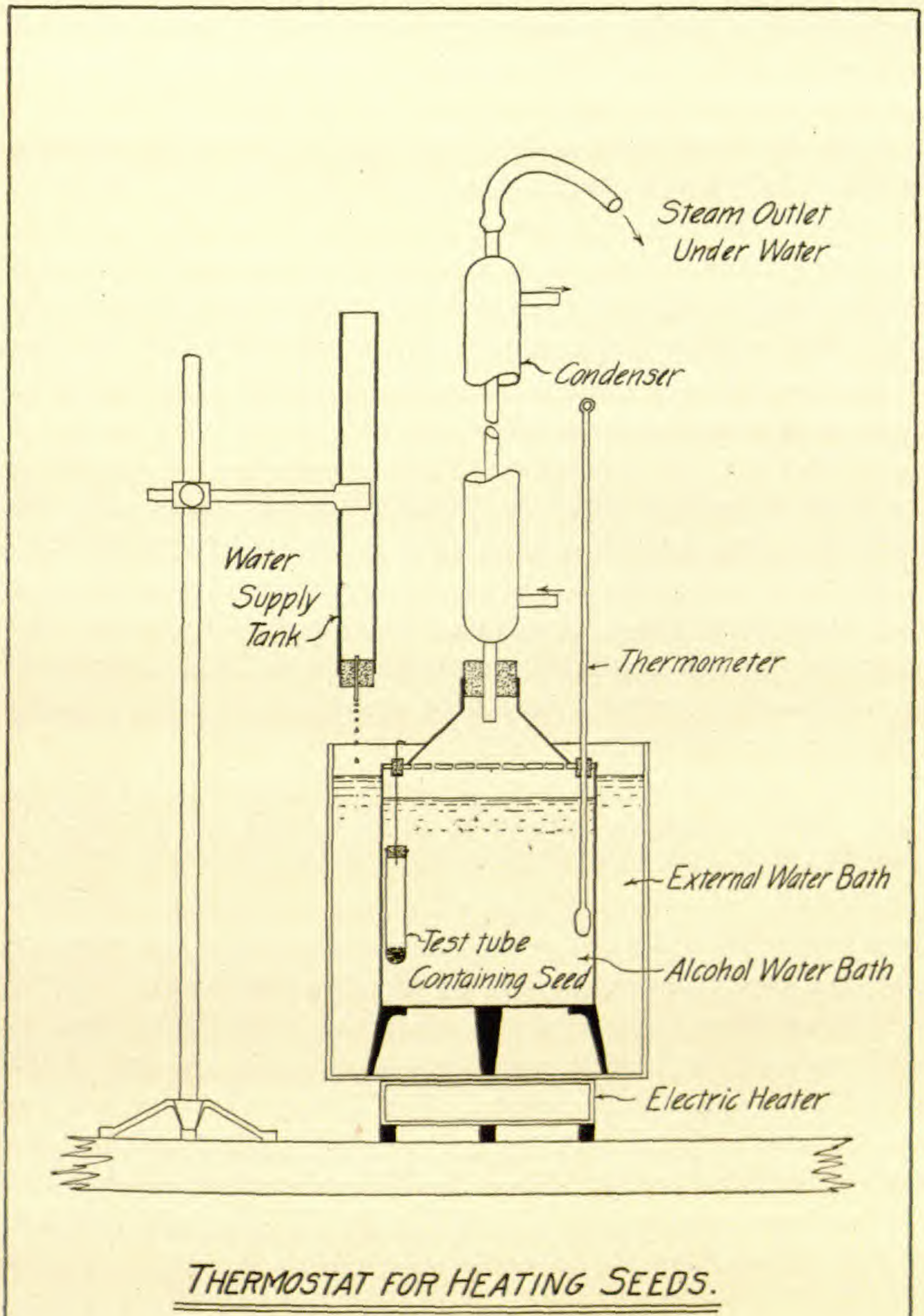
KANITZ (**20**) brings out more clearly the relation of temperature to the rate of life processes by recalculating Q_{10} at the various temperature intervals instead of giving only the average coefficient for the whole temperature range. In this way it is found that in many cases Q_{10} is not a constant at all intervals of temperature, but shows decreasing values with rise of temperature.

Method

In order to obtain constant temperatures for heating the seeds, a thermostat was devised as shown in fig. 1. It consisted of an external water bath heated by an electric stove. In this bath was placed a similar vessel of smaller dimensions which was closed at the top and connected with a water-cooled reflux condenser. Methyl or ethyl alcohol or mixtures of methyl or ethyl alcohol with water was used for temperatures 64–99° C. The temperature during the time of an experiment showed a fluctuation of less than ±0°.1 C. For lower temperatures, where the time was much prolonged, the usual water-jacketed incubator was used. This was well wrapped with heavy woolen blankets. The temperature of the incubator was regulated by the automatic electric apparatus devised by LAND (**21**). It gave a very equable temperature, showing a straight line on the drum of an ordinary recording thermometer.

The seeds were heated in the thermostat by inserting securely corked test tubes, each containing 100 selected seeds, through perforations in the top of the inner vessel. These test tubes were suspended by threads passed through the perforations, and the threads were then secured by corks which closed the openings.

Many of these tubes were inserted at the same time and removed in duplicate at successive intervals. Seeds were heated in the



THERMOSTAT FOR HEATING SEEDS.

FIG. 1

incubator in a similar manner by inserting the tubes through perforations in the top. This avoided the main source of temperature fluctuation in the incubator, opening the door.

After the seeds were heated they were sterilized by washing for 2 or 3 minutes in a $n/50$ solution of silver nitrate. While in this solution the seeds were stirred thoroughly in order to free them of all air bubbles. Then they were washed thoroughly in sterile distilled water to remove the excess of silver nitrate which would injure the seedlings when germinated. SCHROEDER (**28, 29**) has shown that the seed coat of wheat is only slightly permeable to silver nitrate, and many parallel tests with treated and untreated seeds confirmed his conclusions. The importance of sterilizing the seeds is realized when we consider that in some cases the germination was delayed as much as 20 days. Miss MÜLLER (**25**), in her work on germination of heated seeds, found that after 10 days seeds were either germinated or destroyed by mold. By sterilizing the seeds and the germinating dishes and using some care in planting, cultures were kept practically free from fungal growth for several weeks.

After sterilizing and washing, the seeds were germinated in large Petri dishes containing a layer of moist cotton covered with a layer of filter paper. The dishes were sterilized at 140° C., and considerable care was used in planting the seeds to maintain sterile conditions. The dishes were kept in laboratory light and temperature, and their daily progress in germination was noted.

The moisture content of the seeds was tested from time to time, and only very slight variations occurred in any one of the 3 moisture content experiments. The DUVEL (**15**) method was used parallel with the ordinary oven drying method and the two gave concordant results. Since the DUVEL method requires less than an hour to make a test, it was possible to check the moisture content before filling the tubes for each trial.

Results

The effects of heating seeds is well shown in table I, which is a daily record of the germination of a time series heated at 87°.5 C. Seeds were considered normally germinated when both root and

stem had broken through the seed coat. When only the root or the shoot appeared, the seeds were considered partially germinated. Partial germination is represented in this table by the figures in small type. The delay in time of germination as the time of exposure increased is strikingly shown here. The controls usually

TABLE I

Record Sheet No. 21 Turkish Red Wheat
Temp: 87.5°C. Moisture: 12.% April 10, 1914.

Time days	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Control	0	2	2	4	3	3	0	0	0	0	0	0	0	0	0	0	0	0
	92	92	92	93	95	95	98	98	98	98	98	98	98	98	98	98	98	98
7 Min.			0	15	12	7	8	7	8	7	5	3	3	4	4	4	3	2
			2	5	27	41	49	55	61	64	67	70	72	72	72	72	73	74
8 Min.					4	8	8	7	8	7	5	3	1	5	1	1	1	0
					5	10	25	30	35	41	47	52	54	54	58	59	59	60
9 Min.						2	4	4	5	4	5	4	5	2	3	6	5	4
						2	4	8	10	11	18	25	28	32	34	35	37	38
10 Min.		Per Cent Partially Germinated								1	3	4	6	4	3	3	4	5
		Per Cent Germinated								0	0	0	0	4	5	9	11	11
11 Min												1	1	0	0	1	2	1
												1	1	2	4	4	4	5
12 Min.													2	2	2	2	2	2
													0	0	0	2	2	2
13 Min.																		
																0	0	0

germinate in about 2 days, while some of the treated seeds were delayed for 18–20 days. The relation between time of heating and the percentage of germination is shown in the table. There is a gradual decrease in the percentage of germination with increased time of heating. After the delayed seeds germinate their growth is much slower than that of unheated seeds. It should be noted that the effects here of heating are similar to those produced by the aging of seeds stored at room temperature. This indicates

that there may be a similar change in the two cases. The change occurs rapidly at the high temperature, but slowly at the low temperature.

Some investigators have used the time required to kill all seeds as the end point. In this work we have selected the time required to kill 75 per cent of the seeds as the end point. This is more desirable because there seems to be considerable discrepancy in the resistance of a few stronger seeds. This end point for 12 per cent moisture and various temperatures was obtained as shown in table II. While there are some irregularities, there is a definite relation between temperature and time of exposure necessary for killing 75 per cent of the seeds.

The time-temperature formula suggested by LEPESCHKIN (**22**) has been used here to calculate the life duration of the seeds. By determining the time required to kill seeds at any two definite temperatures, the time for killing seeds at any other temperature can be calculated. The formula (referred to as formula 3) is:

$$T = a - b \log Z$$

in which T is the temperature in degrees Centigrade, Z is the time in minutes, and a and b are constants. If the loss of viability of seeds during storage is a matter of coagulation of cell proteins of the embryo, this time-temperature formula for the coagulation of proteins should be applicable as a temperature-life duration formula for seeds. In experiment the life duration determined must be at relatively high temperatures, ranging from 50 to 100° C. for air-dried seeds.

In formula 3 constants a and b may be calculated by substituting the time and temperature of any two trials and solving for a and b in the two equations. This is the method of calculation used by LEPESCHKIN (**22**). In order to weigh all determinations equally, the constants in this paper are calculated by the method of least squares. The values of the constants and the life duration found in each experiment were substituted in the equation and the theoretical temperatures were calculated. The values are shown in table II. A comparison of these found and calculated temperatures shows that a comparatively close agreement exists. The discrepancies are within the limits of experimental error.

Fig. 2 is a time-temperature curve representing the experimental data shown in table II for wheat with 12 per cent

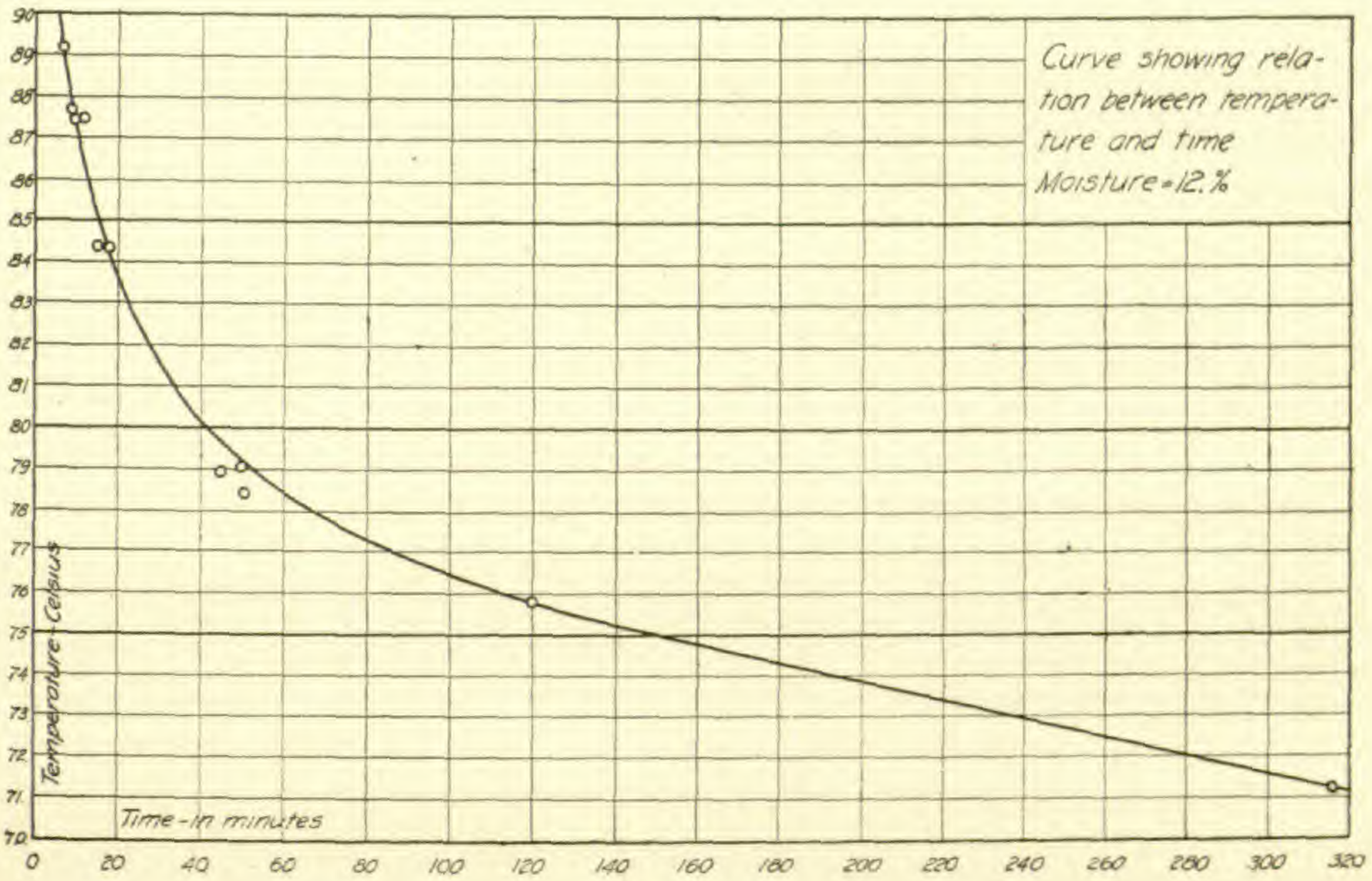


FIG. 2

TABLE II

Germination record of Turkey-red wheat 12 per cent moisture; theoretical temperature calculated by the formula $T = a - b \log Z$; T is temperature (Centigrade); Z is time in minutes; a and b are constants; $a = 96.87$; $b = 10.4$; value of Q_{10} as calculated by method of least squares = 10.14.

Time in minutes	Calculated temperature	Experimental temperature	Q_{10}
7	88.1° C.	89.2° C.	
8	87.5	87.7	
9	87.0	87.5	6.09
10	86.5	87.5	(10.3)
15	84.6	84.4	
18	83.7	84.4	
45	79.7	78.9	
50	79.2	79.1	
50	79.2	78.5	12.94
120	75.2	75.8	(7.6)
315	70.9	71.3	

Predicted life duration: 50° C., about 22.3 days; 25° C., about 15.5 years; 20° C., about 46.9 years; 0° C., about 393 years.

moisture. The ordinates represent degrees Centigrade and the abscissae minutes of time. Except for the irregularities which

occur at 78 and 79°, there appears a marked agreement in the data and the points representing the experimental data approximate a smooth curve. Table II shows the value of Q_{10} for experimental temperatures as calculated by formula 2. In tables II–IV partial germination is represented by the figures in parentheses.

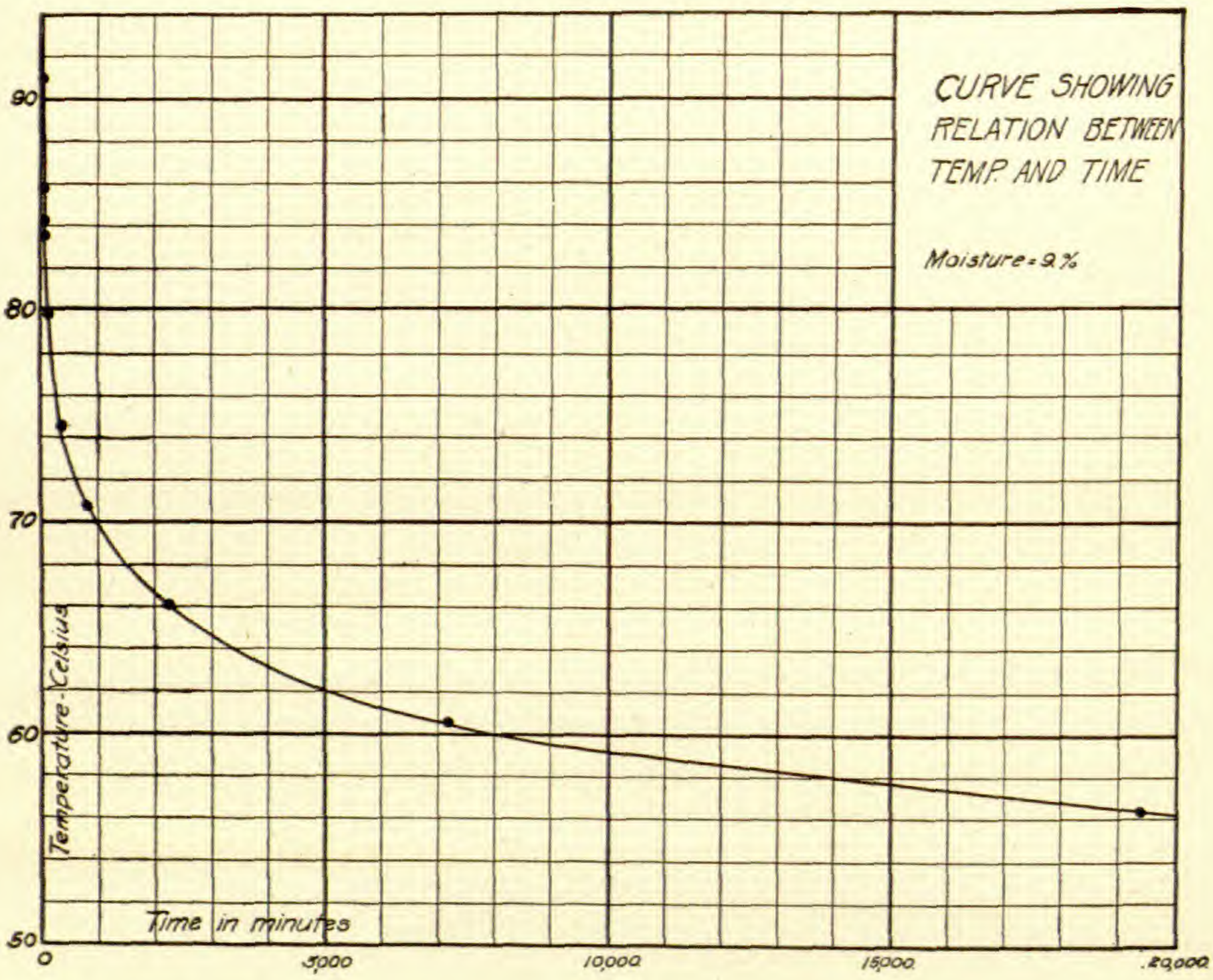


FIG. 3

Table III shows the found and calculated data for wheat with 9 per cent moisture. The calculated temperatures were obtained by the same method as those in table II, and here as before there is a close agreement between the theoretical and observed values. In fig. 3 the experimental data of table III are also expressed in a time-temperature curve. Practically all of the points representing the experimental data fall on a smooth curve. Table III shows the value of Q_{10} for experimental temperatures as calculated by formula 2.

Table IV shows a limited number of data for wheat with 17.5 per cent moisture. As would be expected, the time required to

TABLE III

Germination record of Turkey-red wheat 9 per cent moisture; theoretical temperature calculated by formula $T=a-b \log Z$; T is temperature (Centigrade); Z is time in minutes; a and b are constants; $a=100.8$; $b=10.4$; value of Q_{10} as calculated by method of least squares = 9.23.

Time in minutes	Calculated temperature	Experimental temperature	Q_{10}
8	91.4° C.	90.8° C.	
27	85.9	85.7	13.49
45	83.6	84.2	(11.0)
63	82.1	83.5	
140	78.5	79.8	7.03
435	73.4	74.4	(9.0)
810	70.5	70.8	8.51
2340(1.6 days)	65.7	66.0	(10.2)
7200(5.0 days)	60.6	60.6	
19440(13.5 days)	56.2	56.3	

Predicted life duration: 50° C., about 53.4 days; 25° C., about 37.3 years; 20° C., about 111.2 years; 0° C., about 938.5 years.

kill such seeds at the temperatures used in the former experiments is exceedingly short. The error due to the time required for the

TABLE IV

Germination record of Turkey-red wheat 17.5 per cent moisture; theoretical temperature calculated by formula $T=a-b \log Z$; T is temperature (Centigrade); Z is time in minutes; a and b are constants; $a=81.73$; $b=8.04$; value of Q_{10} as calculated by method of least squares for last 4 temperatures = 16.45.

Time in minutes	Calculated temperature	Experimental temperature	Q_{10}
3.0	77.9° C.	87.1° C.	
3.75	77.1	83.6	2.22
4.0	76.9	83.1	(7.6)
5.5	75.8	79.5	4.90
8.0	74.5	74.7	(9.0)
23.0	70.8	70.5	19.71
140.0	64.5	64.4	(9.9)
440.0	60.5	60.6	

Predicted life duration: 50° C., about 6.1 days; 25° C., about 21.6 years; 20° C., about 64.4 years; 0° C., about 2800 years.

seeds to attain the temperature of the bath is therefore very apparent here. The data of this table are expressed also as a time-

temperature curve in fig. 4. Here again there is close agreement between the found and the calculated values. Table IV shows the value of Q_{10} for experimental temperatures as calculated by formula 2.

In fig. 5 the temperature is plotted against the logarithm of the time for wheat with 9, 12, and 17.5 per cent moisture. Since one of the constants is found to have a common value in the 9 and 12

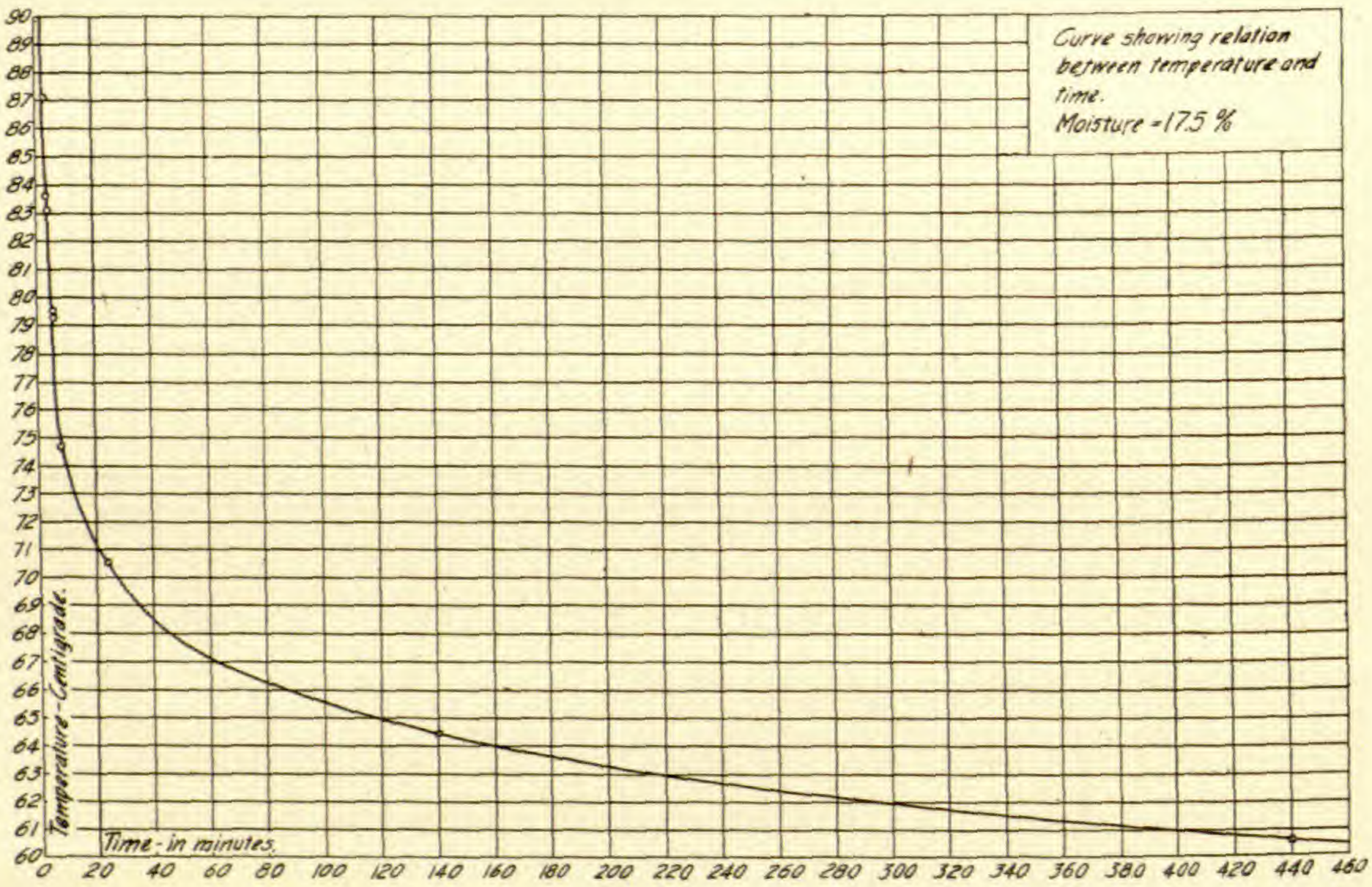


FIG. 4

per cent moisture curves, they are parallel. Since they were obtained under identical conditions, one may surmise that the curves in which temperatures in degrees Centigrade are plotted against time in minutes are parallel. Sufficient data are not available with the 17.5 per cent moisture content at experimentally reliable time intervals to justify a conclusive generalization. The curve for the 17.5 per cent moisture content deviates upward from a straight line in the lower time range. This is due to the fact that a considerable part of the short period of exposure was consumed in heating the seed up to the temperature of the bath.

TEMPERATURE COEFFICIENT

The temperature coefficient (Q_{10}) of the life duration of wheat was found to vary with the moisture content. The average value for 9 per cent moisture, calculated by the method of least squares, is 9.23; for 12 per cent moisture, 10.14; and for 17.5 per cent moisture, 9.83. GOODSPEED (**16**), working with barley grains, found a coefficient varying from 10 to 16 as calculated by KANITZ (**20**). The result obtained by GOODSPEED is marred by the lack

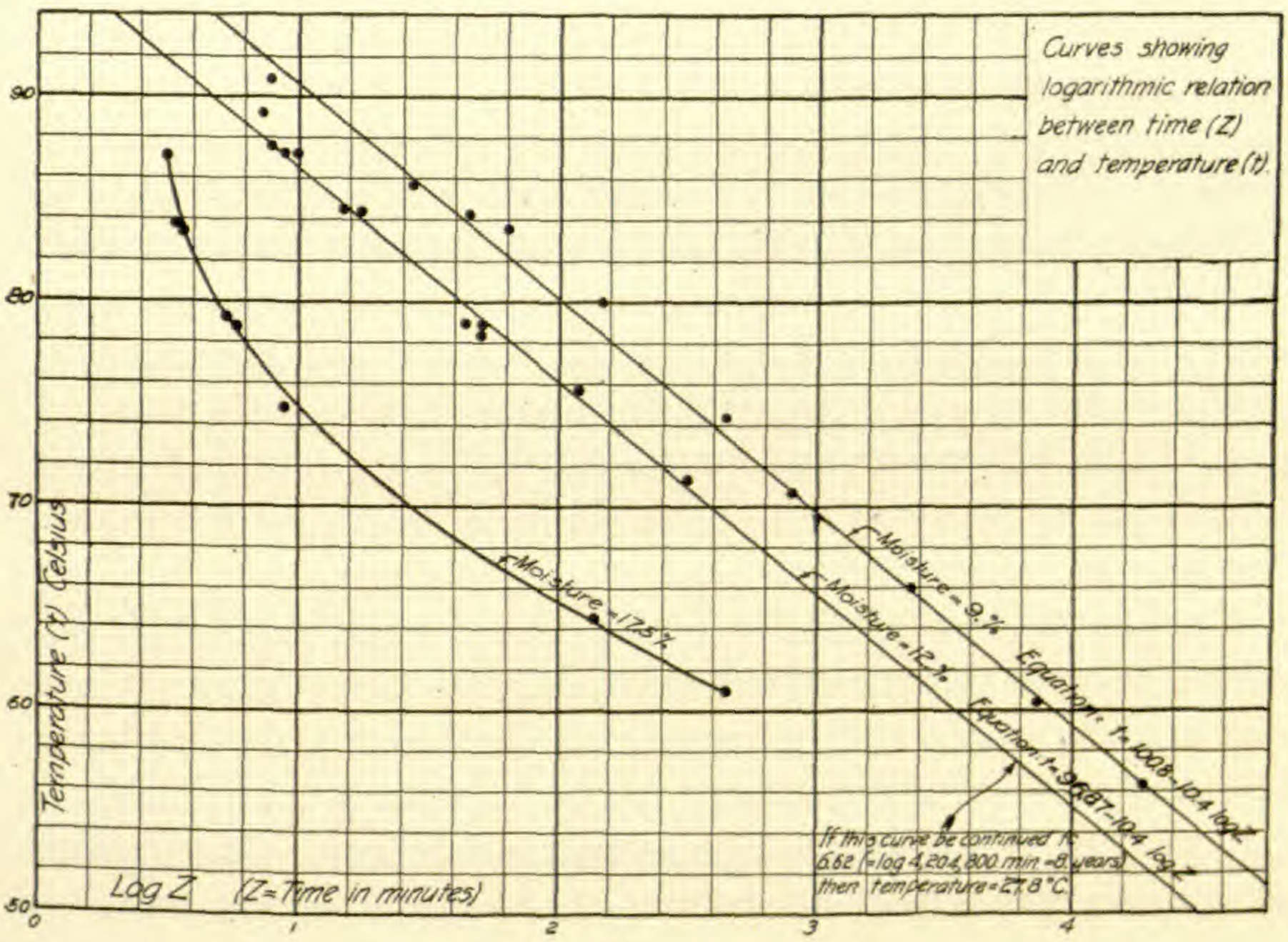


FIG. 5

of determination and control of the moisture content. Since the seeds were soaked for only one hour previous to heating, they had not absorbed the maximum amount of water. Such seeds, when heated in water at various temperatures for various periods of time, show considerable difference in their water content. I find by calculation, according to formula 2, that LEPESCHKIN's data on *Tradescantia discolor* give a value of about 5.0 for Q_{10}, and on *Beta vulgaris* from 2.5 to 10.4. AYRES (**4**), in a recent investigation on one of the red algae (*Ceramium tenuissimum*), found an

average coefficient of 1.47 per degree Centigrade. I have calculated his data according to formula 2 and find the value of Q_{10} to vary from 5 to 88. There is a definite break in his data which prevents them from falling on a smooth curve. This is probably due to some uncontrolled factor in experimentation. I find that the data in other articles cited here, both on life duration and coagulation of proteins, give smooth curves.

The Q_{10} coefficient for life duration of animals, so far as worked, is very much larger than for plants. LOEB (**23**) found a Q_{10} value varying from 240 to 1450 for fertilized sea urchin eggs, while for unfertilized eggs he found a value of 600. MOORE (**24**) found a Q_{10} value varying from 485 to 3900 for the stems of *Tubularia crocea*.

The Q_{10} value for the coagulation of proteins shows a wide variation for the various proteins or different conditions of the same protein as calculated by KANITZ (**20**). CHICK and MARTIN (**12**) found a value of 14 for haemoglobin and 635 for egg albumen. I have calculated the value of Q_{10} for BUGLIA'S (**7**) data and find it to be about 15 for blood serum, 760 for fresh muscle, 45 for neutral albumen, and 240 for neutral concentrated albumen.

The low Q_{10} coefficients found for plants mean that they can endure various supramaximal temperatures for relatively long periods as compared with animals with the larger Q_{10} coefficients. This may be of great importance to plants, since in many habitats they are unable to avoid intense radiant energy. The radiant energy is largely absorbed and the plants attain temperatures as much as 28° C. above the air temperatures, and certainly several degrees above the maximal for growth (**2, 6, 26, 30**). In general the animal is able to avoid such superheating through locomotion. As bearing on this point many more determinations are needed both on animals and on plants of a variety of habits and habitats.

The difference between the temperature coefficients for plants and animals shows itself in an experimental way. There is the possibility of employing a much greater range of temperature in plant material. In the investigations on animals previously cited the range used is about 10° C., while in investigations on plants the range is 20° C. or more. In plants a much greater range

would be possible were it not for the fact that the small coefficients give life durations too long for convenience in experimentation at temperatures not considerably above the maximum.

The difference between plants and animals in the size of the coefficients manifests itself in another way. In the investigations with animals the temperatures used are largely below 45° C., while with plants it is not uncommon to use temperatures above 70° C. and obtain an easily measurable life duration. The maximum temperature usable is determined also in part by other factors, such as percentage of water present and the general attunement of the particular plant to the temperature. The lower the percentage of water in the seed, the higher the temperature that can be used with it. It is probable also that in forms like *Ulothrix* and *Hydrurus*, having maxima below 24° C. (**27**), the possible experimental temperatures would not run so high.

Discussion

The rather close agreement between calculated and found values indicates that the time-temperature formula for the coagulation of protein can be applied as a temperature-life duration formula for seeds, at least under the conditions of these experiments. It is probable that the accumulation of more data will make it possible to find some other equation which expresses more adequately the relationship between the variables involved. In the experiments on wheat of 9 and 12 per cent moisture the average deviation of the observed from the calculated temperatures is less than 1 per cent. The corresponding average deviation for the 17.5 per cent moisture content is about 8 per cent. The unexpectedly large error with the 17.5 per cent moisture content is due to the previously noted fact that a considerable part of the time of exposure is consumed in heating the seeds up to the temperature of the bath. The uniformly increasing deviation of the observed temperatures with short periods of time shows that greater accuracy is possible with long time exposures.

While in many reactions there is a consistent decrease in the value of the coefficient Q_{10} as the temperature increases, we do not find such a trend here. Compared with animal tissue, the value of

the coefficient is small and compares in magnitude with the value found by other workers on plant tissue. The range in the value of the coefficients is small, as indicated by the fact that the data fall on comparatively smooth curves. The coefficient Q_{10} as calculated from the data (for 12 per cent moisture) in table II by the method of least squares is 10.14. When the temperature and time-differences in formula 2 are so small that they are comparable with the errors of observation, then the numerical evaluation of Q_{10} becomes highly inaccurate. But when the time and temperature differences are large enough to render ineffective the errors of observation, then the calculated coefficient Q_{10} is comparable with the value obtained by the method of least squares.

The coefficient Q_{10} for 9 per cent moisture content was found to be 9.23 as calculated by the method of least squares from the data in table III. Similarly, the coefficient for 17.5 per cent moisture content was found to be 16.45 when calculated by the same method, using the 4 highest time observations in table IV. The 4 lowest time observations were ignored on account of the inaccuracy introduced by the time required for the seeds to attain the temperature of the bath, as previously explained.

A number of longevities have been calculated by formula 3 for the low temperatures at different moisture contents. With the relatively short range of temperatures used in these experiments, considerable error may appear in predicted longevities, especially at low temperatures. When such calculated longevities are compared with observed values, they are found usually to be considerably too large, indicating that other processes may also be effective in causing loss of viability. Since hard-coated seeds have long vitality records, it seems quite possible that this is related to the absence of oxygen and low water content.

Much more work is needed to determine how nearly one can thus approximate longevities from measurements made at high temperatures. Determinations should be made on the life duration of seeds with low moisture content. Also similar determinations should be made for a long-lived seed, such as sweet clover, for which we have reliable records of longevity, as well as short-lived

seeds, such as *Drosera*, willow, and poplar. A series of determinations should also be made on seeds at constant temperature with variations in moisture content to ascertain the relations existing between moisture content and life duration.

The data show that the LEPESCHKIN formula applies as a temperature-life duration formula for seeds at the temperatures used in these experiments, but there are several considerations that may limit its application at lower temperatures, including ordinary storage temperatures. (1) Increase of acidity of seeds will hasten the coagulation of the cell proteins; such a change is known to occur in the seeds of certain Rosaceae (**15a**), at least if stored in the imbibed condition. (2) LEPESCHKIN (**22**) found that in active plant cells a redispersal of cell proteins is going on coincidentally with coagulation. As a consequence, at high temperatures where the coagulation was rapid, the found and calculated life durations agree closely; while at lower temperatures, where redispersal is prominent, the calculated life durations are much shorter than the found values. In seeds the calculated values are usually much greater than records of longevity at room temperatures. This indicates that the redispersal process is not going on in relatively dry seeds, or, if it is, it is more than counteracted by some other process. (3) A slight error in the value of the constant *b* in formula 3 will give a relatively large absolute error for a life duration at low temperatures such as 0° C. At higher temperatures the absolute error becomes less. (4) The lower the water content of seeds, the more heating they withstand and the greater the longevity at moderate and lower temperatures. This law has its limits, for excessive drying is itself injurious. In seeds that will endure desiccation, injury sets in with a reduction of the water content considerably below 2 per cent, while in forms like *Drosera* it appears before air-dry condition is reached. The formula, of course, is limited to degrees of desiccation less marked than those producing injury. (5) It is possible that slow oxidation may limit the longevity of seeds. If this be true, hard seeds with their coats impervious to gases along with their constant low percentage of water are in an especially favorable condition for the

marked longevity which they show. Wheat seeds stored in absence of oxygen might give longevities more comparable with calculated values.

Summary

1. The life durations of wheat with 9 per cent moisture at the various temperatures are:

Life durations in minutes	8	27	45	63	140	435	810	2340	7200	19440
Temperatures in degrees Centigrade	90.8	85.7	84.2	83.5	79.8	74.4	70.8	66.0	60.6	56.3

2. The life durations for 12 per cent moisture are:

Life durations in minutes	7	8	9	10	15	18	45	50	50	120	315
Temperatures in degrees Centigrade	92.2	87.7	87.5	87.5	84.4	84.4	78.9	79.1	78.5	75.8	71.3

3. The life durations for 17.5 per cent moisture are:

Life durations in minutes	3.0	3.75	4.0	5.5	8.0	23.0	140.0	440.0
Temperatures in degrees Centigrade	87.1	83.6	83.1	79.5	74.7	70.5	64.4	60.6

4. The application of the LEPESCHKIN formula at high temperatures as checked by actual measurements gives an average error of 0.6 per cent for 9 per cent of moisture; 0.8 per cent for 12 per cent of moisture; and 8.25 per cent for 17.5 per cent of moisture.

5. The data available for testing the application of the formula at storage temperatures are exceedingly limited. WHITE found that 25 per cent of wheat would grow after being stored for 8.5 years. Assuming that they were exposed to an average temperature of 20° C. and had an average moisture content of 12 per cent according to formula 3, applied to the experimental data of this paper, they should have a life duration of 15.5 years. However, since the variations and averages of temperature and moisture, together with other conditions, are not known, we are not justified in pushing comparisons too far.

6. No definite trend appears in the value of the coefficient Q_{10} and its range is confined to rather narrow limits. For wheat with 9 per cent moisture the range varies from 5.6 to 16.9 with an average of 9.23; for 12 per cent moisture the range varies from 4.8 to 12.6 with an average of 10.14; while for 17.5 per cent the range varies from 2 to 20 with an average of about 10 for the whole scope of the experiment.

7. Since the range of temperature used in these experiments is comparatively short, we are not justified in placing too much emphasis on predicted longevities at low temperatures. Such longevities as have been calculated by formula 3 are large when compared with observed longevities by WHITE and others.

8. This work shows possibilities of throwing some light on the nature of the processes of the loss of viability in seeds in storage conditions. It also makes possible a quantitative statement of the significance of various storage conditions, especially moisture content and temperature, upon the longevity of seeds.

I wish to acknowledge many helpful suggestions by Dr. WILLIAM CROCKER and Dr. SOPHIA ECKERSON, under whose direction this work has been done. I am also indebted to Mr. L. L. THURSTONE of the Carnegie Institute of Technology for aid and advice in making mathematical calculations.

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LITERATURE CITED

1. ACTON, E. H., Changes in reserve material of wheat on keeping. Ann. Botany **7**:383–387. 1893.
2. ASKENASY, E., Über die Temperatur, welche Pflanzen im Sonnenlicht annehmen. Bot. Zeit. **33**:442–455. 1875.
3. ASPIT, J., and GAIN, E., Les graines tueés par anesthésie conservent leur propriété diastasique. Compt. Rend. Acad. Sci. **149**:58–60. 1908.
4. AYRES, A. H., The temperature coefficient of the duration of life of *Ceramium tenuissimum*. BOT. GAZ. **52**:65–69. 1916.
5. BECQUEREL, P., Recherches sur la vie latente des graines. Ann. Sci. Nat. Bot. **5**:193–320. 1907.
6. BLACKMAN, F. F., and MATTHAEI, A., Quantitative study of CO_2 assimilation and leaf temperature in natural illumination. Proc. Roy. Soc. London B **76**:402–460. 1905.

7. BUGLIA, G., Über die Hitzegerinnung von flüssigen und festen organischen Kolloiden. Zeitsch. Chem. und Ind. der Kolloide. **5**:291–293. 1909.
8. BROCQ-ROUSSEAU, and GAIN, E., Sur l'existence d'un peroxydiastase dans les graines sèches. Compt. Rend. Acad. Sci. **145**:1297–1298. 1907.
9. ———, Sur la durée des peroxydiastases des graines. Compt. Rend. Acad. Sci. **146**:545–548. 1908.
10. ———, Oxydases et peroxydiastases des graines. Rev. Gen. Bot. **21**:55–62. 1909.
11. ———, Sur la présence de l'amylase dans les vieilles graines. Compt. Rend. Acad. Sci. **148**:359–361. 1909.
12. CHICK, H., and MARTIN, A. J., On heat coagulation of proteins. Jour. Physiol. **40**:404–430. 1910.
13. DIXON, H. H., Germination and high temperatures. Notes from Bot. Sch. of Trinity Coll. Dublin **5**:176–186. 1902.
14. DUVEL, J. W. T., Vitality and germination of seeds. U.S. Dept. Agric., Bur. Pl. Ind. Bull. no. 58. 1904.
15. ———, A moisture tester for grain and other substances and how to use it. U.S. Dept. Agric., Bur. Pl. Ind. Cir. 72. 1910.
15a. ECKERSON, SOPHIA, A physiological and chemical study of after-ripening. BOT. GAZ. **55**:286–299. 1913.
16. GOODSPEED, H. T., Temperature coefficient of the duration of life of barley grains. BOT. GAZ. **51**:220–224. 1911.
17. HÖHNEL, F., Welche Wärmgrade trokens Samen ertragen, ohne ihre Keimfähigkeit einzubüssen. Wissenschaftlich-praktische Untersuchungen auf dem Gebiete des Pflanzenbaues. **2**:77–88. 1877.
18. JODIN, M. V., Sur la resistance des graines aux temperatur élevées. Compt. Rend. Acad. Sci. **129**:893–894. 1899.
19. JUST, L., Über die Wirkungen Temperaturen auf die Keimfähigkeit der Samen von Trifolium. Bot. Zeit. **33**:51–52. 1875.
20. KANITZ, ARISTIDES, Temperatur und Lebensvorgänge. Gebrüder Borntraeger. Berlin. 1915.
21. LAND, W. J. G., An electrical constant temperature apparatus. BOT. GAZ. **52**:391–399. 1911.
22. LEPESCHKIN, W. W., Zur Kenntnis der Einwirkung supramaximaler Temperaturen auf die Pflanze. Ber. Deutsch. Bot. Gesells. **30**:703–714. 1913.
23. LOEB, J., Über Temperaturkoeffizienten für die Lebensdauer kaltblütiger Thiere und über die Ursache des natürlichen Todes. Archiv. Ges. Physiol. **124**:411–427. 1908.
24. MOORE, A. R., The temperature coefficient of the duration of life in *Tubularia crocea*. Arch. Entwicklungsmech. **29**:287–289. 1910.
25. MÜLLER, G., Untersuchungen über die von Weizensamen und Weizenkeimlingen ertragenen höchsten Temperaturen. Zschr. Pflanzenkrankheiten. **23**:193–198. 1913.

26. PEARSON, H. H. W., Observations on the internal temperatures of *Euphorbia virosa* and of *Aloe dichotoma*. Annals Bolus Herb. **1**:41–66. 1914.
27. PFEFFER, W., Plant physiology, Eng. ed. Vol. I. pp. 76. 1913.
28. SCHROEDER, H., Über die selecktivpermeable Hülle des Weizenkörnes. Flora **102**:186–208. 1911. See Review BOT. GAZ. **52**:79–80. 1911.
29. ———, Über die Einwirkung von Silbernitrat auf die Keimfähigkeit von Getreidekörnen. Biol. Centralbl. **35**:8–24. 1915.
30. SMITH, A. M., On the internal temperature of leaves in tropical insolation. Ann. Royal Bot. Gard. Peradeniya **4**:229–298. 1909.
31. THOMPSON, A., Zur Verhalten alter Samen gegen Fermentlösungen. Gartenflora **45**:344–345. 1896.
32. WAUGH, F. A., Enzymatic ferments in plant physiology. Science N.S. **6**:950. 1897.
33. WHITE, J., Ferments and latent life of resting seeds. Proc. Roy. Soc. B **81**:417–442. 1909.

A REPORT ON SOME ALLOCTHONOUS PEAT DEPOSITS OF FLORIDA[1]

PART II: MORPHOLOGICAL

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(WITH PLATES X AND XI)

Previous to the eighteenth century the question concerning the origin of coal was not debatable, since it was taken for granted that it had arisen as a result of special creation, or during the Noachian deluge by sedimentation (**14**). About 100 years ago, however, the increasing value of coal in the industrial world led many investigators to seek a scientific solution for the problem. One of them, VON BEROLDINGEN, came to the conclusion in 1778 that coal was transformed peat similar to that now found in swamps. After this first step in the right direction, many other students formulated theories as to how the process had taken place. Out of the resulting heterogeneous mass of contentions only two tenets have survived, namely, the allocthonous and autocthonous modes of peat and coal formation. All modern students of the problem are agreed that ancient beds have been produced by an accumulation of organic detritus derived from the old lycopod flora, but there are important differences of opinion still as to the method by which this has been accomplished.

Since detailed and elaborate presentations of the drift and *in situ* hypotheses have found a place in many publications upon the subject, more than a brief review of them would be superfluous in this connection (**8, 9, 10, 11, 14, 15**). Those favoring the first doctrine maintain that these accumulations of much comminuted plant débris, commingled with the more resistant elements, such as spores from vascular cryptograms, carbonized wood (the "mother of coal"), cutinized parts of plants, etc., were deposited very slowly in the bottoms of permanent and open bodies of water, similar to

[1] Contribution from the Laboratories of Plant Morphology of Harvard University.

the method characteristic of lacustrine peat beds. Those opposing this doctrine, the autocthonists, reject the idea that this represents a sedimentation of plant derivatives in open water, but contend that it consists of a gradual amassing of successive generations of lowland plants by prostration *in situ*. The strata, thus exposed, were preserved from decay by a permanent though concealed water supply, as is true and characteristic of the upper stratum of peat deposits in our swamps.

The solution of this vexatious problem, as to which of the two processes is the more probable, has been attempted for the most part by geologists, and naturally enough they have sought explanation topographically. To be sure, the results obtained by numerous investigators in this direction have furnished many valuable data relative to the formation of coal beds, although many of the proofs upon which their conclusions are based are open to serious objection. For example, the presence of stigmarioid roots in coal beds and the supporting shales has been hailed frequently as valuable testimony for an autocthonous origin of the strata in which such structures are found. A broader survey of the problem shows that these rootlike organs are by no means conclusive ground for this deduction, since they also occur quite commonly in cannel coal (a type universally agreed to have been formed in open water), and consequently, a statement which argues equally for either process is unreliable. In this same connection it might be well to mention the quality of the so-called "fire clays" usually found below coal beds. Those who believe that the majority of our coal seams were laid down *in situ* see in the material conclusive proof that this inorganic layer was at one time the subsoil of swamps, owing to an absence of certain minerals which, in their opinion, could have disappeared in no other way than through extraction by growing plants. They fail, moreover, not only to show that this chemical state could not have been brought about by prolonged leaching, but also to account for similar strata in regions which reveal no evidence that they at one time supported forests. In like manner, other topographical features might be shown to present similar objections in favor of sedimentation or an accumulation in place, but this will suffice to illustrate that megascopic investigation alone

is inadequate for reaching substantial conclusions, and therefore it becomes necessary to seek some other means of attack, such as the microscopical study of the material itself. It is evident that the anatomy of coal and peat must be determined before the process whereby they have been formed can be demonstrated clearly.

Studies of the formation of peat, and consequently coal, may be divided into 3 main classes: (1) topographical,[2] (2) ecological, and (3) anatomical.

Topographical features have been considered sufficiently to show that they, especially in relation to coal, are not entirely reliable as a final factor in determining the origin of all classes of organic deposits. The ecology of peat forming plants likewise is limited in its application, and accordingly will receive only a brief consideration, for two reasons. In the first place, literature is quite complete in its descriptions of the usual zones of growth in swamps, including careful enumerations of all species of plants characteristic of them (**2, 6, 10, 11**). In the second place, these plants as such enter but little into the formation of the major part of our peat deposits. Even in swamps only a very small proportion (4–6 per cent) has been derived directly from this flora growing *in situ*. As will be shown later, by far the greater portion of our peat deposits represents a sedimentation of macerated plant material in open bodies of water, and as such is not dependent upon any one zone of growth, but rather upon all indiscriminately. For this reason, the two first named branches of the discussion will not be considered further, except for an occasional reference in connection with the microscopical studies of several characteristic peat beds.

Before discussing any special bog, however, it may be well to introduce a brief description of allocthonous and autocthonous peats as they appear under the microscope. It is possible, of course, to discern with reasonable certainty the methods by which any of our present peat deposits have been accumulated, since one has but to choose his material from clearly defined areas; that is, samples selected from modern lakes present detritus which

[2] FORSAITH, C. C., A report on some allocthonous peat deposits of Florida. Part I: Topographical. BOT. GAZ. **62**:32–52. 1916.

has been deposited in open water, while the very upper stratum of swamps is as equally typical of a cumulative origin in place. A microscopical examination of preparations from open lake deposits shows much minute material, both organic and inorganic. The inorganic constituent may be quite variable, depending upon the character of the surrounding country. If the shores and bottom of the pond are of a sandy nature, and the environment much broken by hills or mountains, a condition favorable for rapidly flowing streams, much sand may be found. On the other hand, if the land is quite level and densely forested, the buoyancy of inflowing streams is much reduced, and the inwash from the shores does not carry any large amount of inorganic material on account of a turflike protection. Consequently, the peat found in such regions will be more or less free from earthy inclusions. This difference in the mineral content of peat in rugged and level tracts is significant, and may throw light upon the topography of coal beds during the period of deposition. All available evidence indicates that the external characters of coal beds were very similar to those just mentioned, inasmuch as the land was flat and heavily forested.

Other inclusions found in peat are the calcareous remains of *Chara*, limy silts, diatomaceous tests, and the shells of mollusks. In addition to these mineral substances, which are small in amount, there occurs the more strictly organic material, derived from more or less macerated portions of plants and minute organisms of sedimentary origin. Some of the most conspicuous of these elements, as well as the most important from the scientific standpoint, are pollen grains of the Abietineae and catkin-bearing angiosperms, and spores from ferns, fungi, etc., representing bodies quite analogous to the microspores and megaspores so habitually found in coal. It is especially important to note that normally autocthonous peats do not show the characteristic spore content so universally found in open water formations. In addition to this microspore material, one finds upon an examination of lacustrine samples a rather large volume of amorphous material. Imbedded in the flocculent matter, there appear ingredients the form of which is more intact, such as woody and herbaceous plant fragments, idioblasts from water lily stems, strips of cutinized epidermis, etc.

Besides these plant remains there are certain animal derivatives characteristic of allocthonous peat; for example, ejecta from fish and small aquatic animals, often containing pollen, diatoms, and bacteria; chitinized portions of insects; spicules from fresh water sponges; infusorial bodies; and shells of mollusks and protozoans.

In contrast to the usual inclusions in allocthonous peat (pollen, diatoms, spicules, idioblasts, etc.) there is the strictly autocthonous peat composed of more or less disorganized plant débris. A superficial examination of this material shows a light brown fibrous or dark brown granular texture, depending upon whether or not the included plants are herbaceous or woody. If the substance is more completely decayed, owing to prolonged maceration and the action of fungal enzymes (unhindered by a constant water covering as is true of allocthonous layers), the fallen plants may become so structureless that they resemble humus rather than peat. Under the microscope this form appears quite homogeneous in contrast to the more fibrous and less decayed *in situ* peats, but seldom do the distinguishing features of lacustrine peat appear, only a tangled mass of roots, stems, and leaves in all stages of decay.

Thus it is apparent that there are two distinct types of peat, presenting structures each peculiar to itself, dependent upon the mode of deposition. If a specimen of coal, therefore, can be shown to present a structure analogous to either of these two more recent formations, it is but natural to assume that its composition is due to similar processes of deposition. Strangely enough, this has not been the usual mode of reasoning. Although it is authoritatively asserted that by far the greater number of the peat deposits in the United States are allocthonous in origin, a diametrically opposite view is maintained in respect to the genesis of coal beds. Consequently it will be the object of this paper to show (1) that these two types of peat are microscopically distinct; (2) that some of the bogs (especially swamps) are not, as is usually believed, of *in situ* derivation, but filled lakes in which the peat mainly represents the lacustrine or open water phase; and (3) that coals in general show clearly the organization of allocthonous peat.

The methods used in carrying out these investigations were as follows: Samples of different types of peat were carefully chosen

from localities throughout a wide range extending from eastern Canada to Florida. The numerous deposits were so selected that all stages in the formation of peat beds were inspected, including large and small deep lakes with sandy shores; filled lakes where the zones of growth have entirely covered the former body of water with a layer of accumulated vegetation; large and small shallow lakes; and swamps and river estuaries. At every station a vertical series of samples was secured at 1 ft. intervals. The probings were made over a sufficient area to allow an estimation of the depth and extent of each deposit. The specimens were obtained by the use of a probe devised by DAVIS (3), and stored in cloth sacks. A careful record was made concerning the topography of the region, the gross character of the material, and the depth from which it was taken. These specimens were later studied microscopically in order to determine the correlation between the grosser structures and the minute anatomy in respect to the mode of deposition.

Turning to the more detailed consideration of the several progressive steps in bog formation, Lake Weir in Florida may be considered as an example of the first stage. Sandy shores surround this body of water, and probings show that there are no accumulations of organic detritus nearer than 100 yards off shore; while beyond this there appears a quite extensive stratum of lacustrine peat. A gross examination of the material showed a consistent homogeneous mass, the grayish color of which is due to a calcareous silt. In addition, there appears a very large amount of diatomaceous and limy remains of extinct plants and water animals. The more peatlike content manifests itself as pollen of abietineous and amentiferous derivation, amorphous matter, root fragments (the stigmarioid rootlets so characteristic of certain samples of coal), and herbaceous and ligneous elements from the higher plants.

Attention may now be directed to the more organic peats in order to establish their relation to coal more definitely. In the first place, I shall consider two forms, the one modern and the other ancient, the origin of which is undoubted, namely, lake peat and cannel coal. Samples of lacustrine "muck" were found in the centers of Lakes Newman, Orange, Griffin, Harris, Apopka, Eustis, and many others in Florida, New Hampshire, Massa-

chusetts, and eastern Canada. In general, the samples obtained by probings were deep brown and plastic. They were fine and uniform in texture, without large or fibrous inclusions. As the topographical features around Lake Harris, in Florida, dispel any doubt as to the allocthonous genesis of the stratum there found, preparations from it will be discussed in detail. Fig. 1 represents a sample taken from near the top, and a careful study of it shows clearly pollen grains imbedded in an amorphous mass of drifted and windblown floatsam, ejecta from water animals, etc. A small spore may also be observed. Sponge spicules and idioblasts from water lily stems likewise appear.

A deposit very similar to the one just described was found in Lake Dot, a small dumb-bell shaped body of water near Eustis. This lake is very interesting as an example of those deep bowl-like depressions, known as "lime sinks" (12), which are caused by a subterranean solution of the underlying limestone, so that the roof, becoming too thin to support its own weight, falls. Fig. 2 illustrates a section 3 feet from the top of a 9 ft. layer, and the characters pictured were found by a study of the entire series to be uniform throughout. It will be seen that this sample presents the usual structureless material, ejecta, idioblasts, and pollen. In fact, such structures as are usually encountered in lake "mud," but absent from autocthonous deposits.

The central layers of the peat in Lake Eustis furnished the material shown in fig. 3, which shows several diatoms of the *Stauronesis* and *Navicula* type, in addition to spicules from decayed fresh water sponges. The section also shows 3 specimens of the amoeboid *Arcella*. Other features already found to be characteristic of lake precipitations are idioblasts, pollen, etc. Fig. 4 represents a sample of peat much like those just considered, except that there are more plant fragments. The section from which this sample was taken depicts the type of peat found in Lake Orange a mile off shore, and the topography of the region, as well as the microscopical structure of the material, shows it to be of undoubted lacustrine origin. Although many other deposits throughout a wide range were studied, these 4 illustrations are sufficiently characteristic of all deep water formations, as well as the lower layers of bogs, to

demonstrate the distinctive features of all such lacustrine accumulations.

It is at least significant that cannel coal, which is universally considered to be of open water derivation, should manifest the same structures so generally found in lake peat. Both of these fuels, when microscopically examined, present a considerable spore content. In fact some of the cannels (especially tasmanite) as well as their modern homologues were found by JEFFREY (7) as a result of studies of a great number of carefully prepared sections to be almost entirely sporiferous. A somewhat clearer idea of this correlation may be obtained by a reference to fig. 5, which exhibits the organization of Kentucky cannel coal as it appears under the microscope. Scattered throughout the section there may be seen numerous light bodies, which are the flattened spores of vascular cryptogams (homologues of the spores and pollen shown in the illustrations of allocthonous peat). The long grayish bands are indicative of metamorphosed bits of wood. Separating these spores and lignitoid fragments are dense black masses of amorphous organic material. In comparing this illustration with fig. 4, it is apparent that both the recent and prehistoric deposits show a striking anatomical similarity. Thus it would appear that whenever a peat and coal show like organization it has been brought about by the same methods of deposition. It will consequently be assumed in the sequel that similarity of structure, as between peat and coal, implies an identical mode of origin.

Since the next stage in peat formation is illustrated by those areas where the zones of growth are starting to form around the shores, an example will be given. The shores around Lake Orange in Florida show this fringe of water plants quite well. The peat derived from this vegetation consists of fragments of amphibious angiosperms, among which rushes, water lilies, pondweeds, etc., are common. Although the plants which are found in this zone vary systematically in wide ranges, the peat formed by them is uniform. Since the parts of plants which enter into the composition of this peat are very minute, specific differences are not of importance, and consequently any enumeration of them is omitted. Although it is allocthonous, samples taken from this deposit are of

a light brown spongy nature. Fig. 6 presents a preparation from this material, and it will be seen that there are many parts of plants in a perfect state of preservation due to a perpetual covering by water. Other structures more definitely related to lake peats are idioblasts and parts of insects.

Florida is especially favorable for studies of this type of peat, owing to an abundance of "saw grass" (*Cladium*) marshes about Lakes Harris, Griffin, Apopka, and in fact generally throughout the Everglades. If one were to rely solely upon a superficial examination of this material, representative of the later stage in herbaceous marsh development, he would reach the conclusion that these deposits have been formed by a growth of herbaceous plants *in situ*. A detailed examination of samples from different depths, however, shows that this is not a correct interpretation. On the contrary, these paludal accumulations, with the exception of the uppermost layers, are obviously allocthonous. A sample secured 3 ft. from the bottom of the marsh bordering Lake Harris is pictured in fig. 7. This illustrates conclusively that the material has not been formed *in situ* by a gradual amassing of fallen plants, but rather by a floating together of drifted and wind-blown matter similar to that characteristic of deeper lake deposits, as indicated in figs. 1–4. The usual structures found in lacustrine peat, shown in fig. 7, are pollen grains, idioblasts, plant fragments, ejecta, and formless drift. Although no sponge spicules and diatoms appear in the illustration, it should be added that they are of common occurrence. This kind of peat is usually encountered in the lower four-fifths of "saw grass" marshes, as determined by vertical series of samples. The upper layer, nevertheless, has been accumulated in a different manner, since the microscope reveals only the tangled remains of fallen herbaceous plants, and the structures usually found in open water deposits are conspicuously absent. It is probable that this distinct change in the process of deposition was accomplished at some time when the material had so collected that the mass was above water, for a part of the year at least, so that plants perishing in place were allowed to become more or less reduced owing to exposure, and not permitted to float away and become precipitated among the usual sedimentary detritus. It is

apparent that this peat, a very common type in the Florida lake region, is not, as is ordinarily supposed, of autocthonous derivation; but, on the contrary, is almost entirely allocthonous. It would thus appear that the development of this form of deposit is in accord with the general principle of sedimentation for peat and coal in general.

One of the most interesting phenomena in relation to the formation of peat beds is that illustrated by completely or nearly filled lakes. As has previously been stated, there are several distinct steps in the process, beginning with an open lake surrounded by sandy shores, of which condition Lake Weir served as an example. The next is seen where the herbaceous zone has crept in from the shores, as illustrated by the "saw grass" marshes around Lake Harris. The third stage is the conversion into a bog as a result of drainage and the introduction of woody plants, which marks the end of the process. Consequently, the value of this last formation as a peat builder has in all probability been overestimated, since the detritus formed by it directly comprises but a small proportion of the whole, especially in the more tropical areas where perpetual exposure is favorable to destructive activities. A series of samples from one of these beds, if studied only superficially, shows two distinct types of material: the upper layer consisting of a tangled mat of fallen plants and roots, and the lower layer consisting of a somewhat homogeneous mass of minute débris. This older plastic material is believed by many writers to have resulted from a more prolonged period of reduction of detritus similar to that found in the upper part of the bed. This conclusion, derived from gross examinations alone, is nevertheless misleading, and on this account it seems advisable to refer to microscopic investigations. A bog near Leesburg will serve to illustrate. A topographical study of this area showed that it was at one time either an arm of Lake Harris or a connecting link between Lake Harris and Lake Griffin. At the present time the filling processes have reached completion, and the entire area is now dry land bearing a dense forest of coniferous and deciduous trees. Probings in several localities showed about 15 ft. of peat resting upon a stratum of bluish clay (the initial stage of "fire clays" usually found under coal beds). Above this lamina

there occurs a layer of fine black peat, similar in form to that now found in the open lakes. This is the "completely decomposed stratum" just mentioned. Fig. 8 shows a microscopical section taken 3 ft. from the bottom, and further studies of the figure reveal in addition to the usual structureless drift, woody and herbaceous plant fragments, pollen, spores, spicules, etc., all of which have been preserved from decay by a perpetual water cover and natural acidity. It is manifest that this material does not represent the final stage in the reduction of fibrous peat, but rather an accumulation of drifted and wind-blown matter which was precipitated at some time when lacustrine conditions prevailed. This relation is still more obvious when it is demonstrated to be similar in the most exacting detail to that already shown to be characteristic of present lake deposits and illustrated in figs. 1–4. Above this dark amorphous mass, there appears a light brown fibrous material like that already described for the "saw grass" marshes. Fig. 9 shows photomicrographically the true nature of the substance. In addition to root fragments across the illustration, there appears the usual disorganized material, pollen, spores, and spicules, all of which indicate an allocthonous origin. An even clearer idea of the lacustrine nature of this peat may be obtained by reference to fig. 10. In the upper right hand corner of the figure is a much distorted fragment from some herbaceous plant, amorphous matter, and ejecta. The most noticeable, as well as one of the most significant, features, however, is a fern sporangium and a sponge spicule which could not occur in juxtaposition except through sedimentation in open water.

Microscopical studies of this vertical series indicate that about the time when the last of the herbaceous material had been deposited, the accumulated mass was above water level, thus furnishing a somewhat drained soil for the growth of more woody plants. Consequently the amphibious species were forced to move on, and their place was taken by woody trees and shrubs. This later growth in turn built up a layer of autocthonous peat which shows the remains of comminuted material, but none of the structures so characteristic of the allocthonous layers below. In securing these samples some difficulty was experienced in forcing the probing

instrument through the tangled cypress logs and roots, which resisted decay more than the dicotyledonous trunks and settled through the oozelike mass below. Although these structures are not general in peat beds, they are by no means uncommon, and in all probability have homologues in coal beds, a fact which has led to the idea that they are indicative of an autocthonous origin for coal. Like all other megascopic evidence, however, the interpretation of these structures is open to question, since conditions like those in the bog just mentioned might have prevailed in the past, and fallen logs settled through the unresisting lake peats below the growing stratum.

It must be apparent that allocthonous peats in this region are vastly predominant over those laid down in place, which is quite in accord with the statement of DAVIS (15), namely, "the fact [is] that at the present time peat deposits of this type [lacustrine] are numerically more important than any other in regions where peat formation is common." The even more pronounced dearth of accumulated generations of plants *in situ* in this region than is usual in the more northern bogs is without doubt due to climatic conditions, which in warmer localities are more favorable to the destructive action of fungi. Since Florida now has a climate similar to that generally ascribed to the coal-forming periods, it seems logical to infer that strictly *in situ* depositions were equally scarce during ancient times. This phenomenon is well illustrated, in fact, by several swamps in Florida where the sandy floors do not present any quantity of autocthonous peat. For example, in an extensive swamp near Gainesville there appears a dense growth of cypress, pine, and dicotyledonous trees growing up through an almost impenetrable tangle of fallen trunks in all stages of decay. One can hardly imagine a more favorable location for the accumulation of autocthonous peat, but in spite of this, an examination showed but a few inches of humus-like substance derived from comminuted plants.

Although these Florida peats present conditions of environment more like those which formerly prevailed over the entire earth, some attention should be paid to the more northern deposits, as in all discussions of the problem of coal formation, they are mentioned

as the counterpart of "autocthonous" coals. A bog of this type near Fresh Pond in Cambridge, Massachusetts, will serve as an example. Samples were taken in the usual way throughout the entire 30 ft. of the deposit. With the exception of the extreme upper stratum, the samples present a uniformly brown plastic consistency, similar to that found in open lakes. A subsequent examination revealed that the lower 28 ft. were singularly constant in respect to structure, and composed of the usual amorphous material in which were imbedded pine, larch, and amentiferous pollen; spores and sporangia of ferns and fungi; vast quantities of diatomaceous tests and sponge spicules; minute fragments of roots, stems, and leaves of the higher plants; and animal derivatives such as insect parts, water organisms, and ejecta from aquatic creatures. All of these structures are very similar to those shown in figs. 1–4, with the exception of unimportant northern and southern floral differences. There also appeared in this layer some indications of carbonized woody fragments which had been washed into this former lake from a region swept by a prehistoric forest fire, and there deposited. This fact is significant, since such structures are of common occurrence in coal sections in juxtaposition with unburned material, precluding the possibility of deposition *in situ* (7). These inclusions, together with the wonderfully perfect preservation of the débris even in the very lowest strata, dispel any doubt that it is of an allocthonous origin, and not one brought about by an accumulation of fallen plants which later decay to a structureless mass (the "completely decomposed peat" of many writers).

The next swamp to be considered is a so-called "*Sphagnum* bog" in Auburn, New Hampshire. This deposit is found in depressions between long irregular ridges, in the form of the letter Y, about 2 miles in length and half a mile in breadth. In the central portion there occurs a chain of more or less circular ponds surrounded by the usual zones of growth, the inner zone of which is distinctly sphagnoid. A series of tests showed a layer of lacustrine peat about 27 ft. in depth. Above this and near the ponds there is a floating "mat" of *Sphagnum* and other plants about 1 ft. in thickness. Back from the shores there appears a tangled mass of roots and fallen plants above this mossy stratum. Excepting

this thin autocthonous deposit, all the microscopical sections showed "muck" formed by sedimentation similar in composition to that already described for the bog in Cambridge and in more southern regions. Material from several lakes in eastern Canada was minutely examined, and structural evidences of allocthonous peat were found to correspond so closely to those in the United States that any further discussion of them is unnecessary.

Since the shallow or intermittent lakes are not favorable to an accumulation of any appreciable amount of peat, owing to periods of drought and constant agitation by waves, they will receive but brief consideration. Many of this type were observed around Zellwood and Lake Tohopikaliga in Florida, and several small ponds in New Hampshire. In all there was little peat, especially in the south, where there are distinct wet and dry seasons favoring the destruction of whatever material may have gathered during periods of inundation.

There now remains only one distinct kind of fresh water peat to be considered, namely, that found in river estuaries. One example at Pablo Creek near Jacksonville, Florida, will be discussed. Topography indicates that the space between two elevations was once occupied by a river a mile in breadth. This broad stream gradually filled its bed with organic material until the mass had sufficiently accumulated so that the entire depression was covered by an allocthonous layer of peat, excepting the channel of a meandering river. Tests showed a uniform deposit about 12 ft. in depth. Fig. 11 shows the microscopical character of the material, and studies of the entire vertical series indicate a general uniformity. It will be observed that coniferous woody fragments are very abundant, as indicated by a uniseriate ray in tangential section and an absence of vessels. There are also present the more evident lacustrine derivatives, such as a broken sponge spicule, pollen, amorphous material, and a group of spores. These structures indicate that the deposit, like those found in the now open and filled lakes, has arisen by similar processes of deposition in open water.

The peat illustrated in fig. 11 is especially favorable for comparison with thin sections of the more lignitoid coals (bituminous)

as shown in fig. 12. This pictures a microscopical section of bituminous coal from Perry County, Ohio. Crinkled bands of compressed wood are especially distinct in the upper and left hand parts of the figure, and evidences of an allocthonous origin for this coal are present as flattened spores, appearing as light bodies imbedded in a dense black amorphous matrix. Both the lignitic coal and the woody peat show a large and varied amount of xyloid material in addition to the more obviously lacustrine derivatives, such as spores, etc., which have been shown to be characteristically absent in "swamp" peats. It seems logical to conclude, therefore, that these substances so alike in structure must have arisen by a similar process, and for this reason any coal showing a high spore content should be considered as having been formed in the same manner which obtains in present deposits; that is, in open water, and not by an accumulation of fallen plants *in situ*, as stated by the older geological publications upon this subject (**1, 5, 11, 14, 15**).

Since it is generally admitted that natural factors, such as climate and topography, have been instrumental in the formation of our coal beds, it is obvious that a correlation between past and present phenomena is essential for a precise understanding of ancient and modern peat deposits. In regard to climate, competent investigators are quite agreed that there was a somewhat warm and humid atmosphere over the earth during the Carboniferous and later peat-forming epochs. This supposition is corroborated by observations of fossil remains characteristic of the different periods which show a usual lack of annual rings. The nearest parallel to these climatic conditions of growth is now found to prevail only in semitropical and tropical regions. Because of the importance of these considerations, the writer has chosen many of his illustrations from the semitropical peat deposits of Florida, since they present a closer analogy to coal beds than do the more northern organic strata. It has already been pointed out that there is a surprising lack of autocthonous accumulations in this locality in contrast to an abundance of lacustrine deposits. This dearth of land-formed peat is clearly dependent upon the rapid decay of exposed land plants in zones without a winter season. Accelerated disintegration under these conditions is sufficiently pronounced to

prevent any appreciable amassing of vegetable matter other than that protected by a continuous covering of water. Studies of coal sections indicate that similar processes were as effectual in the past, for there is a universal deficiency of strictly autocthonous coals as revealed by the microscope (8).

Although this prehistoric lycopod flora, growing on the low-lying shores of ancient lakes, was different from that which now enters into the formation of peat, the process by which fragmentary material was derived from this cryptogamic growth was undoubtedly the same. JEFFREY (8), as a result of his studies of sections of coal from all over the world, has found that all categories from cannel to anthracite show spores of arboreal cryptogams in varying amounts, just as the peats of today show different proportions of pollen. In addition to the many spores carried into these carboniferous lagoons by the wind, sluggish streams brought microscopic débris in all stages of decay. This detritus was precipitated, and the allocthonous peat was augmented by an age-long process of sedimentation. A continuance of such conditions finally raised the mass above water when the bordering forests, in zones like the present, marched toward the center and established swamps. This bog-loving flora did not, however, add in any appreciable degree to the substance already accumulated, owing to their rapid decay in a fallen state, both as a result of a warm climate and its less resistant organization. In fact, all microscopical evidence points to a condition very similar to that already described for recent peat deposits, the major part of which is quite conclusively shown to be of drifted derivation.

Another fact which supports the allocthonous theory of coal formation, is the vast predominance of lacustrine peat over *in situ* deposits at the present time. This fact has been well illustrated by the several strata already mentioned, such as those found in open lakes, filled lakes (swamps), and river estuaries. The phenomena are especially noticeable in warm localities, where autocthonous peats are quantitatively almost negligible.

Thus it is apparent that the mode of peat formation, as illustrated by its anatomical structure and topographical features, shows strikingly similar analogies in coal. It must be assumed, therefore,

that the major part of our coal beds, like peat deposits, does not represent a gradual accumulation of successive generations of fallen plants in swamps, but rather a long continued and peaceful sedimentation of wind-blown and drifted plant fragments and minute organisms in the depths of open bodies of water.

In conclusion, the writer wishes to express his sincere thanks to the Committee of Sheldon Traveling Fellowships of Harvard University for the granting of a fellowship, the stipend of which has made possible these investigations; to Professor R. THAXTER of Harvard University for samples of peat; and to Professor E. C. JEFFREY for advice during the course of the work.

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LITERATURE CITED

1. CHAMBERLIN, T. C., and SALISBURY, R. D., Geology. 3 vols. New York. 1904–1906.
2. DAVIS, C. A., The ecology of peat-forming plants in Michigan. Report Mich. State Geol. Survey. Lansing. 1907.
3. ———, The uses of peat for fuel and other purposes. U.S. Bur. Mines, Bull. no. 16, pp. 214. Washington. 1911.
4. FORDYCE, W., A history of coal. London. 1860.
5. GEIKIE, A., Textbook of geology. London. 1893.
6. HARPER, R. M., Preliminary report on the peat deposits of Florida. Florida State Geol. Survey, 3d Ann. Rept. pp. 206–375. Tallahassee. 1911.
7. JEFFREY, E. C., On the composition and qualities of coal. Economic Geol. **9**:730–742. 1914
8. ———, The mode of origin of coal. Jour. Geol. **23**:218–230. 1915.
9. ———, The nature of some of the supposed algal coals. Proc. Amer. Acad. **46**:273–290. 1910.
10. POTONIÉ, H., Die recenten Kaustobiolithe und ihre Langerstätten. Berlin. 1908.
11. RENAULT, B., Sur quelques Microorganismes des combustibles fossiles. Extr. Bull. Soc. Ind. Minérale **14**:1–460. 1900.
12. SANFORD, S., and MATSON, G. C., Geology and ground waters of Florida. U.S. Geol. Survey, Water Supply Paper no. 319, pp. 50. 1913.
13. SCOTT, D. H., Studies in fossil botany. London. 1900.
14. STEVENSON, J. J., Formation of coal beds. Proc. Amer. Phil. Soc. **50**:1–116, 519–643; **51**:423–553; **52**:31–162. 1911–1913.
15. WHITE, D., DAVIS, C. A., and THIESSON, R., The origin of coal. U.S. Bur. Mines, Bull. no. 38, pp. 390. 1913.

EXPLANATION OF PLATES X AND XI

PLATE X

FIG. 1.—Composition of allocthonous peat from Lake Harris in Florida; grayish background represents amorphous mass of organic derivation in which there are imbedded abietineous pollen grains, appearing as oblong bodies with 2 laterally attached air sacs; an idioblast from a water lily is pictured in lower left hand corner as a series of spinelike appendages radiating from a common center; other inclusions are dense black ejecta from amphibious animals, and spindle-shaped fresh water sponge spicules.

FIG. 2.—Sample of similar constituents from Lake Dot.

FIG. 3.—Material from Lake Eustis in which there are idioblasts, pollen, spores, spicules, ejecta, and structureless matter; in upper left hand corner there appear 3 specimens of the amoeboid *Arcella;* diatoms of *Stauronesis* and *Navicula* type occur in upper and lower portions of figure respectively.

FIG. 4.—Magnified view of peat from Lake Orange 1 mile off shore; besides characteristic structures, strips of epidermis and an herbaceous plant fragment appear, cells of which are still intact.

FIG. 5.—Organization of Kentucky cannel coal, ×250; scattered throughout the section are numerous light bodies, flattened spores of vascular cryptogams (homologues of the spores and pollen shown in the illustrations of allocthonous peat); the long grayish bands are indicative of metamorphosed bits of wood; separating spores and lignitoid fragments are dense black masses of organic matter.

FIG. 6.—An herbaceous peat from Lake Orange near shore; fragments of roots, etc., manifest cell structure clearly; evidences of drifted material are present, as an idioblast and the mouth part of some insect.

PLATE XI

FIG. 7.—Sample of "saw grass" (*Cladium*) peat 3 ft. from bottom of a marsh bordering Lake Harris; exemplifies the usual inclusions characteristic of allocthonous peat.

FIG. 8.—Preparation of peat 3 ft. from bottom of a bog near Leesburg, Florida, in which are pollen, spores, spicules, ejecta, and other allocthonous inclusions; also woody and herbaceous fragments of plants, cells of which are still intact.

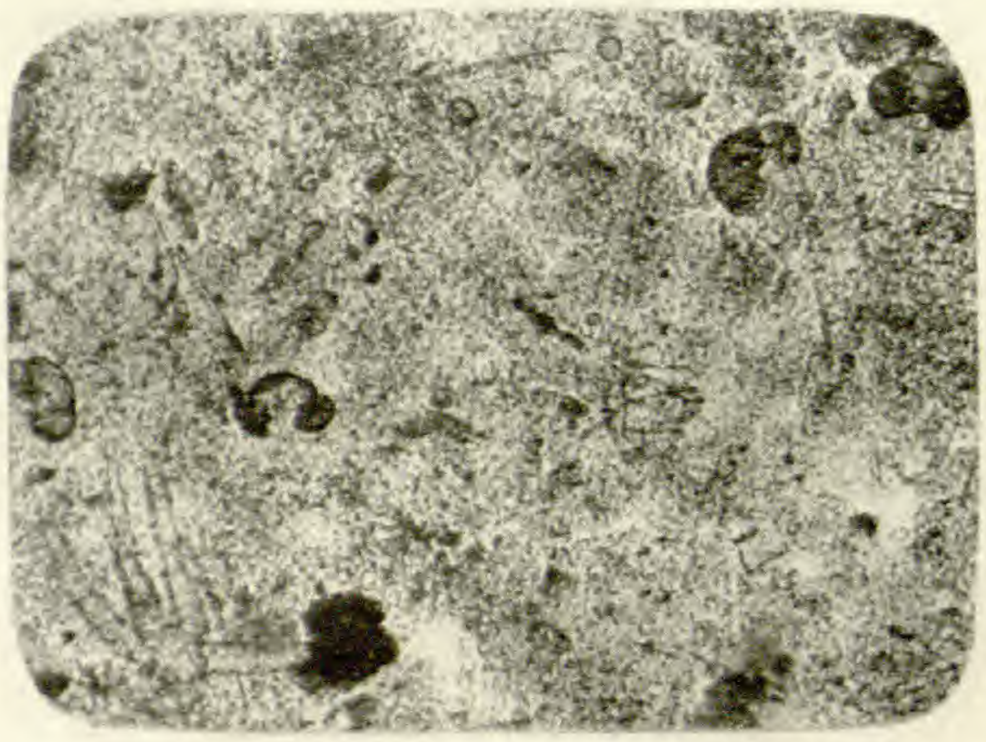
FIG. 9.—Nature of peat above the deep brown plastic layer illustrated in fig. 8; in addition to usual sedimentary matter, intact fragments of herbaceous plants may be seen.

FIG. 10.—Another sample from the same horizontal plane with a much distorted plant fragment in upper right hand corner; below and to left of this, a sponge spicule and fern sporangium may be seen in juxtaposition.

FIG. 11.—A woody peat from Pablo Creek near Jacksonville, Florida, almost entirely composed of lignitoid plant fragments; evidence of a coniferous

origin for this wood is furnished by a uniseriate ray in tangential section and an absence of vessels; indications for a sedimentary origin for this stratum are manifest as a sponge spicule, abietineous pollen, and a group of spores in upper right hand corner.

FIG. 12.—A bituminous coal from Perry County, Ohio, ×250; crinkled bands of compressed wood are especially obvious in upper and left hand parts; evidences of an allocthonous origin for this coal occur as flattened spores, appearing as light bodies imbedded in a dense black amorphous matrix.



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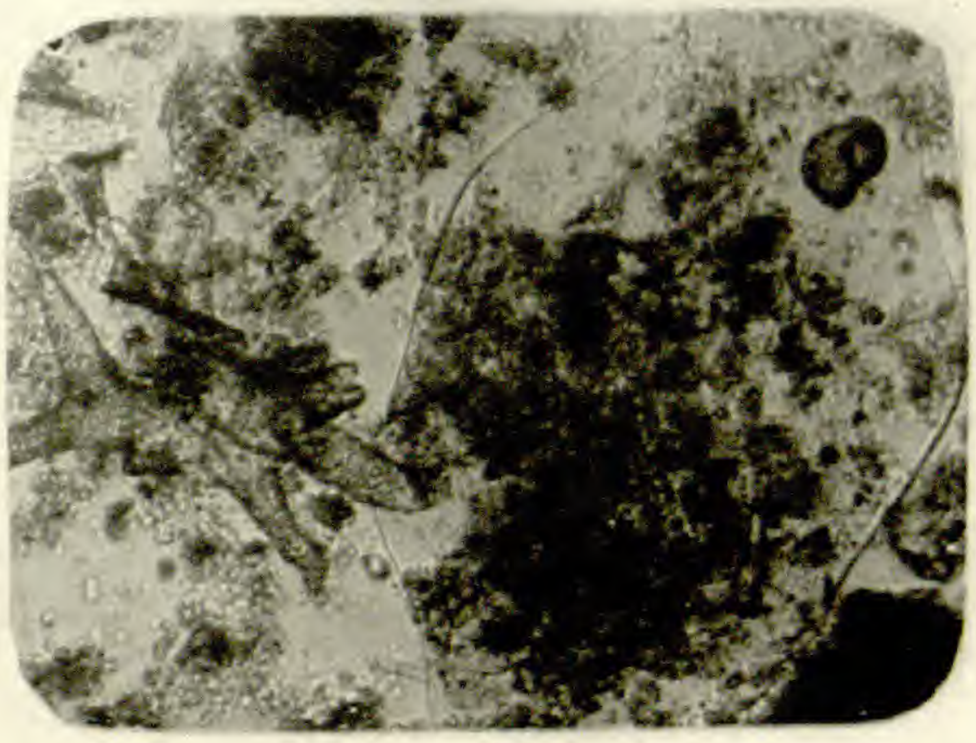

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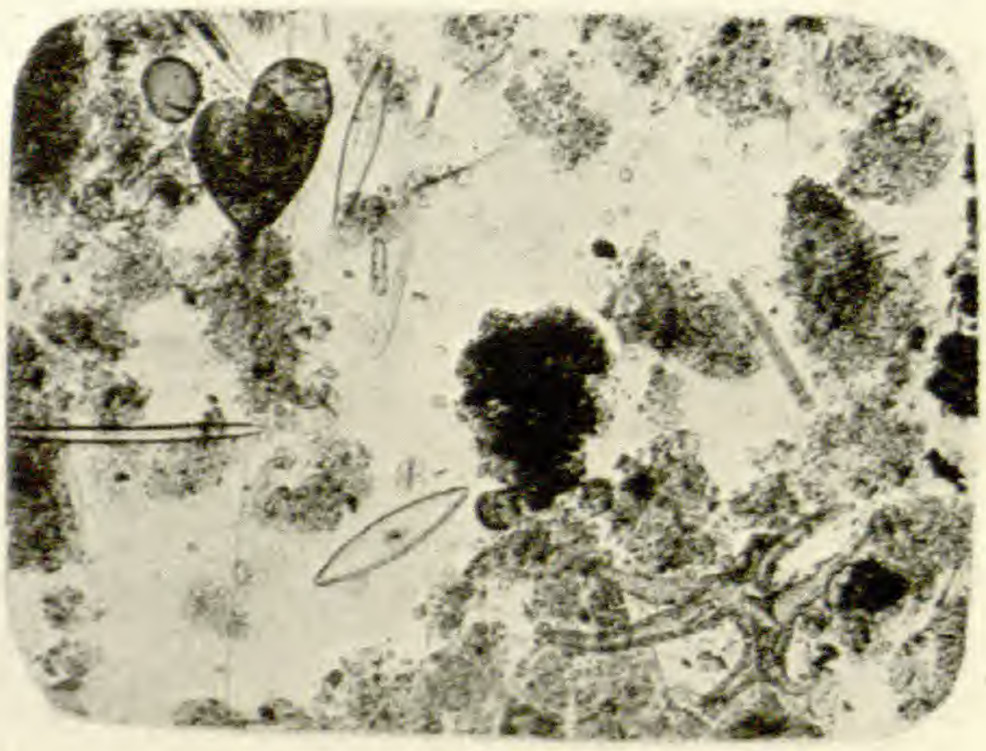

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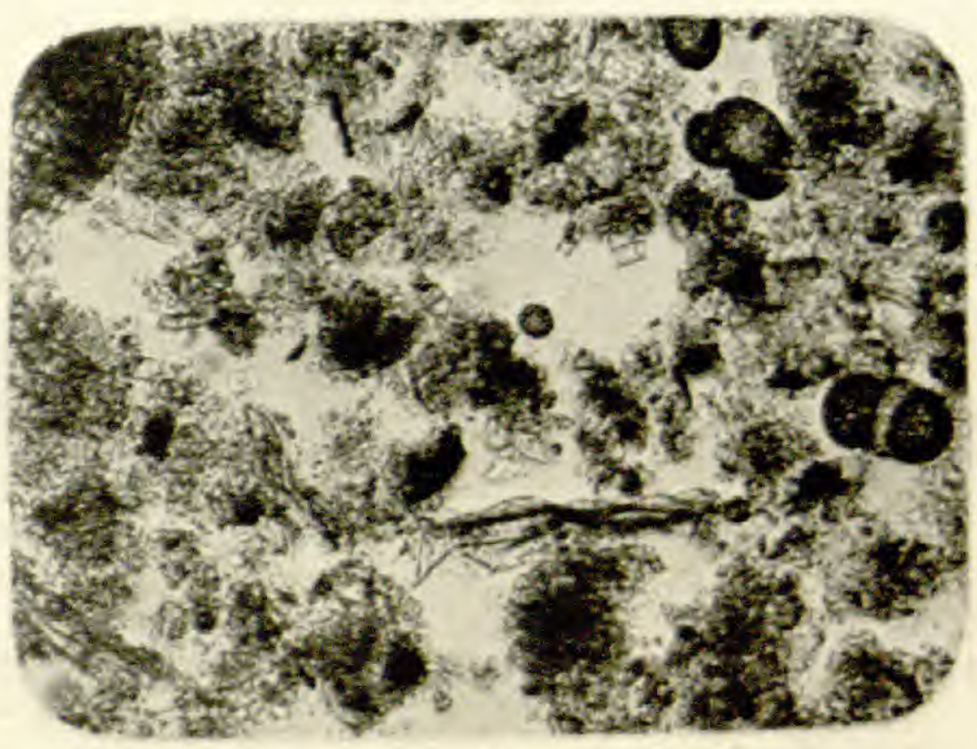

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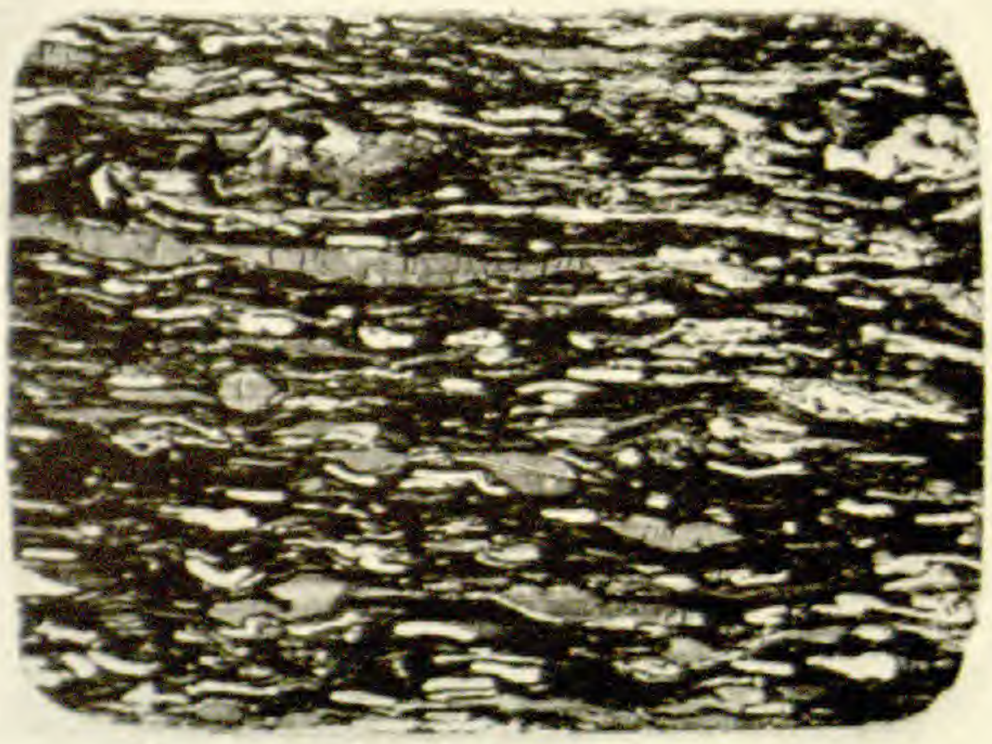

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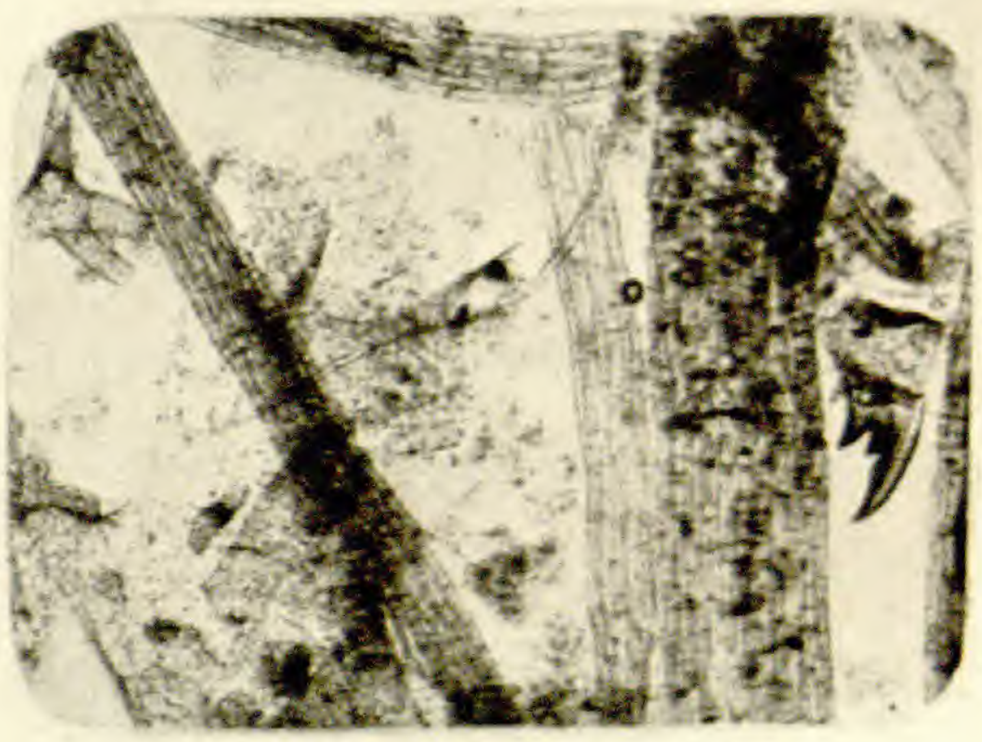

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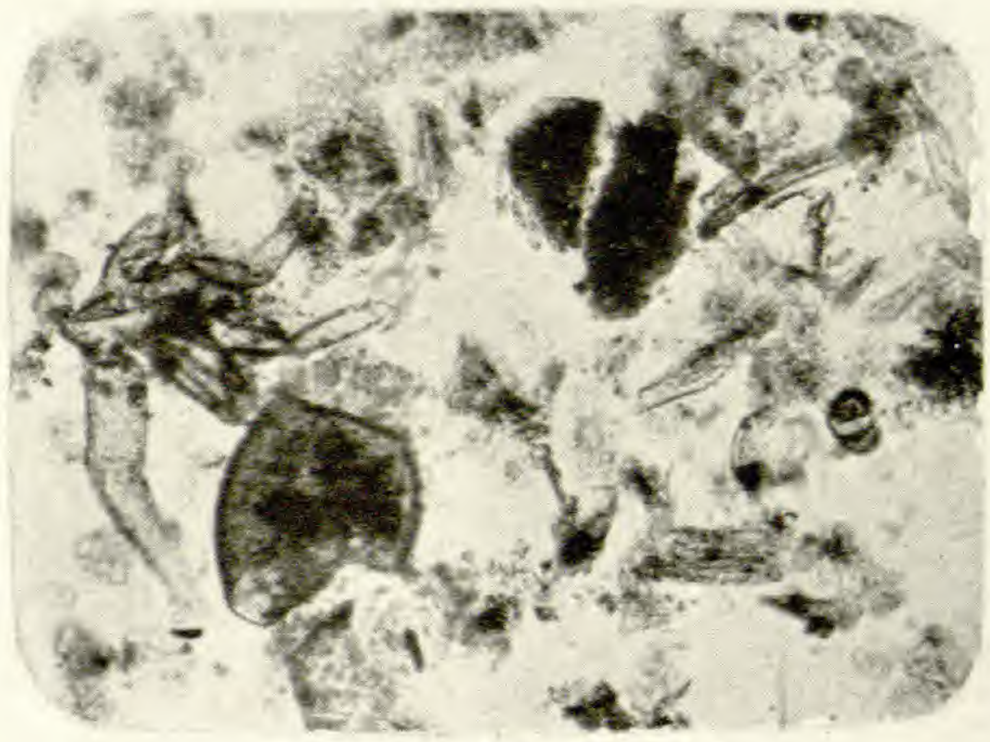
FORSAITH on PEAT



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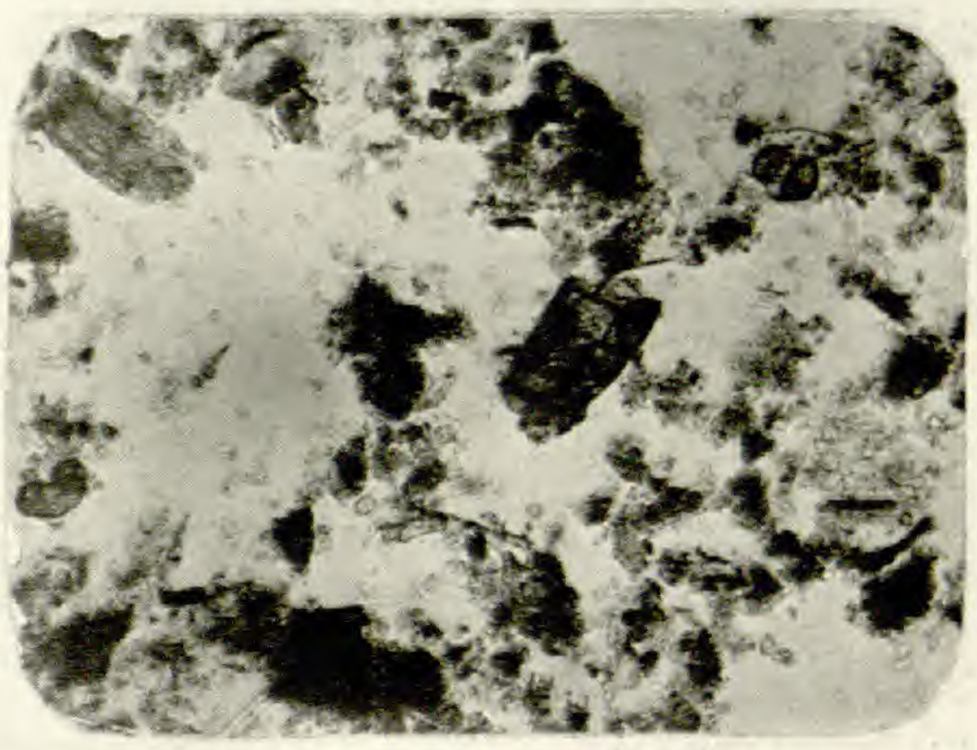

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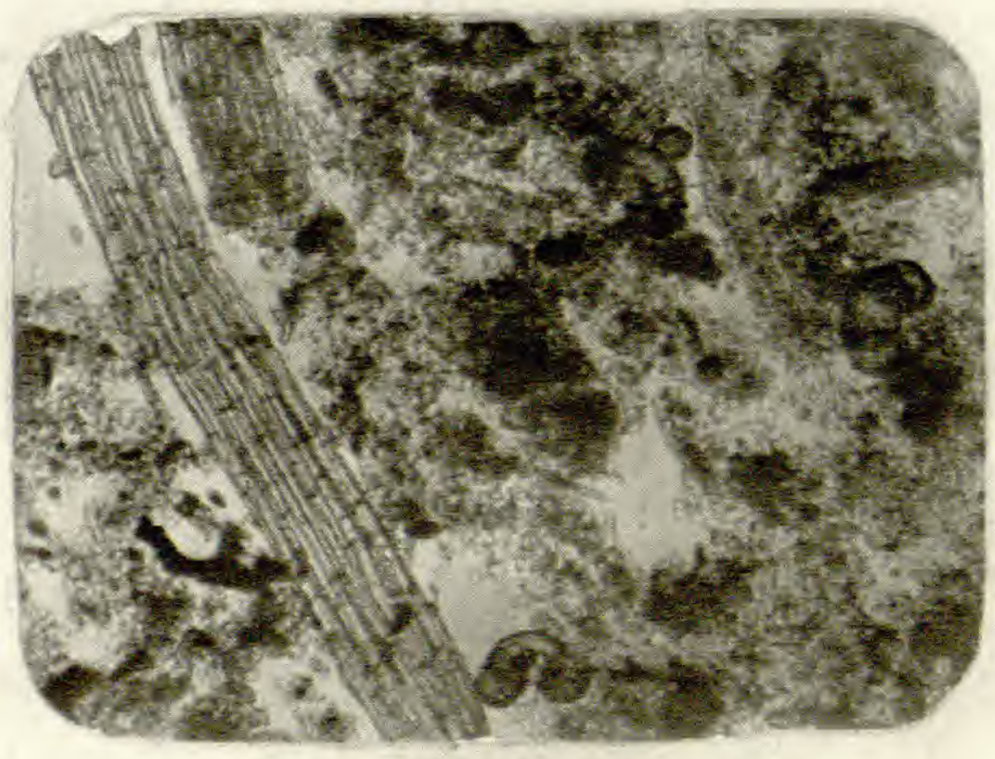

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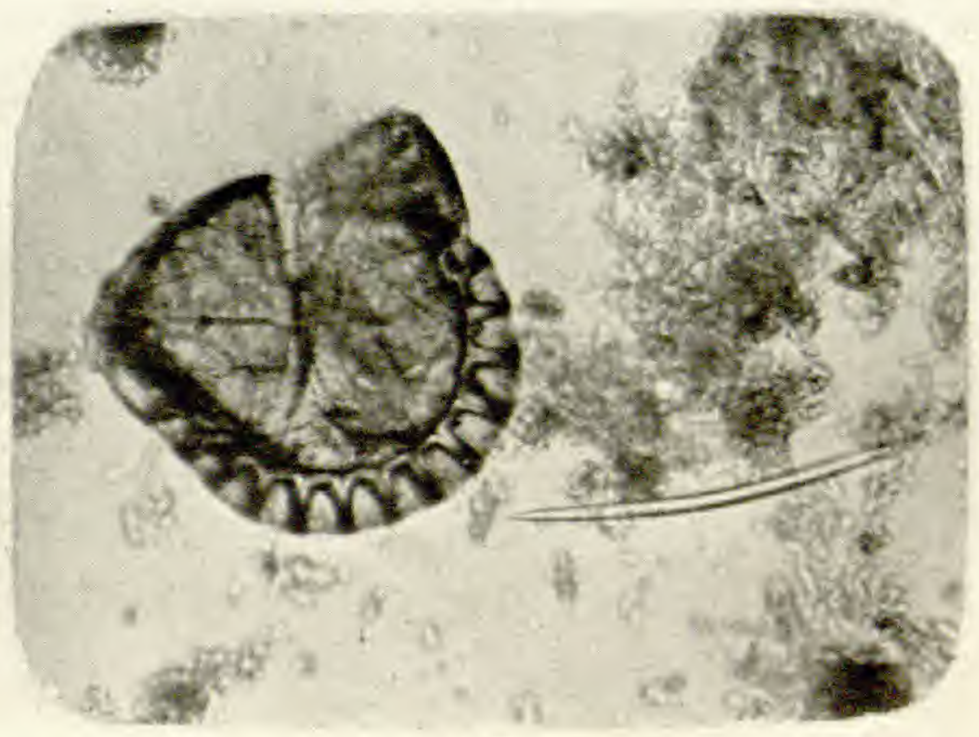

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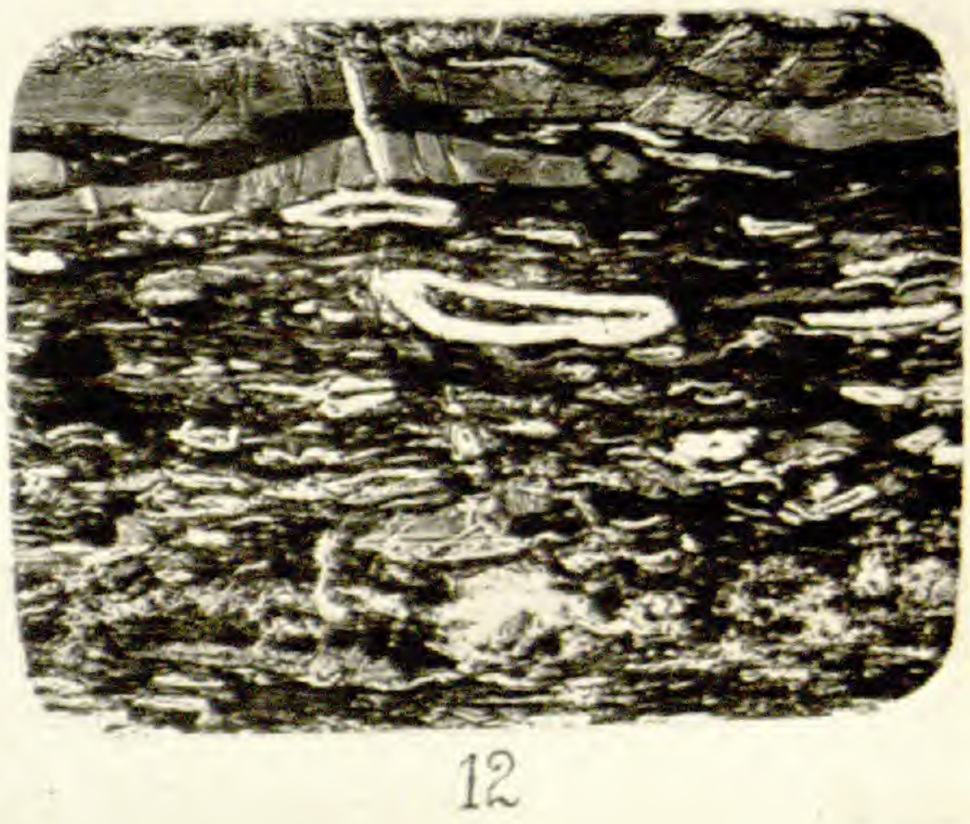


12

THE RESPONSE OF PLANTS TO ILLUMINATING GAS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 227

SARAH L. DOUBT

(WITH SIX FIGURES)

Introduction

Owing to increasing loss of plants in greenhouses, and of shade trees along city streets, it has seemed worth while to work out simple accurate methods by which gardeners, florists, and foresters may detect gas injury. This study falls into two divisions: (1) injury to greenhouse plants due to presence of gas in the air; and (2) injury to trees and bedding plants due to leakage in the soil. It is hoped that as a result of this work any florist or gardener may be able to determine readily the presence of illuminating gas in the air. The presence of gas in the soil as shown by injury to trees is more difficult to determine.

Considerable work has been done in this laboratory on the effect of illuminating gas and its various constituents upon plants. CROCKER and KNIGHT (1) showed that the buds and flowers of carnations are extremely sensitive to traces of illuminating gas in the air. Three days' exposure, 1 part in 40,000, killed young buds and prevented the opening of those which already showed the petals. The flowers were closed by 12 hours' exposure to 1 part in 80,000. One part ethylene to 1,000,000 parts of air prevented opening of the buds, and 1 part in 2,000,000 caused the flowers to close. Their work showed that ethylene is the constituent which is most toxic to plants. HARVEY and ROSE (6) showed that the relatively high toxicity of ethylene holds for many different species of plants. CROCKER, KNIGHT, and ROSE (2) found etiolated sweet-pea seedlings to be extremely sensitive to gas, and suggest the use of them as test plants for traces of gas. HARVEY (8) suggests the use of the castor bean for the same purpose.

Little is known about the effect of gas on trees. STONE (9) has made a number of observations in the field, which led him to

conclude that many trees which are seriously injured by gas finally succumb to attacks of fungi or of insects. He believes these secondary causes are often blamed for the total injury.

Rather striking formative effects have been noted by a number of writers. STONE observed abnormal tissue in the cortex of stems of Carolina poplar, proliferation tissue in the lenticels of willow, and increased root development in cuttings of the willow exposed to gas. HARVEY and ROSE (6) found that gas and ethylene cause tubercle-like growths on roots of *Catalpa* and *Ailanthus*.

Methods

The gas used in this investigation was that of the Peoples Gas Light and Coke Company of Chicago. It averages about 4 per cent ethylene and about 25 per cent carbon monoxide. The ethylene used was generated from absolute alcohol and concentrated sulphuric acid and washed in the usual way. It contained 90 per cent ethylene and 10 per cent air impurities. The carbon monoxide was generated from oxalic acid and concentrated sulphuric acid, washed and analyzed. Each of these was mixed with air to give it the same volume percentage as exists in illuminating gas, and the air-gas mixtures were checked against the illuminating gas.

Three general methods of exposing plants to gas were used: (1) the flowers only were treated with gas; (2) potted plants were sealed in wardian cases and measured amounts of gas added; and (3) root systems were treated with a slow stream of gas in the soil.

1. To determine the limit of toxicity for the flower of the calla lily (*Zantedeschia ethiopica*) the method employed by CROCKER and KNIGHT (1) upon carnations was used. A 20 liter carboy was inverted over the flower bud and a rubber stopper fitted about the petiole and made gas-tight with vaseline. Gas was forced into this 20 liter bottle through a tube inserted in a second hole in the stopper. A measured amount (800 cc. or 4 per cent) of illuminating gas was forced in, and the pinchcock closed. A control plant was put under identical conditions, except that no gas was used. When the bottles were removed 11 days later, both flowers

opened and were of normal size. The one treated with gas showed slight discoloration on the spathe. Four per cent of illuminating gas, then, slightly injures the inflorescence of the calla lily. Further results on the calla lily are given later.

2. Exposure of entire potted plants was made in two wardian cases (each of about 1000 liters capacity); one case was used for exposure to gas and the other case was used for the control plants. The plants were placed in the cases by the removal of a pane of glass which was later sealed into place with the vaseline-clay mixture used by CROCKER and KNIGHT. The plants were kept under moisture conditions as nearly optimum as possible. During the winter season the temperature varied between 15 and 20° C. with day and night. The experiments were carried on from January to July. In April the temperature became so high in the cases as to injure the plants. The cases were then given a coat of whitewash. As the temperature again rose, the cases were moved outside and partly shaded. Plants were treated for 2 days, then the cases were opened, the plants removed, and the cases aired by means of an electric fan. After watering, the plants were returned to the case and following renewal of gas were left 2 days more. They were then removed and the immediate and the after effect of the gas noted. Since the control plants were in no case injured, it is clear that the response of the other plants was due to the gas.

Types of responses

1. *Leaf fall.*—In certain concentrations of ethylene or of illuminating gas, *Mimosa*, *Lycopersicum*, *Salvia*, *Datura*, *Coleus*, and *Hibiscus* dropped their leaves after a few hours' exposure. The abscission layer was probably formed. The older the plants, the less gas was required to cause the older leaves to drop. The youngest leaves were least affected.

2. *Rigor.*—*Coleus*, *Ricinus*, *Datura*, and *Mimosa* showed rigor when subjected to large amounts of gas. *Mimosa* showed imperfect rigor, lost sensitiveness to touch, but was somewhat injured by the gas. *Coleus* was completely anesthetized, with no ill aftereffects. FITTING (5) found that heat rigor or rigor from lack of oxygen will prevent gas injury to plants.

3. *Epinasty of petioles.*—Suitable concentrations of illuminating gas and of ethylene produced epinasty in petioles or flower stalks. In *Lycopersicum* and *Salvia* this response is often so marked as to produce complete spiral coils. The petioles of *Ricinus*, *Datura*, *Coleus*, and *Hibiscus*, and the flower stalk of calla lily also showed epinasty in traces of these gases. The bending may be near the blade or the bud, as in calla lily leaf and flower; all along the petiole, as in most younger leaves; or very near the stem, as in most older petioles (figs. 1–5).

4. *Proliferation tissue in lenticels, leaf scars, etc.*—In the presence of traces of illuminating gas or of ethylene, soft spongy tissue developed in the lenticels (*Hibiscus* and *Sambucus*), at leaf scars (*Lycopersicum*), or at more or less extensive regions along the stems. In the roots of the apple and pear the abnormal tissue developed just outside the vascular cylinder, but it is not determined whether it was produced by the cortex or the pericycle. Deep longitudinal cracks developed in the bark of the stem. These appeared on the apple, pear, ash, and *Hibiscus*, and to a less degree in *Sambucus*, *Grevillea*, and cottonwood.

5. *Root tubercles.*—Traces of these gases produced tubercle-like growths on the roots of *Grevillea*, *Sambucus*, *Populus*, apple, pear, and *Hibiscus*. In the tomato similar tubercles are produced by nematodes.

Results of treatments

In the following records, plants are arranged in the order of their sensitivity to gas. All amounts of gas indicated are in parts per million of air (ppm).

Lathyrus odoratus.—With 1000, 100, 75, and 50 ppm illuminating gas, the leaves turned yellow and died. Ethylene 8 ppm caused the leaflets to fall off; 5 ppm caused the leaves to become yellow and die; 2 ppm caused death of the older leaves; and 0.1 ppm still caused noticeable injury, although less than in the other cases.

Salvia splendens.—With 25,000 ppm illuminating gas, the older leaves fell off, while the younger ones showed epinasty; 9000 and 8000 ppm caused epinastic response of the petioles;[1] 1000 ppm

[1] In all cases of epinasty the leaves drooped, but the blades and petioles remained rigid. In some cases the halves of the blades folded together somewhat (fig. 4).

caused the oldest leaves at the base of the stem to fall off and the younger leaves showed epinasty; 100, 50, and 25 ppm still caused marked epinasty. With ethylene, 5 ppm caused some leaf fall, epinasty was marked, and some petioles showed a complete spiral coil (figs. 3, 4); 2 ppm caused epinasty but no leaf fall; 0.2 and 0.1 ppm still caused epinasty. With carbon monoxide, 50 and 12.5 ppm caused no response.

1 2

FIGS. 1, 2.—Fig. 1, *Lycopersicum esculentum:* plant at left has been treated for 12 hours with 50 ppm carbon monoxide; plant at right has been treated for 12 hours with 8 ppm ethylene; the former appears perfectly normal, while the latter shows the distinct epinastic response characteristic of gas poisoning; note spiral coiling of one petiole; fig. 2, *Lycopersicum esculentum:* control at left; plant at right has stood for 18 hours in atmosphere containing 1000 ppm illuminating gas; a few hours longer would cause leaf fall, but as they stand the leaves show strong epinastic response; they are bent down, but are stiff.

Mimosa pudica.[2]—With illuminating gas, 60,000 ppm caused imperfect rigor;[3] 100 and 50 ppm caused folded leaflets and pulvinal movement; after a day the leaflets turned yellow and fell off; then some petioles fell; some of the youngest leaves were

[2] With *Mimosa* all amounts of gas used caused the plants to lose their sensitiveness to touch. After recovery they regained it.

[3] The leaflets folded and the leaves drooped as they do at night or after stimulation, but recovery was complete after removal.

uninjured. With ethylene, 8 and 5 ppm caused the same response as the preceding; 2 ppm caused some leaf fall but the injury was less; 0.2 ppm resulted in the fall of a few leaflets, but all leaves showed sensitiveness by folding together; 0.1 ppm caused some leaflets to fall. With carbon monoxide, 50 ppm caused a clear response; leaflets folded and petioles drooped; no leaflets fell; the plant lost its sensitiveness to touch; recovery was complete after two days in air.

Ricinus communis.[4]—With illuminating gas, 60,000 ppm caused imperfect rigor, some leaves falling; 100 ppm caused falling of the older leaves and epinasty of all others; 50 ppm caused marked epinasty but no leaf fall. With ethylene, 8 and 5 ppm caused most of the leaves to fall, the youngest showing epinasty; 2 ppm caused no leaf to fall, but all the leaves showed epinasty; 0.2 and 0.1 ppm caused a less marked response, but epinasty was still evident (fig. 5). With carbon monoxide, 50 and 12.5 ppm caused no response.

Datura Stramonium.—With illuminating gas, 60,000 ppm caused partial rigor; 4000 ppm caused all the leaves except the youngest to fall; 500 ppm caused falling of the oldest leaves; epinasty of the younger leaves was very similar to that of *Ricinus;* 50 ppm caused epinasty of the older leaves. With ethylene, 8 ppm caused the older leaves to fall, the younger leaves showing epinasty; 5 ppm caused less leaf fall, but the remaining leaves showed epinasty; with 2 ppm there was no leaf fall, but evident epinasty; with 0.2 and 0.1 ppm there was evident epinasty. With carbon monoxide, 50 ppm gave no visible response.

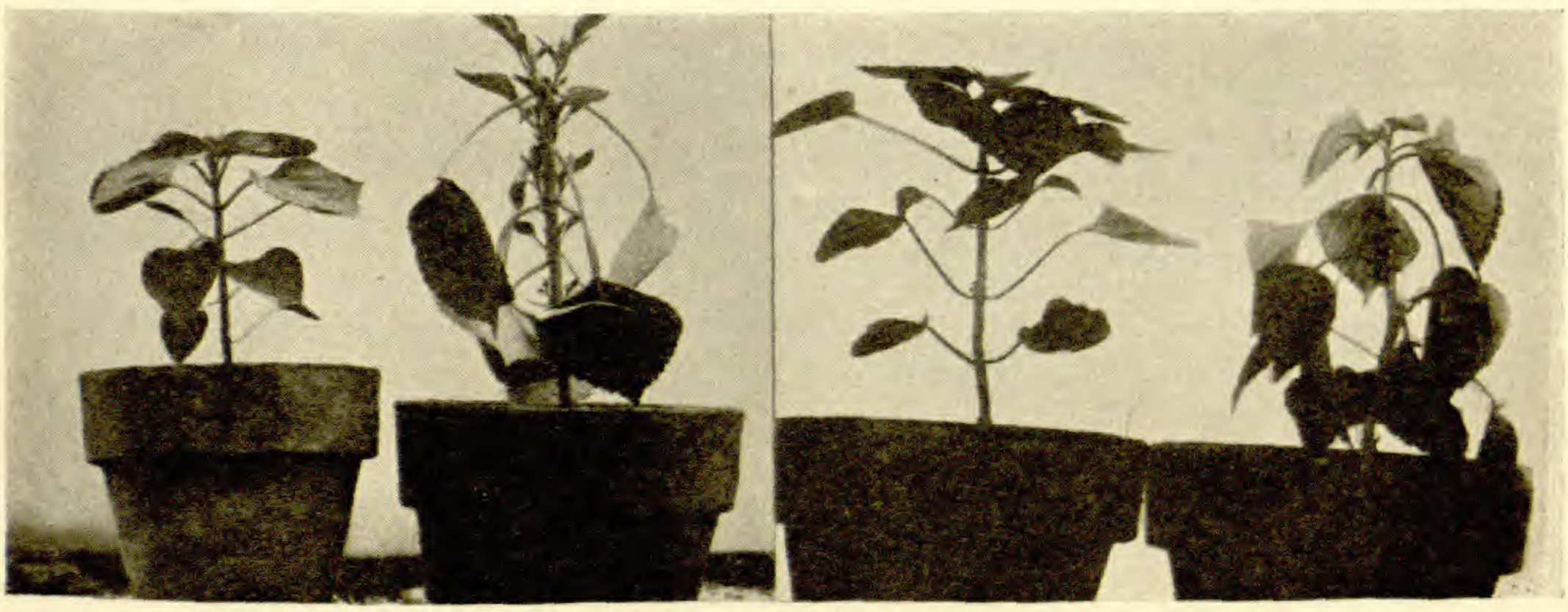
Lycopersicum esculentum.—With illuminating gas, 35,000 ppm caused the older leaves to fall, the root growth was stimulated on the stem above the ground, and epinasty occurred;[5] 26,000, 1000,

[4] The epinastic response is very striking in this plant. The cotyledons, leaf blades, and petioles, all show the characteristic turning. The petioles droop about 90° from their normal position, so that instead of making an angle of about 45° with the stem above the leaf, they droop until they make an angle of about 45° with the stem below the leaf. The blades and petioles are rigid after turning, and usually recover their normal position after a couple of weeks with no gas present (fig. 5).

[5] On the older leaves this was near the blade; on the younger leaves it was near the stem. In some cases this growth caused a spiral coil of the petiole (fig. 1).

75, and 50 ppm caused the same kind of response, but the degree was lessened somewhat. With ethylene, 8 and 5 ppm caused fall of the older leaves, the younger leaves showing epinasty (fig. 2), and proliferation tissue developed on the leaf scars; 2 ppm caused a few leaves to fall, but this amount was about the limit for causing leaf fall; 0.2 ppm caused evident epinasty; 0.1 ppm caused no response. With carbon monoxide, 12.5 ppm caused no response.

Coleus sp.—With illuminating gas, 35,000 ppm caused rigor, no leaves fell, and after removal from the cases recovery was com-



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FIGS. 3, 4.—Fig 3, *Salvia splendens:* plant at right was treated with 2 ppm ethylene; after 12 hours it showed distinct epinastic response; plant at left, appearing normal, received 12.5 ppm carbon monoxide; fig. 4, *Salvia splendens:* control plant and one which has been treated for 18 hours in 1000 ppm illuminating gas; epinastic growth of petioles is clear and leaf blades show a folding together of the sides, which is characteristic of presence of considerable gas.

plete; 25,000 ppm caused all the leaves to fall at the end of 24 hours exposure; 6000 ppm caused falling of about half the leaves, the older being the ones affected; 1000 ppm caused the oldest to fall, the younger showed epinasty, and the youngest were unaffected; 100 ppm caused slight epinasty of the younger leaves, and this is close to the limit of response. With ethylene, 5 ppm caused the oldest leaves to fall, while the younger, except those at the tip, showed epinasty; 2 ppm caused no leaf fall, and epinasty was slight; 0.2 ppm caused no response. With carbon monoxide 12.5 ppm caused no response.

Hibiscus rosa-sinensis.—With illuminating gas, 9000, 8000, and 4000 ppm caused all leaves to fall; new leaves developed in 2–3 weeks after removal from the case; the lenticels on the stem were filled with spongy white tissue; with 1000 ppm the older leaves fell, and the younger leaves showed epinasty; with 100 ppm only slight epinasty was evident. With ethylene, 8 ppm caused distinct epinasty; 2 ppm caused slight epinasty, but this is near the limit for response. With carbon monoxide, 12.5 ppm caused no response.

Acalypha tricolor.—With illuminating gas, 25,000 ppm caused some leaf fall, and other leaves showed epinasty; 8000 and 1000 ppm caused no leaf fall, but distinct epinasty.

Acacia horrida.—With illuminating gas, 8000 ppm caused fall of many leaves; 1000 ppm caused fall of some leaves several days after treatment.

Euonymus japonicus.—With illuminating gas, 20,000 ppm caused most of the leaves to fall; 8000 ppm caused the older leaves to fall; 1000 ppm caused a few of the older leaves to fall.

Citrus decumana.—With illuminating gas, 20,000 and 8000 ppm caused most of the leaves to fall after removal from the case; 1000 ppm caused the older leaves to fall.

Zantedeschia ethiopica.—With illuminating gas, 40,000 ppm caused the flower spathe to become somewhat discolored, but the plant seemed uninjured; 10,000 ppm caused epinasty in the young leaves, the petioles being arched next to the blade; 9000, 8000, and 1000 ppm caused the youngest leaf and the peduncle to show epinasty as above. With ethylene, 5 ppm caused slight epinasty of the youngest leaf; 2 ppm caused no response.

Pelargonium zonale.—With illuminating gas, 25,000 and 4,000 ppm caused no visible response during 4 days of treatment, but all leaves fell in 3–6 days after treatment had stopped;[6] 100 ppm caused no response. With ethylene, 8 and 2 ppm caused no response. With carbon monoxide, 12.5 ppm caused no response.

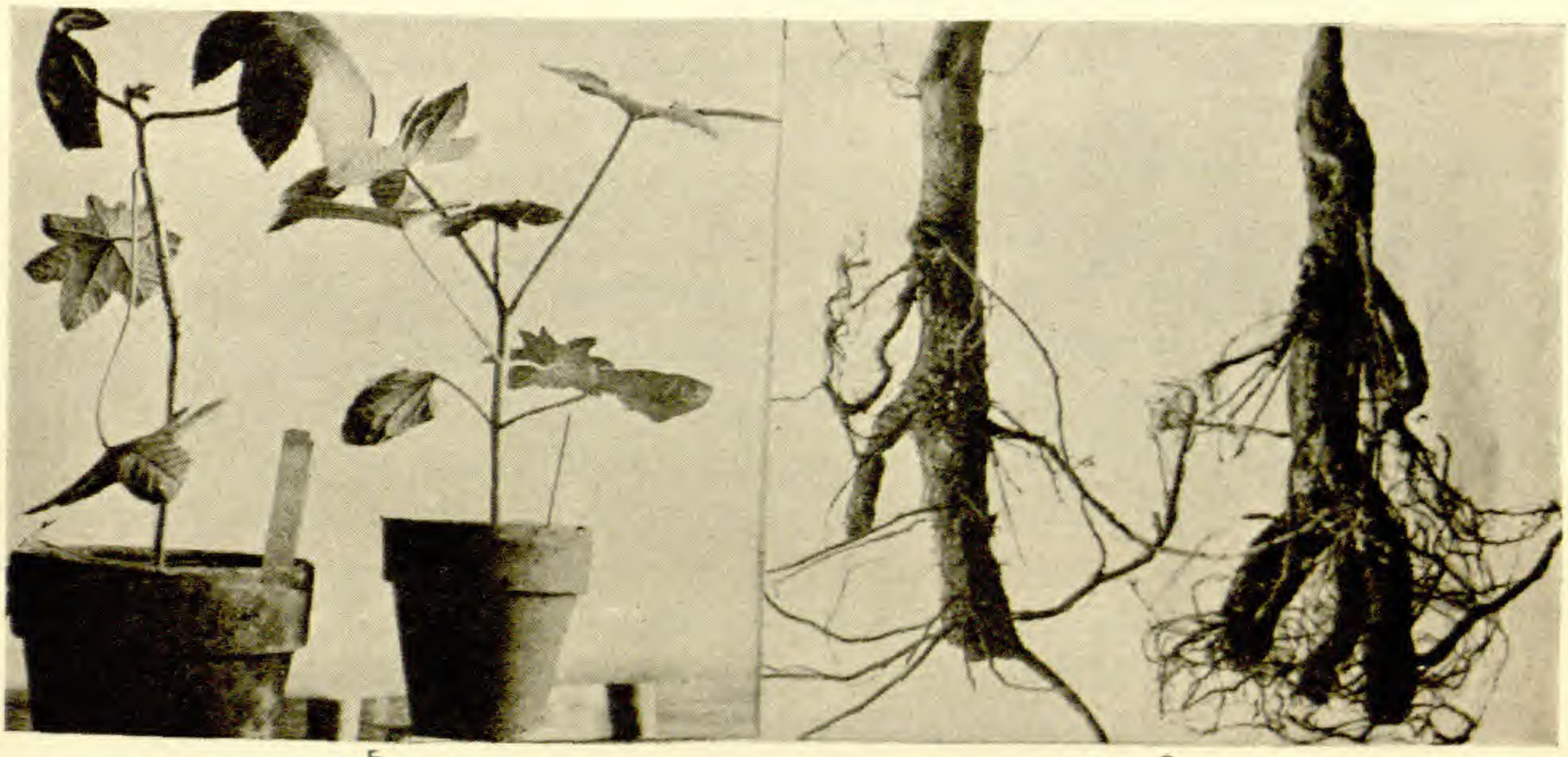
Begonia luminosa.—With ethylene, 8 ppm caused some epinasty at the base of the leaf blade. With carbon monoxide, 50 ppm caused no response.

[6] When new leaves developed, they were without the variegated zone.

Fuchsia speciosa.—With illuminating gas, 20,000 ppm caused some of the older leaves to fall; 8000 ppm caused epinasty.

Populus deltoides.[7]—With illuminating gas, 35,000, 25,000, and 10,000 ppm caused some leaf fall, and after removal from the cases other leaves died and fell off; 8000 ppm caused some leaf fall.

Ficus elastica.—With illuminating gas, 20,000 ppm caused the older leaves to fall during treatment; 8000 ppm caused some of the older leaves to fall about a week after treatment.



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FIGS. 5, 6.—Fig. 5, *Ricinus communis:* plant at right, appearing normal, stood for 2 days in 50 ppm carbon monoxide; plant at left stood in 8 ppm ethylene for 2 days, and has dropped one leaf, all other petioles showing strong epinastic response; note that direction of leaf blades is altered as well as that of petioles; fig. 6, Roots (*Pyrus communis* at right; *P. Malus* at left): the 3-pronged root of pear has had 100 liters of illuminating gas forced into the soil during 40 days; notice swollen condition of underground parts, also numerous "tubercles"; the root of apple received 160 liters of illuminating gas in 62 days, its response, swelling and tubercles, being very similar to that of pear.

Croton tiglium var. Sanders.—With illuminating gas, 20,000 ppm produced some leaf fall of the older leaves; 8000 ppm caused slight epinasty.

Tulipa (several varieties).—With illuminating gas, 10,000 ppm caused injury of the flower buds and the tips of the younger leaves rolled up; 4000 ppm caused no visible injury.

[7] These were cuttings rooted in sand and grown in flower pots.

Hyacinthus (several varieties).—With the same quantities of illuminating gas, the responses were identical with those of the tulip.

Carica Papaya.—With illuminating gas, 20,000 ppm caused the older leaves to fall and the younger leaves to show epinasty.

Caladium esculentum.—This showed no response with 75 ppm illuminating gas, 8 ppm ethylene, or 50 ppm carbon monoxide.

Lupinus perennis.—This showed no response with 8 ppm ethylene or 50 ppm carbon monoxide.

Eriobotrya japonica, *Phoenix canariensis*, *Conocephalus* sp., *Canna* (King Humbert and other varieties), *Achyranthes Lindini*, *Cytisus canariensis*, and *Alternanthera* sp. showed no response with 20,000 ppm illuminating gas.

Polypodium, *Aspidium*, and *Asplenium.*—With illuminating gas, 60,000, 8000, and 4000 ppm caused no response.

The preceding data are summarized briefly in table I. The plants are grouped according to their sensitiveness to gas: very sensitive, less sensitive, and resistant. The minimum concentration necessary to produce a response is given in each case.

The following plants showed no response to illuminating gas or to ethylene in the concentration used: *Caladium esculentum*, *Lupinus perennis*, *Eriobotrya japonica*, *Phoenix canariensis*, *Conocephalus*, sp., *Canna*, *Achyranthes Lindini*, *Alternanthera* sp., *Cytisus canariensis Polypodium*, *Aspidium*, and *Asplenium.*

Root treatment of trees

Two or three year old trees were used for the root treatment. They were treated in flower pots during the winter and early spring, and then the work was carried on out of doors upon young trees which had been growing in the soil for a year or more.

The potted plants were set on tripods and glass tubing was run through the cork plug in the bottom of the pot. Connection was made with a wash bottle and the rate of gas flow through this wash bottle was controlled by means of a brass stopcock. The gas was forced out from the inverted carboys by means of water from a raised tank. All rubber connections with glass tubing were as short as possible, gas tight, and the gas was "water sealed" in

the inverted carboys. By means of the brass stopcocks and glass tubes drawn out to a fine point in the wash bottles, the rate of flow of the gas could be regulated at will. To keep the soil from plug-

TABLE I

Plant	Gas used and amount in parts per million (ppm)	Response
VERY SENSITIVE PLANTS		
Lathyrus odoratus.....	Illuminating gas, 25	Leaflets died and fell off
Salvia splendens.....	Illuminating gas, 25	All leaves showed epinasty
Mimosa pudica.....	Illuminating gas, 50	Some leaflets fell; others showed epinasty
Ricinus communis.....	Illuminating gas, 50	Epinasty shown by the leaves
Datura Stramonium.....	Illuminating gas, 50	Epinasty shown by the younger leaves
" "	Ethylene, 0.1	Close to the limit for the response
Lycopersicum esculentum	Illuminating gas, 50	Epinasty
" "	Ethylene, 0.2	Epinasty shown by the leaves
" "	Ethylene, 0.1	No response
LESS SENSITIVE PLANTS		
Coleus sp.....	Illuminating gas, 100	Slight epinasty shown by the younger leaves
Hibiscus rosa-sinensis....	Illuminating gas, 100	Epinasty shown by the older leaves
Acalypha tricolor.....	Illuminating gas, 1000	Epinasty
Acacia horrida.....	Illuminating gas, 1000	Some leaves fell
Euonymus japonicus.....	Illuminating gas, 1000	Some leaves fell
Citrus decumana.....	Illuminating gas, 1000	Some leaves fell
Zantedeschia ethiopica ...	Illuminating gas, 1000	Youngest leaf arched at the base of the blade
Pelargonium zonale.....	Illuminating gas, 4000	Leaves fell several days after treatment
Begonia luminosa.....	Ethylene, 8	Slight epinasty
Fuchsia speciosa.....	Illuminating gas, 8000	Epinasty
Populus deltoides.....	Illuminating gas, 8000	Some leaves fell
Ficus elastica.....	Illuminating gas, 8000	Some leaves fell
Croton tiglium.....	Illuminating gas, 8000	Slight epinasty
Tulipa (several vars.)....	Illuminating gas, 10,000	Flower buds somewhat injured
Hyacinthus (several vars.)	Illuminating gas, 10,000	Flower buds somewhat injured
Carica Papaya.....	Illuminating gas, 10,000	Older leaves fell, the younger showed epinasty.

ging the glass tube inserted in the pot, a small vial with a slit along the side of the cork was fitted over the end of the tube inside the flower pot. The pot was then dipped into melted paraffin in order to prevent too much escape of gas through the lower part of the pot.

When the trees were treated in the open ground, glass tubes 12–24 inches long, depending upon the size of the trees, were buried close to the side of the tree. The same precaution was used here to prevent clogging.

The following are the results for each tree or plant treated, the length of time treated, and the amount of illuminating gas passed into the soil. In no case could the odor of the gas be detected on a handful of the soil or escaping in the air. The temperature range was 12–20° C.

Acer Negundo.—A young tree was treated for 45 days and given 60 liters of gas. There was no visible effect above or below ground.

Acer saccharinum.—Treated 42 days and given 140 liters of gas, the parts above ground were unchanged. The stem below ground, however, was swollen, soft, and cracked longitudinally. A section showed proliferation tissue produced just outside the vascular cylinder.

Chrysanthemum hortorum.—One plant, being treated 42 days and given 80 liters of gas, was killed, no proliferation tissue being produced or other visible changes. A second plant was treated 28 days and given 60 liters of gas. Some roots grew up out of the ground, probably due to loss of geotropic response.

Fraxinus americana.—Treated for 42 days and given 120 liters of gas, the parts above ground were unchanged. Below ground the stem was swollen, soft, and cracked longitudinally. Sections showed that abundant proliferation tissue was produced just outside the vascular system.

Grevillea robusta.—One specimen was treated 33 days and given 40 liters of gas. After 2 weeks gummy matter exuded from a slight crack in the stem just above the ground. A second plant was treated 48 days and given 40 liters of gas; and a third was treated 31 days and given 19 liters of gas. The roots of all three showed spongy white masses of tissue at short intervals, with no epidermis. Many roots were dead. The underground parts of the stem were swollen, due to the development of spongy, white tissue.

Hibiscus rosa-sinensis.—A plant was treated 15 days and given 40 liters of gas. The leaves showed epinasty for 2 days and then fell off. After 4 days' treatment, white spongy tissue developed

in the lenticels just above ground. The underground parts were enlarged to three times their normal size. The cortical tissue was white and spongy. The bark split longitudinally and dropped off. Small tubercles developed on many roots. The xylem and phloem appeared normal. These results with *Hibiscus* agree with those of HARVEY and ROSE (**6**).

Lycopersicum esculentum.—One plant was treated 24 days and given 20 liters of gas, while a second plant was treated 18 days and given 20 liters of gas. After a few hours' treatment, the lower leaves began to show the epinastic response, falling after 2 days. Many more roots developed on the stem above ground than on the control plant. Roots grew up out of the ground, probably due to loss of geotropic sensitiveness. Tubercles occurred on the roots. The control plants showed some tubercles, but the number was greatly increased upon the treated plants. Nematodes were present in many of these tubercles.

Poa pratensis.—One sod was treated 38 days and given 60 liters of gas; a second 25 days with 60 liters; a third 5 days with 40 liters; and a fourth 8 days with 40 liters. There was no response in any case.

Populus deltoides.—Treated 81 days and given 60 liters of gas, the roots developed many small "tubercles," being swollen to twice the normal size at these points. The tissue was soft and spongy. The stem showed no visible effect above ground; below ground it was swollen and rigid.

Pyrus communis.—Treated 40 days and given 100 liters of gas, there was no visible response above ground, but all underground parts were swollen. Longitudinal cracks appeared, in which was soft spongy tissue. All the roots were irregularly enlarged, all the proliferation tissue being in the cortex (fig. 6).

Pyrus Malus.—Treated 62 days and given 160 liters of gas, the response was very similar to that of the pear (fig. 6).

Ricinus communis.—Treated 45 days and given 80 liters of gas, all leaves except the youngest fell. The underground part of the stem was swollen and cracked longitudinally.

Salvia splendens.—One plant was treated 18 days and given 20 liters of gas, and another plant was treated 42 days and given

80 liters of gas. Some leaves fell and others showed epinasty; but the underground parts showed no effect.

Sambucus canadensis.—Treated 60 days and given 60 liters of gas, the roots were killed. Some roots which were still living showed "tubercles" similar to those upon *Populus*. The stem below ground was somewhat swollen, due to the development of spongy white tissue in the lenticels.

Ulmus americana.—Treated 90 days and given 180 liters of gas, after 3 weeks the bark cracked vertically just above the surface of the ground. After 6 weeks, 2 small limbs died and were removed. About half the leaves fell during the treatment. Near the close of the treatment the underground parts were dead and cracks extended throughout the bark and cortical tissue. There was a small amount of proliferation tissue just outside the vascular system.

Practical suggestions for florists

To detect illuminating gas in a greenhouse, the florist should provide himself with some vigorous plants of one of the following: tomato, castor bean, scarlet sage, Jimson weed, or sensitive plant. These should be grown in pots so that they may readily be handled, and should have from 6 to 12 or more leaves. They must also be in vigorous condition; otherwise they may not respond should illuminating gas be present. These should be placed at various locations throughout the greenhouse and left 24–48 hours with poor ventilation. All the plants named will respond to traces of illuminating gas within this period at ordinary temperatures.

With only a trace of gas present in the air, the epinastic response of the leaves will be very noticeable if these plants are compared with normal plants without gas. This bending down of the leaves will increase with the concentration of the gas present in the air. All these plants will drop their leaves with a concentration below the limit of the odor of gas. The older leaves fall first, the younger leaves being retained until there is 1 part of illuminating gas to 1000 of air.

Summary

1. The following plants are admirably adapted for use as test plants for illuminating gas in greenhouses: *Lycopersicum escu-*

lentum, *Salvia splendens*, *Mimosa pudica*, *Ricinus communis*, *Datura Stramonium*, and *Dianthus Caryophyllus*. The response of each is definite, striking, and not easily mistaken.

2. Traces of gas (50 ppm of air) cause the epinastic growth of the petioles of all these plants, with the exception of the last. The flower buds of the carnations are blighted by these amounts. One part of illuminating gas per 1000 of air causes leaf fall in the following plants: *Lycopersicum esculentum*, *Salvia splendens*, *Mimosa pudica*, *Datura Stramonium*, *Ricinus communis*, *Coleus* sp., and *Hibiscus rosa-sinensis*. Both the amounts (50 ppm of air and 1 part per 1000 of air) are far below the limit of odor. Repeated trials showed that it was impossible to detect less than 0.25 per cent of illuminating gas (1 part to 400 of air) by the sense of smell.

3. Amounts of ethylene corresponding to the gas mixture gave similar responses; 2 ppm of air caused epinastic growth of the petioles of *Lycopersicum esculentum*, *Salvia splendens*, *Mimosa pudica*, *Ricinus communis*, and *Datura Stramonium;* 8 ppm of air (equivalent to 200 parts of illuminating gas) caused some leaf fall in the 5 plants named.

4. *Poa pratensis* and *Acer Negundo* are very resistant to gas, having shown no response to concentrations injurious to all other forms tested.

5. The following plants are not noticeably injured by gas unless there is enough present to be detected by odor: *Caladium esculentum*, *Lupinus perennis*, *Eriobotrya japonica*, *Phoenix canariensis*, *Conocephalus* sp., *Canna*, *Achyranthes lindini*, *Alternanthera* sp., *Cytisus canariensis*, *Polypodium*, etc.

6. The following trees are rather sensitive to gas escaping into the soil: *Pyrus Malus*, *P. communis*, *Fraxinus americana*, *Ulmus americana*, *Sambucus canadensis*, *Grevillea robusta*, *Catalpa speciosa*, *Populus deltoides*, and *Tilia americana*. Apple, pear, ash, elm, *Catalpa*, and *Sambucus* showed proliferation tissue in the cortex of the stems below the surface of the ground. Elm, ash, and cottonwood showed longitudinal cracks in the bark just above the surface of the ground.

7. The following bedding plants are injured by gas escaping into the soil: *Lycopersicum esculentum*, *Salvia splendens*, *Ricinus communis*, and *Chrysanthemum hortorum*. *Chrysanthemum* is

killed outright; the others drop their leaves or show epinastic growth of the petioles.

8. Young trees at least are injured by leakage of illuminating gas too slight to be detected by odor. The foliage shows no injury, and one would not be likely to suspect gas poisoning from the appearance of the tree above ground. Judging from my results with trees, their killing by illuminating gas is a very slow process, going on for months or years. It is certain that enough gas to cause an odor in the vicinity of trees would be enough to injure them seriously.

I am indebted to Dr. WILLIAM CROCKER for suggestions and help during the progress of the work.

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LITERATURE CITED

1. CROCKER, WM., and KNIGHT, L. I., Effect of illuminating gas and ethylene upon flowering carnations. BOT. GAZ. **46**:259–276. 1908.
2. CROCKER, WM., KNIGHT, L. I., and ROSE, R. C., A delicate seedling test. Science N.S. **37**:390. 1913.
3. ———, A new method of detecting traces of illuminating gas. Science N.S. **31**:636. 1910.
4. ———, Effect of various gases and vapors upon the etiolated seedling of the sweet pea. Science N.S. **31**:635–636. 1910.
5. FITTING, HANS, Untersuchungen über die vorzeitige Entblätterung von Blüten. Jahrb. Wiss. Bot. **49**:187–263. 1911.
6. HARVEY, E. M., and ROSE, R. C., The effect of illuminating gas on root systems. BOT. GAZ. **60**:27–44. 1915.
7. HARVEY, E. M., Some effects of ethylene on the metabolism of plants. BOT. GAZ. **60**:193–214. 1915.
8. ———, The castor bean and laboratory air. BOT. GAZ. **56**:439–442. 1913.
9. STONE, G. E., Effect of escaping illuminating gas on trees. Mass. Exper. Sta. Report pp. 180–185. 1906.
10. ———, Effects of illuminating gas on vegetation. Mass. Exper. Sta. Report pp. 45–60. 1913.

THE SUPPOSED ACTION OF POTASSIUM PERMANGANATE WITH PLANT PEROXIDASES

HERBERT H. BUNZELL AND HEINRICH HASSELBRING

REED[1] has recently reported experiments which he believes throw a new light on the mechanism of oxidation in living tissues. The experiments relate to the reactions involved in the process of oxidation by means of peroxidases. To a horseradish extract, which in itself was incapable of bringing about the oxidation of potassium iodide or of gum guaiac, he added concentrated potassium permanganate solution until the permanganate was no longer reduced. He then added a small excess of horseradish extract to reduce any free permanganate present. On filtration a clear, rather deep yellow solution was obtained, which, when mixed with solutions of potassium iodide, gum guaiac, or pyrogallol, caused rapid oxidations of those substances. REED'S interpretation of these experiments is that the peroxidase of the horseradish extract combines with oxygen from the permanganate, thus forming a new compound which readily gives up oxygen to other compounds. He concludes, therefore, that in oxidation processes catalyzed by peroxidases two reactions are involved: first, a combination of the peroxidase with oxygen from substances acting as oxygenases; and second, a transfer of this oxygen to the substances oxidized by means of peroxidases. Thus he believes the mechanism of oxidation in living tissues is explained.

Contrary to REED'S belief, this interpretation throws no new light on the mechanism of oxidation in living tissues, but is quite in harmony with the older views of TRAUBE, BACH, ENGLER, and KASTLE and LOEVENHART. Moreover, since manganese compounds themselves are capable of bringing about oxidations, the correctness of REED'S view ascribing the oxidations in the mixtures with which he worked to peroxidase activated by oxygen from the postassium permanganate is at least open to question. That the

[1] REED, G. B., The mode of action of plant peroxidases. BOT. GAZ. **62**:233–238. 1916.

presence of peroxidases is not necessary to bring about the observed reactions is shown by the following experiments:

1. Two-tenths of a gram of dried white of egg was dissolved in 10 cc. of water. To this solution 2 drops of saturated solution of potassium permanganate were added. The pale brown filtrate from this mixture gave intense oxidase reactions with guaiac, potassium-iodide-starch, and pyrogallol.

2. One gram of Witte's peptone was dissolved in 20 cc. of water and 5 drops of saturated potassium permanganate solution were added. With the pale brown filtrate the 3 oxidase reactions just mentioned were carried out. All were strongly positive.

3. To about half a gram of tyrosin mixed with water 2 drops of a 5 per cent solution of potassium permanganate were added. The brownish mixture gave a clear brown filtrate which oxidized potassium iodide, guaiac, and pyrogallol.

4. One-half gram of tyrosin was dissolved in hot water. To the boiling solution 3 drops of a 5 per cent solution of potassium permanganate were added. The pale brown filtrate from the mixture gave the 3 oxidase reactions. This filtrate was boiled and allowed to stand overnight, but no further precipitate was formed. The filtrate still gave all the oxidase reactions.

5. To a solution of 1 gram of glucose, 2 drops of a saturated potassium permanganate solution were added. The mixture was warmed until the purple color had given way to light brown. The filtrate gave the oxidase reactions distinctly. Fructose treated in the same way gave strong reactions with guaiac and with potassium-iodide-starch, but none with pyrogallol.

6. To a boiling solution containing 3 drops of a 5 per cent potassium permanganate solution in 10 cc. of water, glycerine was added drop by drop until the purple was replaced by brown. The filtrate from the brown precipitate was pale straw-colored. It gave all the oxidase reactions.

7. One cc. of salicylic aldehyde reduced quickly in the cold 2 drops of a 5 per cent solution of potassium permanganate, forming a brown precipitate. The pale straw-colored filtrate gave reactions with potassium-iodide-starch and with guaiac.

8. Methyl alcohol and ethyl alcohol when gently warmed reduced potassium permanganate to a colorless solution, which when filtered from the brown precipitate gave no oxidase reactions.

9. Formaldehyde and acetaldehyde reduced potassium permanganate in the cold, giving brown or black precipitates and colorless solutions which gave no oxidase reactions. This result was to be expected from the action of methyl alcohol and ethyl alcohol.

10. A trace of manganese peroxide shaken up in water oxidized guaiac, potassium iodide (in neutral solution), and pyrogallol.

In all these experiments, a large excess of the organic compounds was used, so that the solutions would be free from potassium permanganate in the sense in which REED considered his solutions free from unreduced potassium permanganate. The potassium iodide reactions were carried out in solutions of the same strength as those used by REED. In all cases the reagents and control mixtures failed to give the oxidase reactions. The brown filtrates as well as the colorless ones contained manganese.

It appears from these experiments that in the reduction of potassium permanganate by organic substances in neutral solutions, hydrated peroxides of manganese are formed, which are held in solution and which though they are reduction products of permanganic acid are still capable of carrying out oxidations. When the permanganate is still further reduced, the water-clear filtrate which still contains manganese compounds no longer gives the oxidation reactions described. By careful reduction both stages can be obtained with the same compound (glycerine, glucose).

Inasmuch as the brown solutions contain manganese not reduced to its lowest state of oxidation, and since manganese peroxide itself brings about the oxidation of guaiac, potassium iodide, and pyrogallol, it becomes extremely probable that the oxidation phenomena observed by REED were brought about by peroxides of manganese and not by activated plant peroxidases. Moreover, since a number of substances acting on potassium permanganate give mixtures which oxidize other compounds, there is no evidence in REED'S experiments that the reduction of the

potassium permanganate was brought about by plant peroxidases. His conclusions, therefore, drawn from reactions which are common to many organic substances and which are not known to be properties of peroxidases, are too sweeping for the experimental grounds upon which they are based.

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LEAF NECTARIES OF GOSSYPIUM

E. L. Reed

(WITH PLATES XII AND XIII AND ONE FIGURE)

On the midrib and sometimes on the other principal veins on the underside of leaves of *Gossypium*, certain nectar glands are found. All species of cotton, with the possible exception of *G. tomentosum*,

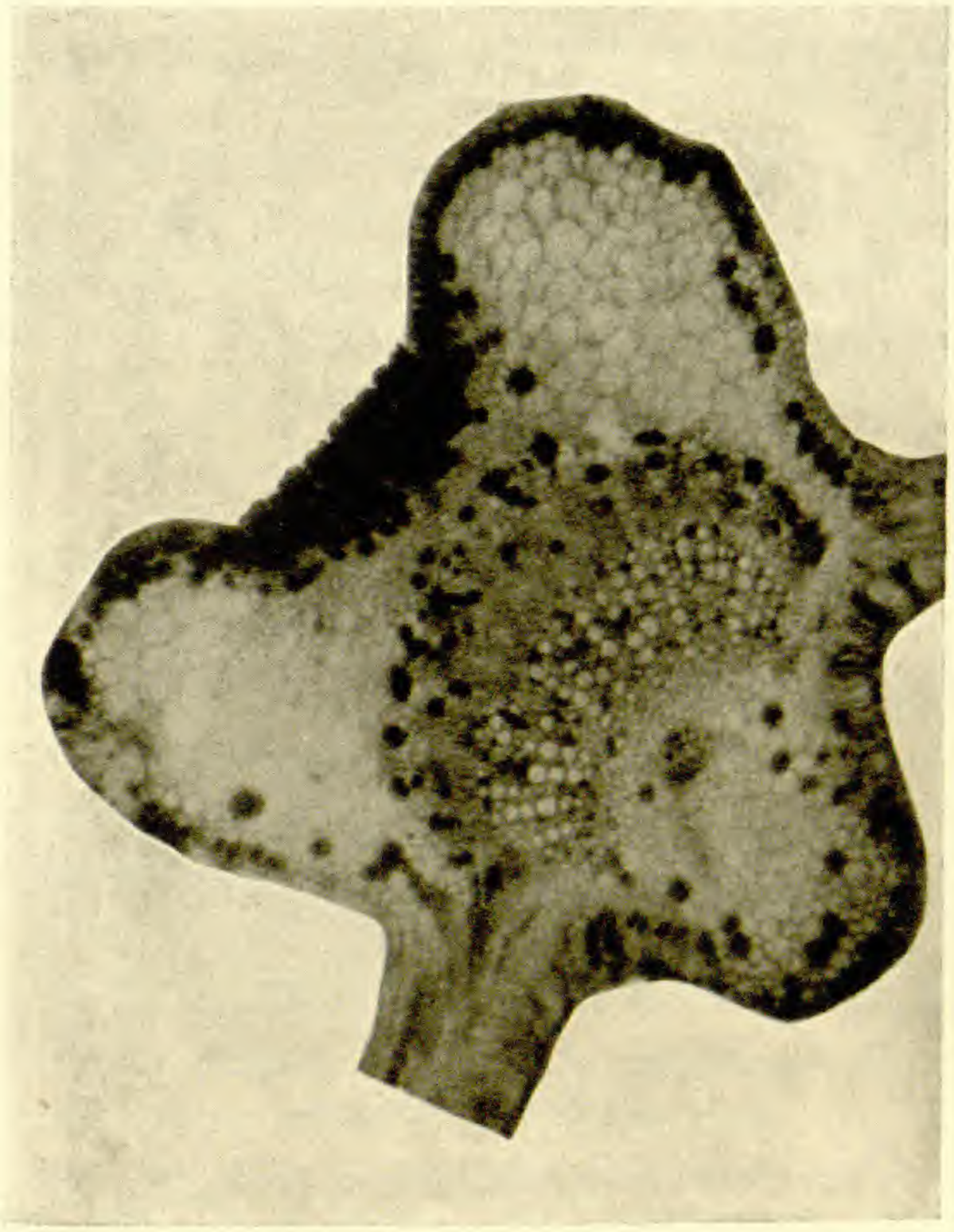

FIG. 1.—Nectar gland on a leaf of *Gossypium hirsutum*

possess these glands, which are usually oval or pear-shaped, sometimes even sagittate in form. TAYLOR (1) says "these glands are usually small, rounded, shallow pits, with a floor of round-topped secreting cells"; and WATT (2) states that "these midrib glands may be elongated and elevated portions of the veins that

become pale colored or assume a pink tinge, and then rupture lengthwise, or they may be circular or oblong warts which open into distinct pits." TRELEASE (3) points out that the glands begin to secrete at about the time the seedling has expanded 4 leaves, and that the nectar is secreted most abundantly at night. SAFFORD (4) states that "they . . . . occur on all leaves of cotton . . . . in the form of vaginated glands." He gives a photograph by HOWARD, of the United States Department of Agriculture, of a cross-section of a nectar gland of the cotton leaf.

The glands described in this paper are from *Gossypium hirsutum*. They are oval-shaped depressions, filled with closely crowded multicellular papillae and surrounded by a thick wall of epidermal cells (text fig. 1 and fig. 7). In all cases observed the glands became visible on the cotyledons about the time the first pair of true leaves developed; they began to secrete a little later. Sections were made of the cotyledons as soon as they were fully expanded; of the second and third pair of true leaves at different stages of development; and also of mature leaves. A section through a gland of a mature leaf is shown in fig. 7 and one through that of a young leaf in fig. 9. These glands are of epidermal origin and consist of numerous multicellular papillae (text fig. 1). Their organogeny is as follows:

The epidermal cells from which the glands arise cease to develop normally and become papillate (fig. 1). The papillae are next cut off by transverse walls (fig. 2). The cells thus formed divide again in the same plane; this may be repeated once or twice, and results in the formation of short pedestals consisting of 2, 3, or 4 cells (figs. 3–6). The terminal cell of each papilla then divides by a vertical wall into two (fig. 10). These in turn divide by walls at right angles to the first cross wall into 4 cells (fig. 10). The latter divide by periclinal walls into 4 central and 4 peripheral cells (fig. 11). Lastly, each of the external cells divides by a wall at right angles to the surface, and thus a peripheral layer of 8 cells is formed (fig. 12). The development of the papillae of these glands bears a remarkable resemblance to that of the antheridia of *Riccia* (5).

COLLEGE STATION
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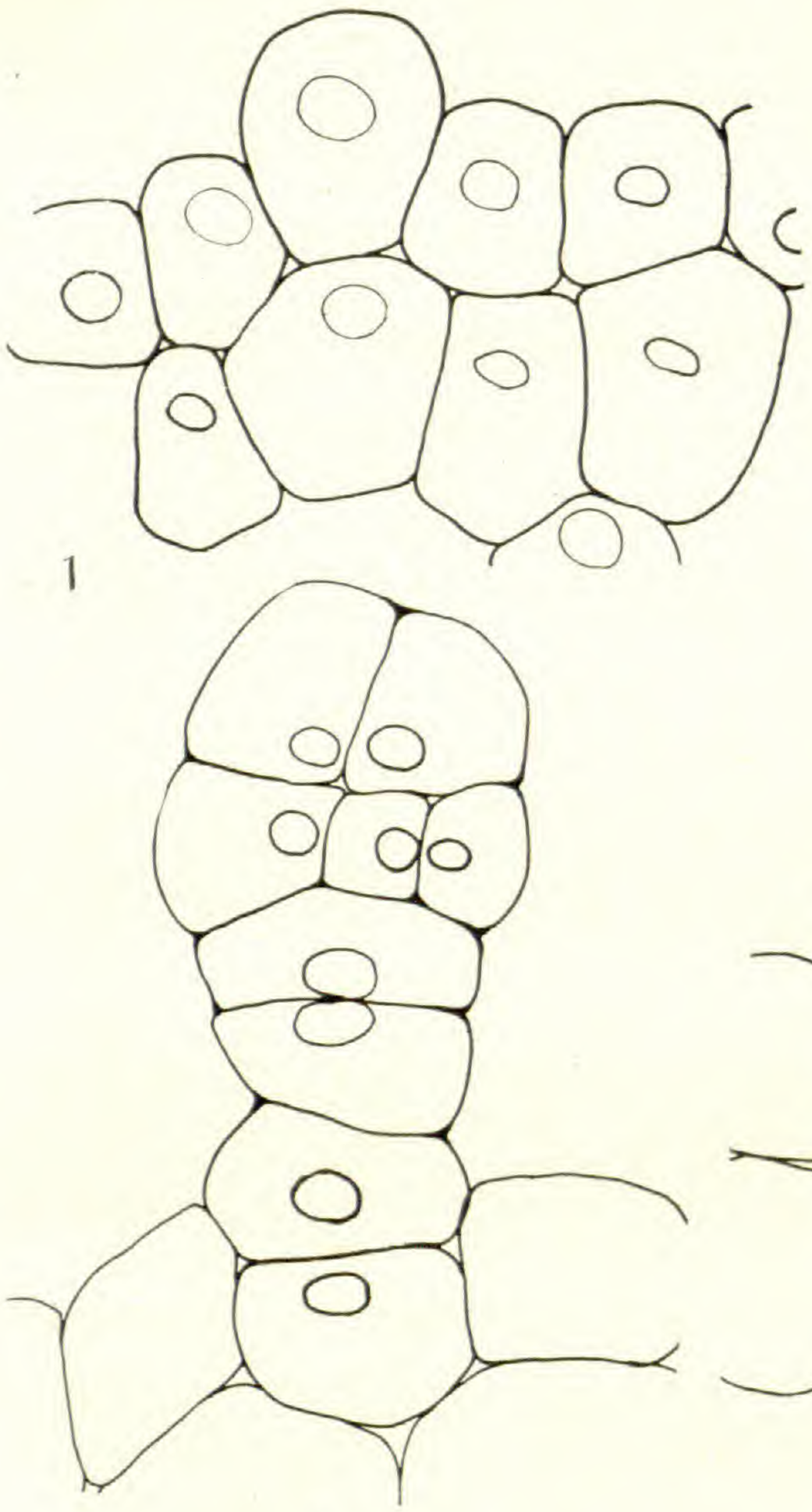

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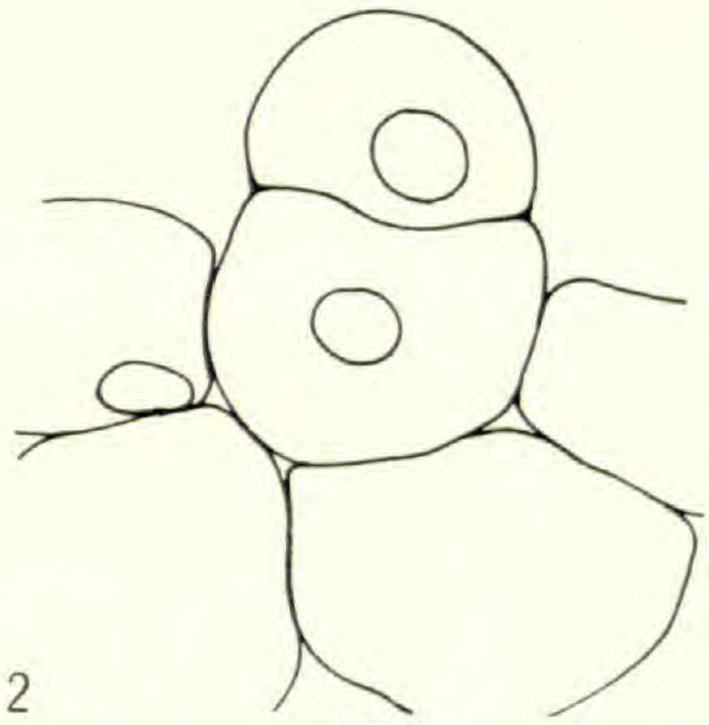

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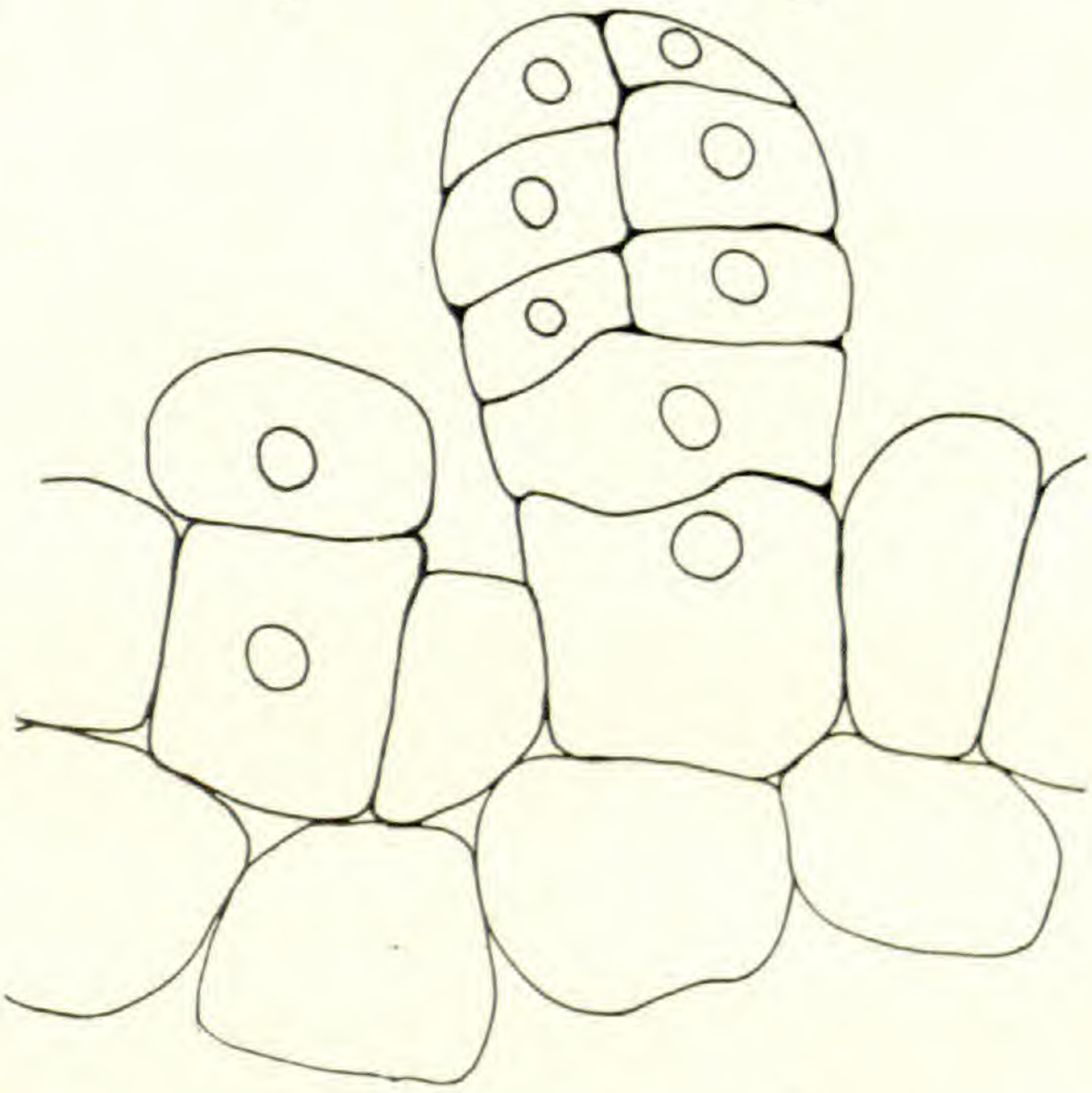

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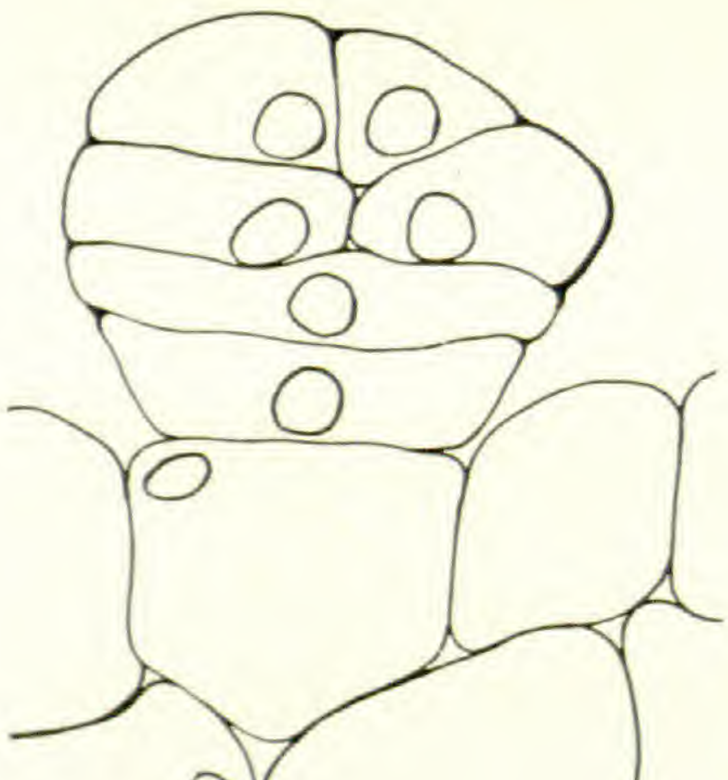

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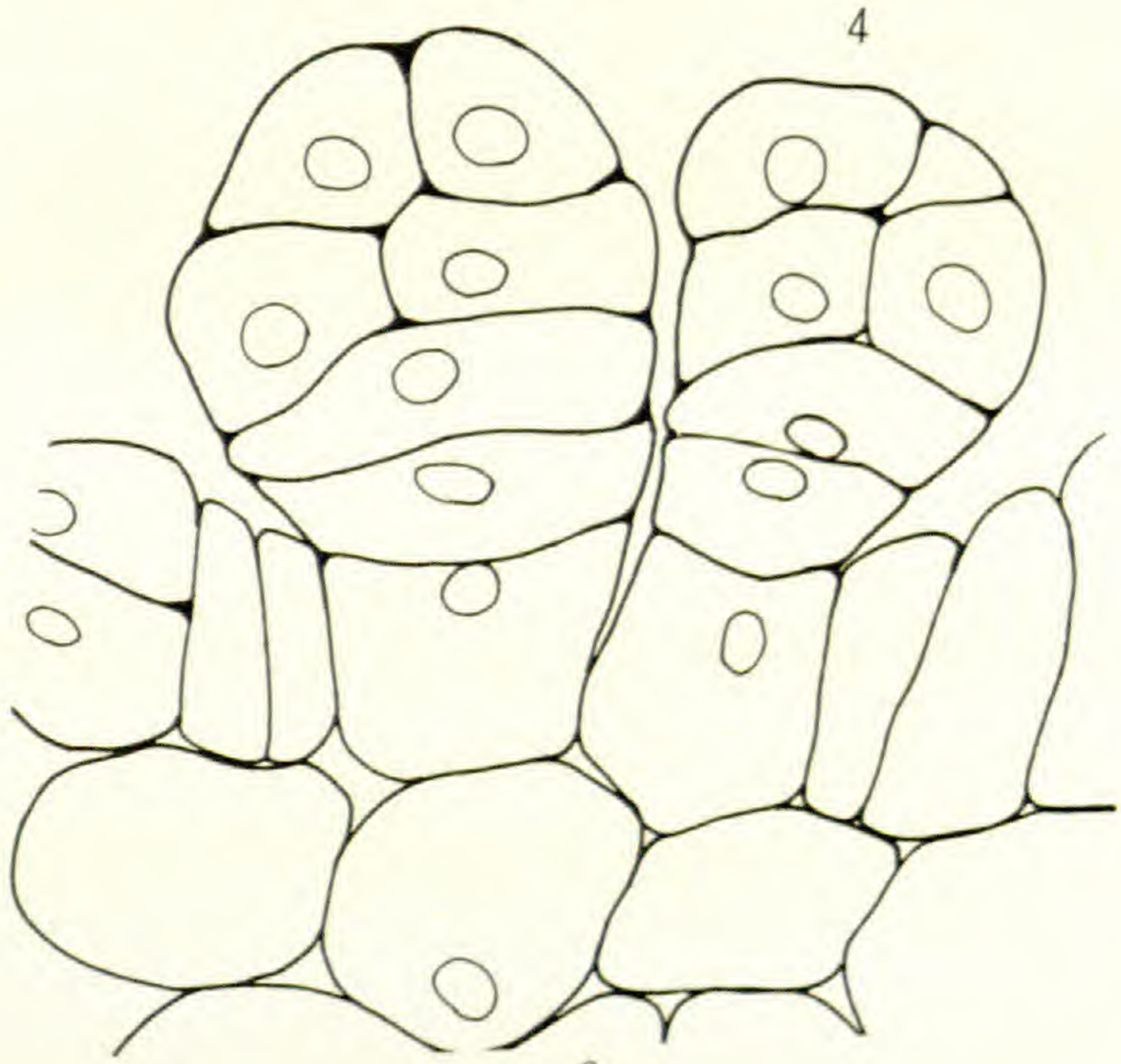

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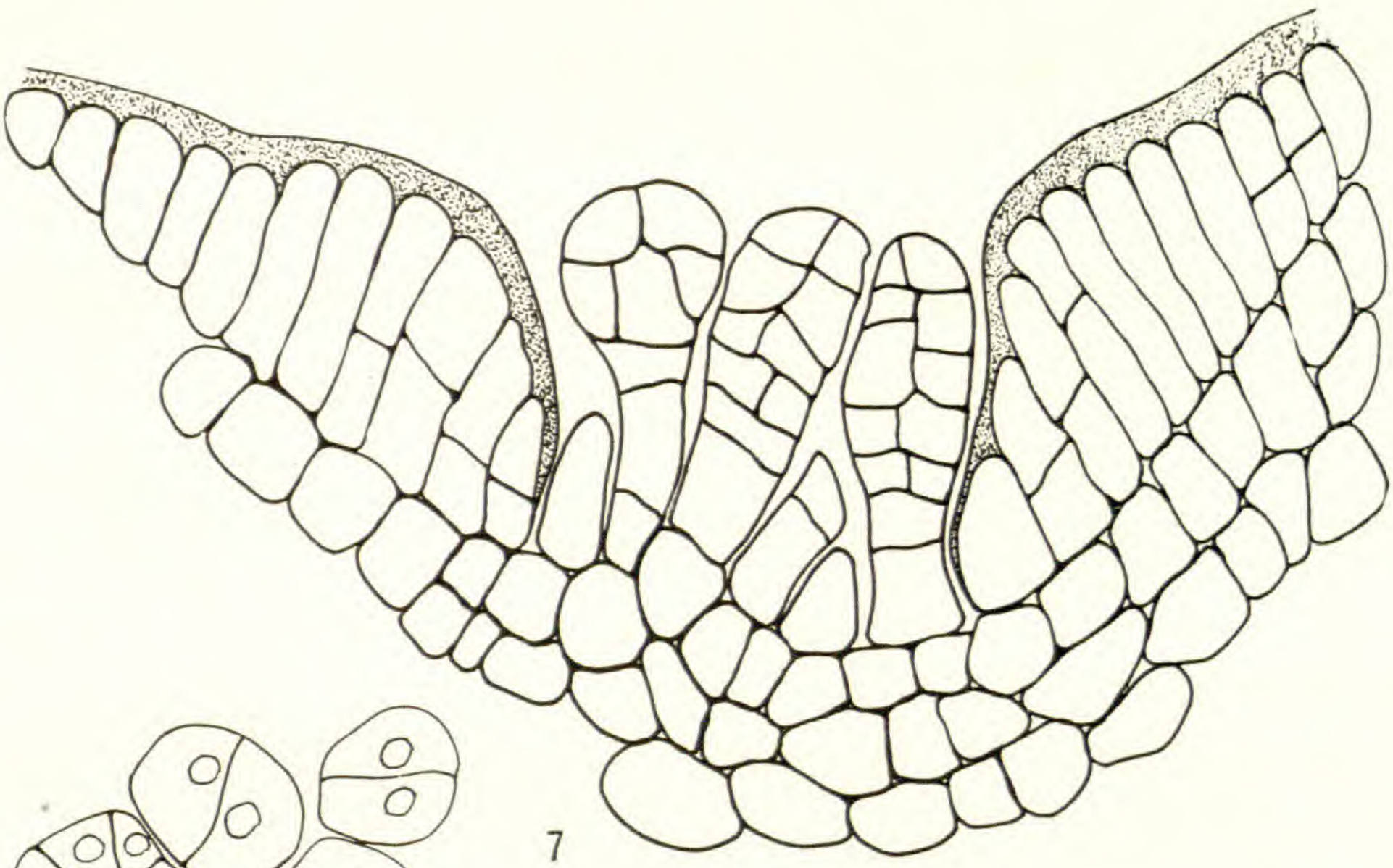
REED on LEAF NECTARIES



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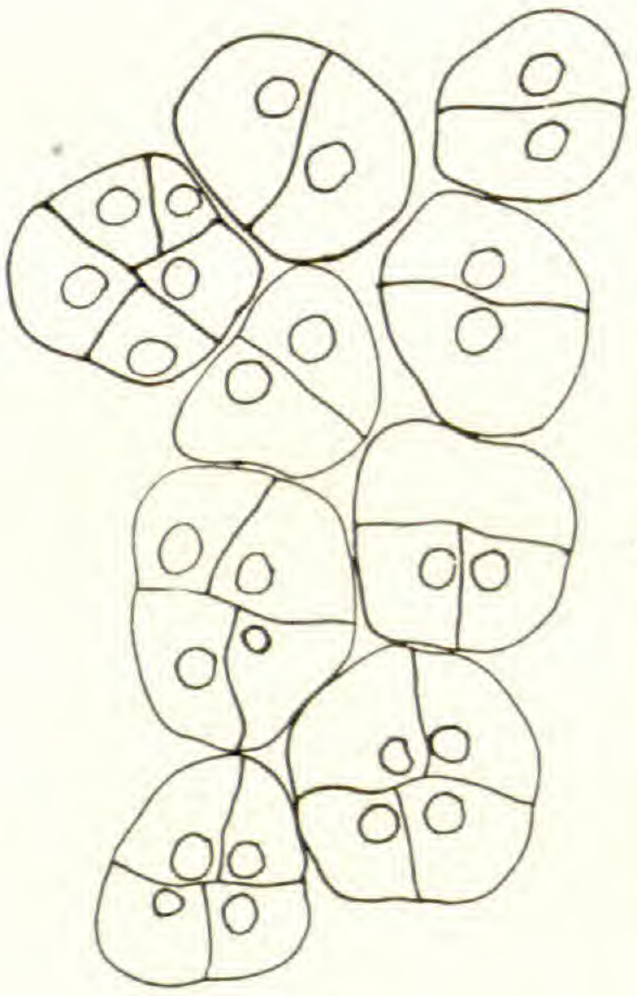

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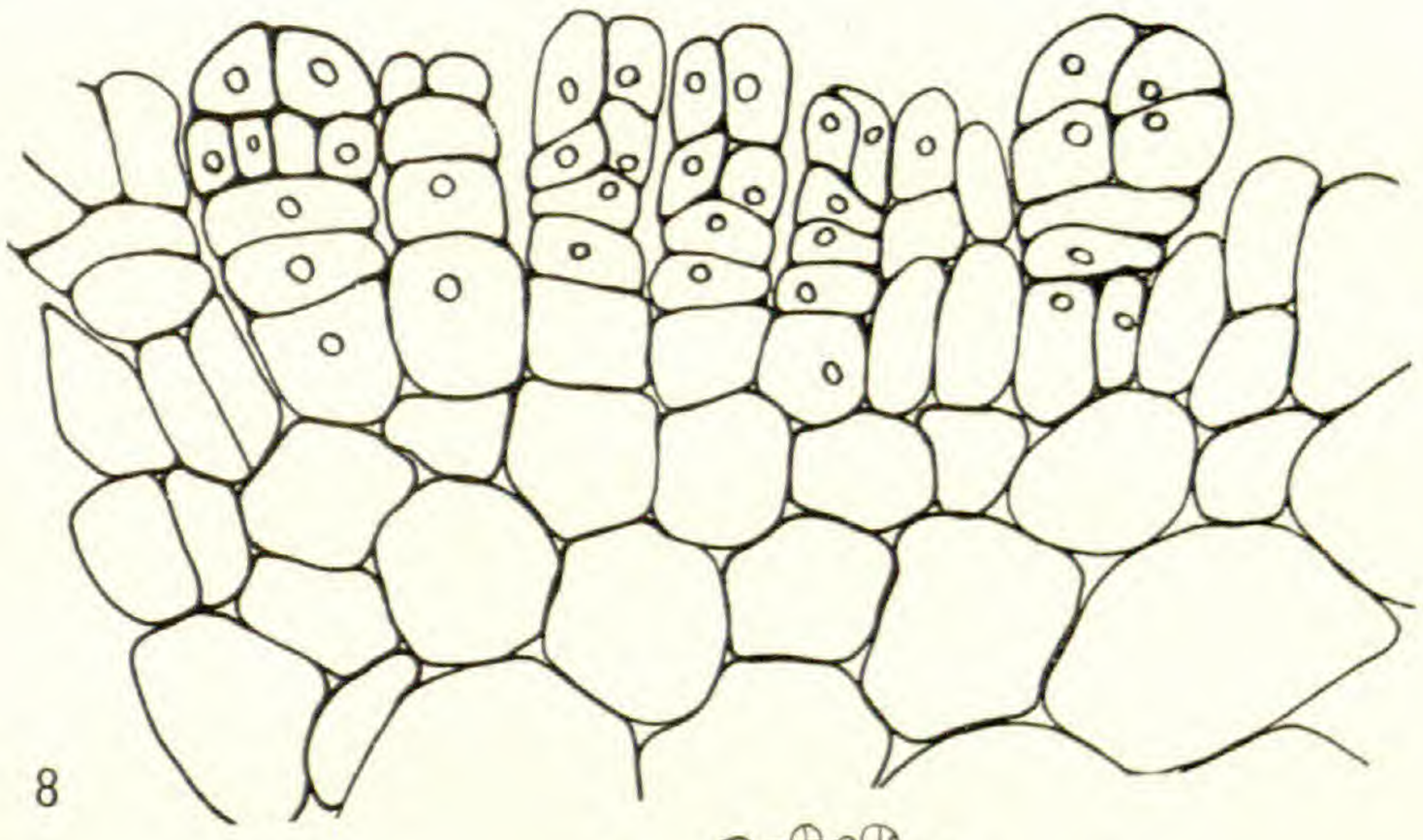

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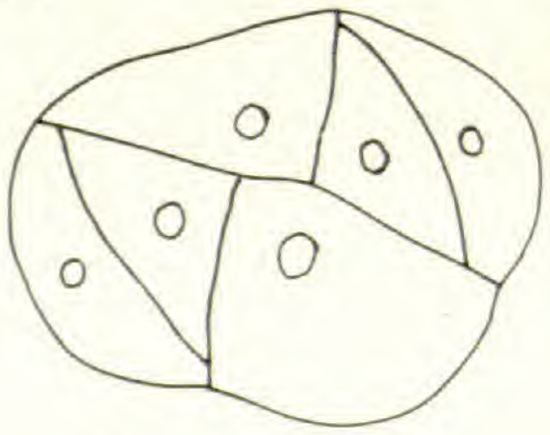

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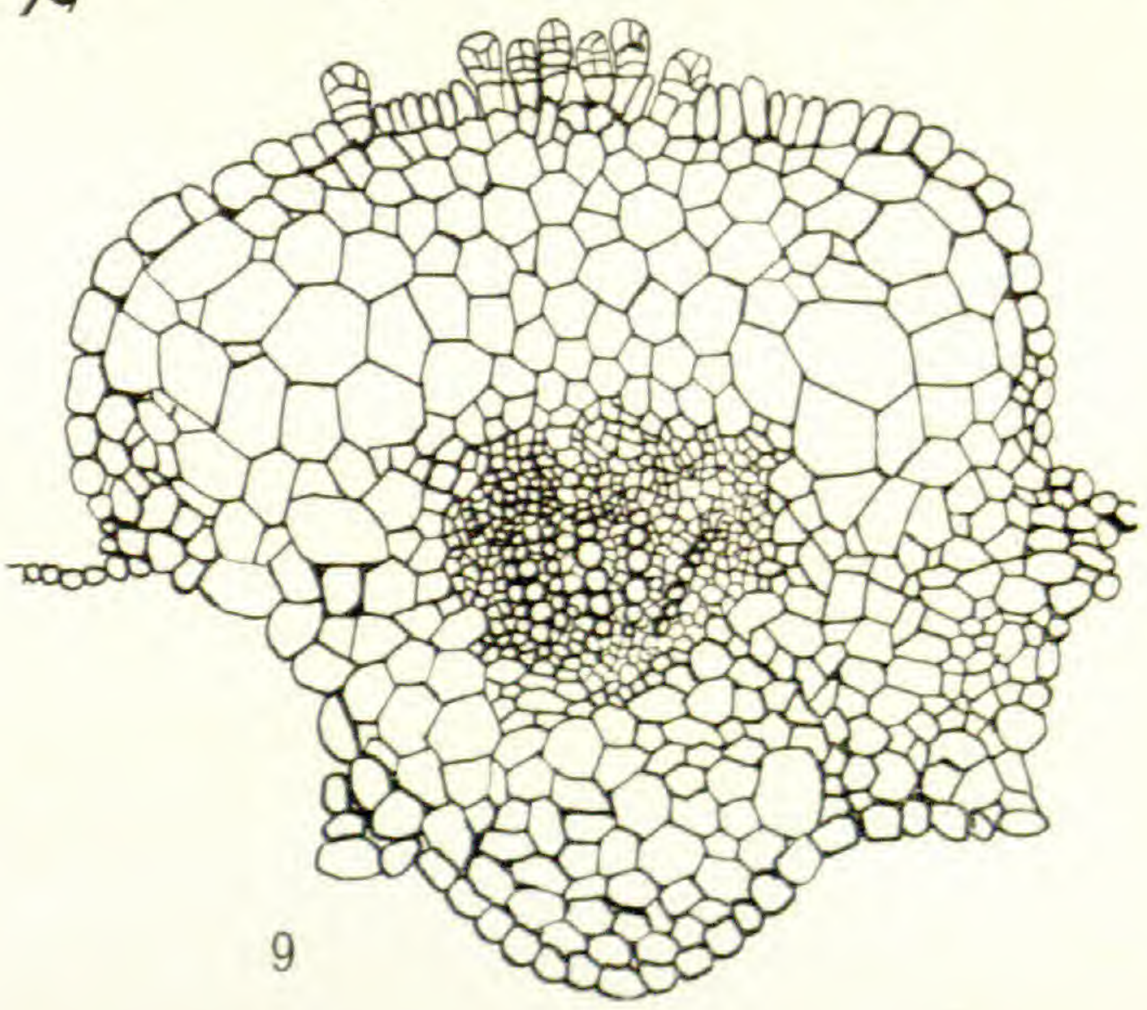

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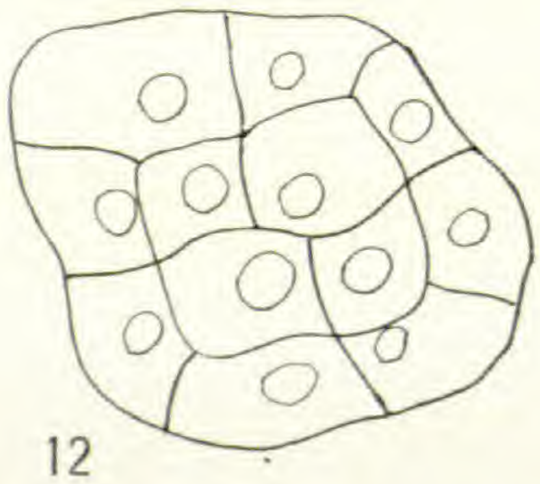

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REED on LEAF NECTARIES

LITERATURE CITED

1. TAYLOR, FREDERICK J., The nectaries of cotton. Bull. no. 131, Part V, Bur. Plant Ind., U.S. Dept. Agric.
2. WATT, GEORGE, Wild and cultivated cotton plants of the world.
3. TRELEASE, WILLIAM, Nectar, its nature, occurrence, and uses. Published in JOHN HENRY COMSTOCK's Report on cotton insects.
4. SAFFORD, W. EDWIN, Useful plants of Guiam.
5. CAMPBELL, D. H., Mosses and ferns.

EXPLANATION OF PLATES XII AND XIII

PLATE XII

FIG. 1.—From transverse section of leaf showing papillate cell.

FIG. 2.—First cell of pedestal cut off by cross wall.

FIGS. 3, 4, 5, 6.—Multicellular papillae showing pedestals with varying number of cells.

PLATE XIII

FIG. 7.—Vertical section through gland near one end showing papillae and elongated cells; some of the latter have divided horizontally, and have formed the wall of the gland.

FIG. 8.—Vertical section through young gland showing first stage in formation of wall (right side of figure).

FIG. 9.—Vertical section through midrib showing young papillae.

FIG. 10.—Cross-section of several papillae showing first and second vertical division.

FIG. 11.—Cross-section of papilla showing periclinal walls.

FIG. 12.—Cross-section of mature papilla showing divisions subsequent to those shown in fig. 11.

THE REACTION OF PLANT PROTOPLASM

A. R. HAAS

The reaction of protoplasm is one of the most important factors of metabolism. It is not, however, the apparent reaction (or total acidity), as shown by titration, which is of chief importance, but the actual reaction, as shown by the gas chain or by indicators. The total acidity includes both undissociated and dissociated acid, while the actual reaction depends only upon the latter.

In the case of a buffer solution[1] the total acidity may be very high, while the actual acidity may be very low. The higher the total acidity in this case the more difficult it becomes to change the actual reaction by the addition of acid or alkali. This applies to protoplasm, which always has the properties of a buffer solution (since it contains carbonates, phosphates, proteins, etc.). Hence if a low actual acidity is advantageous to the protoplasm, a high total acidity may be favorable, in that it preserves this desirable actual acidity.

So far as the writer is aware, no determinations of the actual reaction of plant protoplasm have been made by means of the gas chain (except a single determination of pineapple juice made by REED[2]). Only a few determinations have ever been made[3] by means of indicators.

The writer has investigated the reaction of a number of plants, including some which seemed to be of special interest on account of their unusual acidity. The method employed was to crush the cells and to determine the acidity of the juice by means of the gas chain. The gas chain used was essentially the form described by HILDEBRAND.[4]

[1] Cf. HÖBER, H., Physikalische Chemie der Zelle und der Gewebe (pp. 118, 169). Berlin. 1914.

[2] Unpublished results.

[3] Cf. FRIEDENTHAL, H., Zeit. Allg. Physiol. 1:56. 1902. It is not known whether in these experiments the proper precautions were taken to crush all the cells and to secure plant juices which had as high an acidity as the cell contents.

[4] HILDEBRAND, J. H., Jour. Amer. Chem. Soc. **35**:869. 1913.

The method of obtaining the juice is of considerable importance. It has been shown by MAMELI,[5] by MARIE and GATIN,[6] and by DIXON and ATKINS,[7] that when tissue is crushed the juice which is first expressed contains a much lower concentration of electrolytes than that which is obtained when greater pressure is applied so as to crush more of the cells. It is obvious that when pressure is first applied and sap is squeezed out through the intact plasma membrane, the electrolytes may largely be retained within the cell because they are not able to pass freely through the membrane. It is desirable, therefore, to grind the tissue and rupture all of the cells. This was accomplished by thoroughly grinding the tissue in a mortar. Only a little tissue was ground at a time, and the grinding was continued until microscopic examination showed that all the cells were ruptured.

TABLE I

Material	Actual acidity of CO_2-free undiluted juice as determined by the gas chain	Total acidity as determined by ordinary titration methods
Lemon (fruit)	0.006N	
Cranberries (fruit), fresh material, peeled and unpeeled	0.004N	0.9172N (ripe fruit) 0.3194N (overripe, soft fruit) 0.3493N (overripe, soft fruit)
Grapefruit (fruit)	0.001N	0.1927N
Apple (fruit)	0.0004N	0.0711N
Rhubarb (leaf-stalk) basal part	0.0007N	
Rhubarb (leaf-stalk) intermediate part	0.0005N	0.1578N
Rhubarb (leaf-stalk) green part below leaf blade	0.00022N	0.1681N
Orange (fruit)	0.00016N	0.0941N
Pineapple (ripe fruit), juice standing 2 hours	0.00009N	
Pineapple (ripe fruit), fresh juice	0.000035N	0.1377N
Green pepper (fruit)	0.0000038N	
Eggplant (fruit)	0.000002N	

Before determining the acidity of the juice, the CO_2 was driven off by means of a current of hydrogen. The results of the determinations are given in table I. The results in all cases represent the average of two or more closely concordant determinations.

[5] MAMELI, E., Atti Ist. Bot. Univ. Pavia **12**:285. 1908.

[6] MARIE, C. H., and GATIN, C. L., Déterminations cryoscopiques effectuées sur des sucs végétaux. 1912.

[7] DIXON, H. H., and ATKINS, W. R. G., Proc. Roy. Dublin Soc. **13**:422. 1913.

The figures of the table show that the actual acidity bears no fixed relation to the total acidity, and that great variations are to be found in different plants, as well as in different tissues of the same plant.

The figures for actual acidity are surprisingly high in the case of the lemon and of the cranberry, especially in view of the prevalent opinion that protoplasm demands a neutral or nearly neutral reaction for normal metabolism. It is of interest, therefore, to inquire whether the figures represent the actual acidity of the protoplasm. In the lemon the acid juice is contained in sacs, the walls of which are composed of living cells, while the cavity is produced by the disintegration of cells. REED[8] has shown that the living cells of the walls contain oxidases whose activity is promptly inhibited by the acid contained in the cavity of the sac. Since the oxidases are active in the living cells, it follows that the protoplasm is by no means as acid as the juice in the sacs, and hence the figures given in table I cannot apply to the protoplasm in the case of the lemon.

With the cranberry the case seems to be different. In this fruit there are no sacs such as are found in the lemon; the juice is contained entirely in the cells. It is important, therefore, to ascertain whether these cells are dead or alive. In order to test this, the outer colored layer of cells was removed and the following results were obtained on the colorless cells:

1. Eosin failed to penetrate unboiled peeled cranberries, but penetrated readily into the boiled peeled cranberries. The difficulty due to the precipitation of eosin in acid solutions was obviated by frequent renewal of the eosin solution.

2. Ripe cranberries can be peeled without staining the white tissue beneath, while this is not possible in overripe, soft cranberries.

3. Unboiled, peeled cranberries in a red watery extract of cranberry peeling were unstained after several hours, while the indicator readily penetrated the boiled peeled cranberries.

All these tests go to show that the living cells of the cranberry have an actual acidity which is extremely high. It is quite pos-

[8] REED, G. B., BOT. GAZ. **57**:528. 1914.

sible, however, that the acid sap is contained in vacuoles rather than imbibed in the protoplasm proper.

Summary

1. The actual acidity and the total acidity of a number of plant tissues were determined.

2. There is no constant relation between the two, but great variations occur in different plants and in different parts of the same plant.

3. In one case (cranberry fruits) the surprisingly high actual acidity of 0.004N (as determined by the gas chain) was found in the living cells.

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BRIEFER ARTICLES

A FREEZING DEVICE FOR THE ROTARY MICROTOME

(WITH ONE FIGURE)

A few years ago OSTERHOUT[1] figured and published an account of a simple freezing device to be used in connection with various sorts of sliding microtomes. Later[2] he published an account of a simple freezing microtome in which he made use of a knife of a plane for cutting on account of its rigidity. In each of these devices, the freezing chambers being stationary, they are both adaptable to the use of brine, carbon dioxide, or other substances for freezing. These devices have been of considerable service in the preparation of sections of living tissues. However, if one wishes sections in large quantity and of uniform thickness, and particularly if thin sections are desired, it is found that a sliding microtome of almost any construction is inadequate, and to manipulate it requires considerable dexterity.

It occurred to the writer that the OSTERHOUT apparatus for freezing with brine might be modified in such a way as to make it usable with a rotary microtome of any make, and thereby increase its efficiency and enlarge the usefulness of both pieces of apparatus. The adaptation was made and the results have proven so satisfactory that a brief account of the apparatus seems desirable.

The accompanying photograph of the apparatus will serve as a basis for the description. It is simple and easy to construct, consisting of a 2×10 board 3.5 ft. long for the base, and 2 upright pieces fastened at the base and braced by a cross-piece about one-third of the distance up. A bolt passes through the base and the center of the cross-piece, and another through the upright pieces just above the cross-piece to make the apparatus firm. The upright has been lengthened in this case to receive larger receptacles than were originally used. The wheel is 20 inches in diameter and 1.5 inches thick, making the whole device about 5 ft. high. The wire to hold the pails is firmly fastened in the middle to the grooved wheel. The rubber tubing is of stiff white rubber. When

[1] OSTERHOUT, W. J. V., A simple freezing device. BOT. GAZ. **21**:195–201. *figs. 6.* 1896.

[2] ———, Univ. Calif. Publ. Bot. **2**:73. 1904.

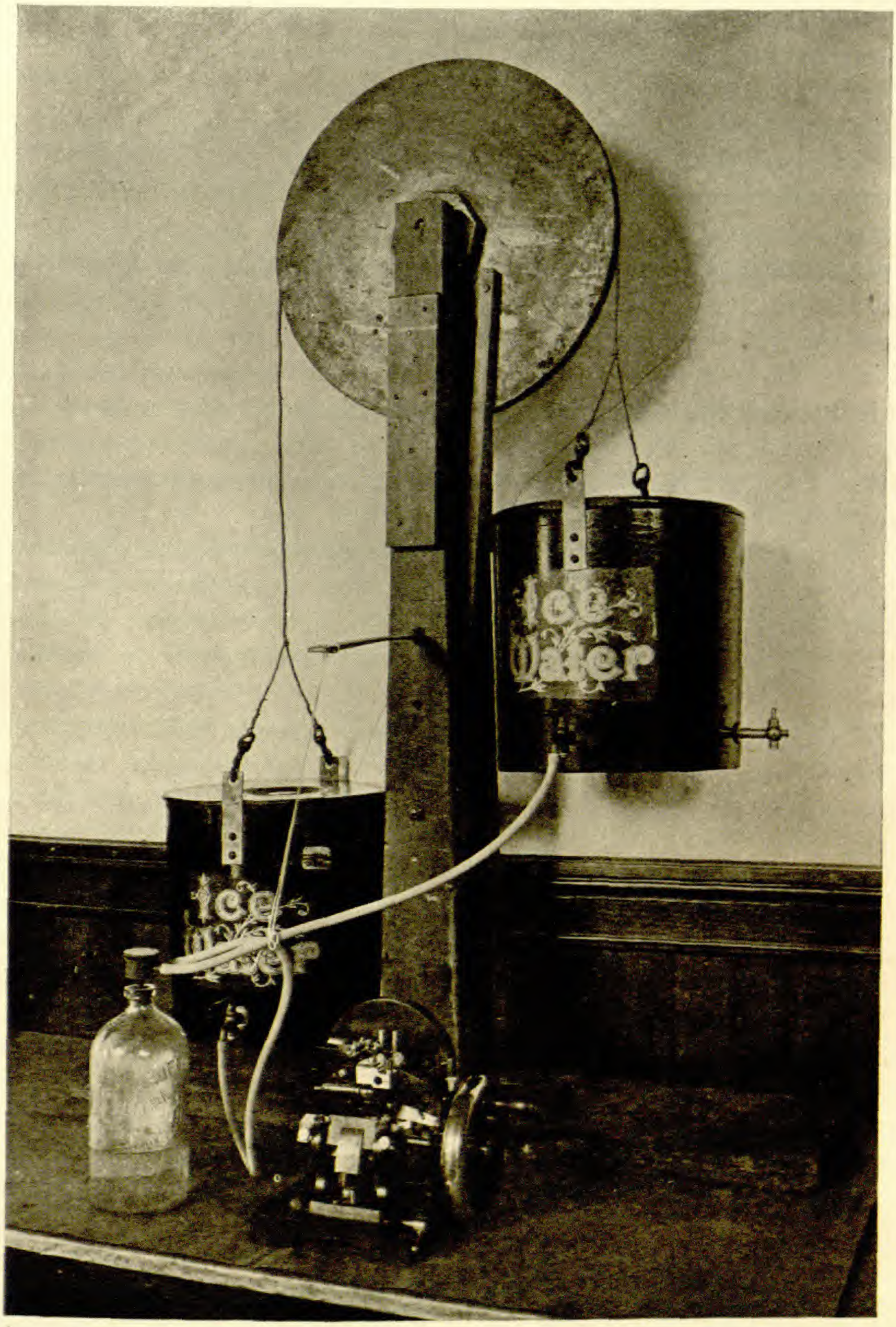

FIG. 1

the freezing mixture is put in, the 2 pails should balance. An important though simple detail is the proper adjustment of the brace to hold one of the pails up until the water runs through the tubes and freezing box into the other pail. This, as is shown in the accompanying illustration, is made of a piece of hard wood about 0.75 inch square, fastened at the upper end to the wheel by a heavy screw, the hole in the brace being large enough so that it may move freely. The brace must be long enough for its lower end to rest on the cross bar when the lower pail is about 3 inches above the base board. When the lower pail receives over half of the water it will move slowly to the base board, the lower end of the brace will pass over the cross bolt and hang perpendicularly by the side of the higher pail. As soon as this pail is emptied it should be lowered, and this should be attended to with promptness, for it is necessary that the water be kept in constant circulation to obtain the maximum freezing efficiency.

A very important part of the apparatus of course is the freezing chamber. This will have to be made to order to fit the particular microtome one is using, and the size depends upon one's needs. The one which seems to be of general use and which is employed by the writer is constructed as follows: A rod of brass about 2.25 inches long is hollowed out about 1 inch deep for the chamber, leaving walls thick enough so that a firm cap can be screwed on. The other end of the rod is trimmed down, making a stem of the desired size to fit the particular microtome. Two tubes with inside diameters 7–8 mm. and 0.75 inch long are welded into the chamber a few millimeters apart on one side. The faucets, one in each pail, should be large enough for a free flow of the water into the tubes and should be shielded on the inside of the pail by copper gauze. A second faucet should be put into one pail to be used to remove surplus water.

The freezing chamber, of course, must be in a horizontal position while the object is being frozen. A half gallon bottle with a hole in the cork large enough to receive the stem as shown in the illustration is very convenient.

It is highly desirable to have a section collector if one is cutting much material. This may be made of a block of wood about 1 inch thick, hollowed out on one side, leaving enough margin on 3 edges around the cavity so that it will fit snugly against the knife, a little vaseline being used to prevent leaking. The box may be clamped on in various ways. If this chamber is nearly filled with cold water, the sections will slide down into it and melt, after which they may all be poured out together

by removing the knife carrier and all attached thereto from the remainder of the machine. Gum arabic of medium consistency is used to freeze the objects in. A layer 2–3 mm. thick should be frozen on the chamber before placing the object on for cutting. By this means it is possible to obtain a large number of sections in a very short time, greatly facilitating the study of algae, fungi, and other soft tissues of either plants or animals in which only cell forms and cell relations are being studied. Likewise it is exceedingly useful in preparing cross and longitudinal sections of leaves, soft stems, etc., for class use. It is also inexpensive. One can run the machine 8 hours with no difficulty on 40 lbs. of ice.

In orienting the material for cutting, 2 methods may be followed. Segments of the material may be piled on top of each other on the freezing chamber and covered with gum arabic and frozen. After trimming to the desired form the material may then be removed, properly oriented, and quickly refrozen to the chamber. This is desirable only in cases in which the material is too delicate to stand on end or on edge if cross-sections are to be made. In the other method one takes the material, for example, segments of leaves a few millimeters long, dampens them in gum arabic, and piles them one upon another on a knife blade, after which the whole pile is tipped over onto smooth frozen gum on the freezing chamber. With a little care the whole pile may be made to stand on edge and may be frozen in position for cutting cross-sections.—N. L. GARDNER, *University of California.*

CURRENT LITERATURE

NOTES FOR STUDENTS

Phenomena of parasitism.—Two further contributions to a series of studies begun by BROWN[1] on the parasitism of *Botrytis cinerea* have appeared. In the first of these, BLACKMAN and WELSFORD[2] describe the microscopical details of the process of penetration of the cuticle by the germ tubes; in the second, BROWN[3] deals more specifically than in his former paper with the action on the cuticle of extracts and exudates of the germ tubes.

BLACKMAN and WELSFORD observed in the earliest stages of penetration a slight indentation of the outer epidermal wall as a result of the action of the germ tube, which is held fast to the cuticle by a mucilaginous sheath whose presence was made evident by means of a suspension of silver particles. The actual penetration of the cuticle is accomplished by a narrow peglike outgrowth from the tip of the germ tube. No swelling of the cuticle or of the subcuticular layers previous to penetration was observed, and in no case was an injury to the epidermal cells or subepidermal cells apparent before the breaking of the cuticle. Soon after the penetration of the epidermis, the cells of the palisade layer begin to disintegrate, and with the advance of the hypha the cells of the spongy parenchyma also are killed. The toxic action of the fungus extends considerably beyond the region actually invaded. After a portion of the leaf tissue had been killed, other hyphae were observed to penetrate through the stomata, probably as a result of the diffusion of food substances from the dead cells, for primary infection though a stomate was never seen.

From their observations the authors conclude that the cuticle is ruptured by mechanical pressure exerted by the germ tube and not by the solvent action of any substance secreted by it. They believe that the germ tube is enabled to exert the pressure necessary for the indentation of the cell wall and penetration of the cuticle by virtue of the gelatinuous sheath which holds the germ tube in place. It is not clear, however, how the germ tube is thus enabled to bring about an indentation of the cell wall over an area more extensive than that covered by the tip of the tube itself, as shown in some cases (notably

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[1] Rev. BOT. GAZ. **61**:79. 1916.

[2] BLACKMAN, V. H., and WELSFORD, E. J., Studies in the physiology of parasitism. II. Infection by *Botrytis cinerea*. Ann. Botany **30**:389–398. *pl. 10. figs. 2.* 1916.

[3] BROWN, WM., Studies in the physiology of parasitism. III. On the relation between the infection drop and the underlying host tissue. Ann. Botany **30**:399–406. 1916.

fig. 8). It appears not improbable that these may be accidental depressions, for in many cases of actual penetration figured such indentations are not evident.

In the study of the action on the cuticle of extracts and exudates of germ tubes, BROWN found that when the extract of germ tubes was placed in considerable quantity on intact leaves and petals of *Viola*, *Petunia*, *Dahlia*, *Vicia Faba*, and *Begonia heraclaefolia*, no effect was produced; but in experiments with *Tropaeolum*, *Geranium*, *Rosa*, and *Fuchsia* a varying number of discolored spots appeared on the surfaces covered by the drops. The action in these cases was attributed to possible wounds in the cuticle. All the extracts were tested also on wounded leaves and petals, and in those cases in which no action was observed, the corresponding experiments on uninjured leaves and petals were rejected. Thus conclusions were drawn only from extracts known to be active.

When spores were sown in drops of liquid on the surface of leaves, the discoloration appeared first around the margin of the drops where the spores germinated earliest. When such drops, containing germinating spores, were displaced slightly on the leaf, the discoloration due to the action of the spores appeared within the area originally outlined by the drop and none in the new area occupied. Infection drops cleared of spores had no action on the most sensitive petals.

With reference to the possibility of the production of oxalic acid in sufficient quantity to cause the death of tissues under the uninjured cuticle, BROWN found that solutions of $n/40$ oxalic acid and of $n/20$ potassium oxalate placed on the leaves had no effect within a period of 12 hours, the time required for the germinating spores to produce discoloration. The maximum concentration in the infection drops, it was shown, could not exceed $n/800$.

These experiments seem to show quite clearly that cuticle-dissolving substances are not present in the extracts made from germ tubes of *Botrytis cinerea*, and that such substances, if they exist, do not diffuse into the surrounding medium to any considerable extent. The conclusion that chemical action is entirely excluded seems somewhat too sweeping, however, for there still remains the possibility of such action at the point of contact of the germ tube with the cuticle by substances which cannot be obtained in extracts in an active state. The possibility that oxalic acid occurs in sufficient quantities to injure cells through the cuticle seems to be definitely excluded.

The observation that the germ tubes of *Botrytis cinerea* exude no substances which are capable of diffusing through the cuticle and killing the cells below corroborates the histological study of BLACKMAN and WELSFORD, according to which the cells underlying the cuticle are not injured before the cuticle has been perforated. In this respect, the behavior of *Botrytis cinerea* differs from that of *Sclerotinia Libertiana*, in which DEBARY observed a killing of the host cells before penetration of the cuticle.—H. HASSELBRING.

Leaf size in plant geography.—RAUNKIAER,[4] whose name is associated with the system of biological types or life forms, has recently submitted another means of quantitative estimation, so far as the unit chosen is a recorder of the biological value of a climate. He regards the size of the leaf as the outstanding character, and using the simple leaf as a standard, has proposed a system of leaf classes (Bladstørrelsesklasser). In the plan submitted there are 6 different classes or divisions: (1) leptophyll, 25 sq. mm.; (2) nanophyll, $9\times25=225$ sq. mm.; (3) microphyll, $9^2\times25=2025$ sq. mm.; (4) mesophyll, $9^3\times25=18225$ sq. mm.; (5) macrophyll, $9^4\times25=164025$ sq. mm.; (6) megaphyll, which is limited by the upper limit of macrophylls. Originally he planned to use the number 10 with 25, but from a large number of trials, both by himself and several of his colleagues, 9 was found to give a better differentiation. In using 9, it is easy to make subdivisions, large, medium, and small, if desired. RAUNKIAER is of the opinion that it is an easy matter to place the various leaves in their right classes, but in order to facilitate matters, a graphical representation of the various limits of surface area is pictured, and by the use of this scheme the leaves may be correctly grouped. Thus, if a leaf has an area which is less than 25 sq. mm., it is a leptophyll; if larger than 25 sq. mm. but smaller than 225 sq. mm., it is a nanophyll, and so on.

In using such a method, RAUNKIAER contends that it is possible to obtain the biological factor for climate as far as it influences leaf size. By the use of such a scheme, comparisons may be made readily between two climates which have varying effects. One may compare formations which vary at different points, and also determine the relation between a series of associations which are somewhat similar. To prove his point he has selected and analyzed several European evergreen shrub formations.

He suggests that the leaf "size classes" are not the only quantitative units to be employed, but shows that these units lend themselves rather readily to the statistical method. A system which would in some way estimate such structural features as stomatal protection, stomatal opening, or hairiness, would also give significant results. The difficulties would naturally be many, but they should not hinder the attempt.

Ecologists and physiologists no doubt will be in hearty sympathy with RAUNKIAER'S move in placing ecology upon a basis that is at least somewhat quantitative. We all are in accord with his concluding sentence (translated somewhat literally): "by such means only will it be possible to pass beyond the tourist plant geographer's superficial and vague determinations."—A. L. BAKKE.

Mountain grassland.—Many of the valleys of the Colorado Rocky Mountains have their comparatively level floors covered with grasslands of somewhat

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[4] RAUNKIAER, C., Om Bladstørrelsens Anvendelse i den biologiske Plantegeografi. Botanisk Tidsskrift **34**:225–240. 1916.

varied types, presenting ecological problems of peculiar interest. The more xerophytic type of such grassland has been studied in South Boulder Park by RAMALEY[5] and by him designated "dry grasslands" in contrast to the more mesophytic "meadow." One of the most interesting problems of the park is the relationship of these two phases of grassland, and one must regret that it has been so slightly touched upon in the present paper. Another deficiency is the limited number of data regarding the environmental factors. Some soil moisture studies seem to show that the growth water is not abundant in any association, although unfortunately the relationship of the soils of which wilting coefficient determinations were made and those whose water content were studied is not clearly apparent. Wilting coefficients ranging from 3.5 to 7.6 indicate to some extent the coarse texture and low water-retaining power of the soil which, combined with such climatic factors as short summers, high winds, and an annual rainfall of 28 inches, tend to retard the development of mesophytic vegetation.

A most interesting seasonal succession is described, ranging from a prevernal period extending from May 1 to June 15 and characterized by the blooming of *Mertensia Bakeri* and *Thlaspi purpurascens*, through well marked vernal, early and late aestival, to an autumnal in which the bloom is almost entirely limited to late grasses and blue gentian.

The series of associations involved in the xerarch succession here in progress proceeds from one characterized by *Erigeron multifidus* and *Selaginella densa* on recently exposed soil, through others in which *Carex stenophylla* associated with certain Leguminosae and Compositae such as *Aragallus Lambertii* and *Chrysopsis villosa* gradually give place to others in which grasses become increasingly abundant and important. The author regards the ultimate grassland vegetation as an association in which the grasses represented by species of *Muhlenbergia*, *Danthonia*, *Poa*, and *Festuca* predominate. Whether this will pass eventually to the more mesophytic meadow, and it in turn be replaced by forest, seems at present to be a probability not demonstrated. In spite of this and other unsolved problems, the present discussion, together with the careful analyses of the same author[6] previously published, very greatly advances our knowledge of these interesting grasslands.—GEO. D. FULLER.

Taxonomic notes.—BERRY[7] has described a new species of *Zamia* (*Z. mississippiensis*) from the Lower Eocene of Missisippi. It has "slender, graceful leaves and much reduced pinnules suggestive of *Z. floridana*."

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[5] RAMALEY, F., Dry grasslands of high mountain park in northern Colorado. Plant World **19**:249–270. *figs. 6.* 1916.

[6] ———, The relative importance of different species in a mountain grass-land. BOT. GAZ. **60**:154–157. 1915.

———, Quadrat studies in mountain grassland. BOT. GAZ. **62**:70–74. 1916.

[7] BERRY, E. W., A *Zamia* from the Lower Eocene. Torreya **16**:177–179. *figs. 3.* 1916.

BLAKE,[8] in "A revision of the genus *Polygala* in Mexico, Central America, and the West Indies," recognizes 137 species, 39 of which are described as new. There are also numerous new combinations and new names, and a general reorganization of the classification.

BRITTON,[9] in connection with an account of the vegetation of "the little known island of Anegada," one of the Virgin Islands, has described a new *Acacia* (*A. anegadensis*) and a new lichen (*Arthonia anegadensis*).

BRITTON,[10] in his eighth paper on West Indian plants, describes a new *Cyperus* from Jamaica; lists the West Indian species (16) of *Stenophyllus*, including a new species; lists the Cuban species (15) of *Galactia*, with 4 new species; lists the Cuban species (5) of *Machaonia*, with 2 new species; presents the Cuban genus *Heptanthus*, recognizing 6 species, 5 of which are new; and publishes 5 new species from Porto Rico, 9 new species from Cuba, and 21 new species from the Isle of Pines, by several specialists.

BURT,[11] in continuing his studies of North American Thelephoraceae, has monographed the genus *Hypochnus*, recognizing 31 species, 13 of which are new species, and 12 are new combinations.

BURT,[12] in his seventh paper on the Thelephoraceae of North America, presents the genus *Septobasidium*. It does not belong to the Thelephoraceae, because its basidia are not simple, but it is included "merely for the convenience of students of the Thelephoraceae." The North American forms include 17 species, 10 of which are described as new.

CHRISTENSEN[13] has described a new genus (*Maxonia*) of ferns founded on *Dicksonia apiifolia* Swartz. The species (*M. apiifolia*) is represented by specimens from Jamaica and Cuba, while a variety (*M. apiifolia duale*) occurs in Guatemala, and is *Nephrodium duale* Donn. Smith.

DIXON[14] has reported upon a collection of mosses from Borneo, showing that our knowledge of the moss flora of the tropics is comparatively meager. The list includes 133 species, 13 of which are described as new. Attention is called especially to the "peculiar ecological distribution of the remarkable and striking genera *Syrrhopodon* and *Calymperes*."—J. M. C.

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[8] BLAKE, S. F., Contrib. Gray Herb. no. 47. pp. 122. *pls.* 2. 1916.

[9] BRITTON, N. L., The vegetation of Anegada. Mem. N.Y. Bot. Gard. **6**:565–580. 1916.

[10] ———, Studies of West Indian plants. VIII. Bull. Torr. Bot. Club **43**:441–469. 1916.

[11] BURT, E. A., The Thelephoraceae of North America. VI. Ann. Mo. Bot. Gard. **3**:203–241. 1916.

[12] ———, The Thelephoraceae of North America VII. Ann. Mo. Bot. Gard. **3**:319–343. *figs.* 14. 1916.

[13] CHRISTENSEN, CARL, *Maxonia*, a new genus of tropical American ferns. Smiths. Miscell. Coll. **66**:no. 9. pp. 4. 1916.

[14] DIXON, H. N., On a collection of Bornean mosses made by the Rev. C. H. BINSTEAD. Jour. Linn. Soc. Bot. **43**:291–323. *pls.* 26, 27. 1916.

Mosaic disease of tobacco.—ALLARD[15] has recently presented good evidence to combat the theory of WOODS and of HEINTZEL that oxidases are responsible for the mosaic disease of tobacco, in which he showed that the disease was dependent upon a specific infection. A more recent paper by ALLARD[16] describes in detail a study of the properties of the so-called "virus" of the mosaic disease of tobacco. Healthy plants were inoculated with the virus after filtration through a Livingston atmometer porous cup, after filtration through powdered talc, after precipitation with ethyl alcohol, after treatment with formaldehyde, with hydrogen peroxide, with precipitates of aluminum and nickel hydroxides, and after subjecting the virus to high and low temperatures. Plants were inoculated also with water extracts of the dried mosaic tobacco, made after extracting with ether, chloroform, and other solvents. The infectious principle was retained by filtration through Livingston atmometer porous cups and by powdered talc, although the filtrates gave intense peroxidase reactions. Alcoholic solutions of 75–80 per cent destroyed the infective principle, while 45–50 per cent solutions did not, but carried down the infectious principle with the precipitate. Virus treated with one part formaldehyde in 800–1500 parts of solution gave an infection. Stronger solutions gave no infection, although they still gave strong peroxidase reactions. Ether, chloroform, carbon tetrachloride, toluene, and acetone failed to extract either the infective principle or the peroxidase from dried material. The virus was killed at temperatures near 100° C., but when subjected to a temperature of −180° C. for 15 minutes it was not weakened. In every case controls were carried out with tap water and with the untreated virus. From the results the author concludes that neither enzymes nor the constituents of healthy sap can be responsible for the disease, and that since the pathogenic agent is highly infectious and capable of increasing definitely, there is every reason to believe that it is an ultramicroscopic parasite of some kind.—H. R. KRAYBILL.

Fossil Osmundaceae.—KIDSTON and GWYNNE-VAUGHAN[17] have described three species of fossil *Osmundaceae*, two of which are respectively from the Tertiary of Spitzbergen and of Queensland. Another species, *Osmundites Carnieri*, between the Tertiary and Jurassic of the Andes of Paraguay, is most interesting. The authors add something to the original descriptions of SCHUSTER from whom they received their material. The stem unfortunately is not well preserved, but the endodermis frequently joins around the margins of the leaf gaps, and an internal phloem was also probably originally present. The

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[15] ALLARD, H. A., The mosaic disease of tobacco. U.S. Dept. Agric. Bull. 40. 1914.

[16] ———, Some properties of the virus of the mosaic disease of tobacco. Jour. Agric. Research **6**:649–674. 1916.

[17] KIDSTON, R., and GWYNNE-VAUGHAN, D. T., On the fossil Osmundaceae. Part V. Trans. Roy. Soc. Edinburgh **50**:469–480. *pls. 41–44.* 1916.

stem strongly resembles that of *Osmundites skidegatensis* from the western coast of Canada (Lower Cretaceous).

In a second paper, GWYNNE-VAUGHAN[18] has described the effect of injury on a narrow stele of *Osmunda regalis*. Tracheids appear in the central region of the stele. The author regards this as evidence of the stelar origin of the pith in the Osmundaceae. The voluntary blindness of British anatomists as regards medullary structures is an interesting phenomenon. They welcome the small amount of evidence which can be brought forward for the stelar origin of the pith, and close their eyes to the overwhelming evidence for its derivation from the fundamental system of tissues. The equitable procedure seems to give the same value to both kinds of evidence, and decides the question on the quantitative basis. We may record here the regret that American anatomists all feel for the untimely death of the junior author GWYNNE-VAUGHAN, whose published work is of such promise. An appreciative obituary has recently been published by SCOTT in the *Annals of Botany*.—E. C. JEFFREY.

Monomeric capsules in Bursa.—The reviewer has shown[19] that the triangular capsule of *Bursa bursa-pastoris* is produced independently by two distinct Mendelian factors (dimery), and has expressed the view (1914) that this is a derivative condition, the original form of this species probably having had only one of these factors. A considerable number of wild plants have been investigated, but as yet only one specimen has been found by the reviewer which had but one of the capsule factors, this case being still unpublished. DAHLGREN[20] has investigated a plant of this species growing in the botanical garden at Upsala, and secured from a cross with *B. Heegeri* (which lacks both of the factors for inflation of the capsules) an F_2 progeny consisting of 71 *B. bursa-pastoris* and 17 *B. Heegeri*. One of these F_2 plants produced in the F_3 16 plants having triangular capsules and 3 with turbinate capsules, thus showing that without doubt the *B. bursa-pastoris* used in this cross had monomeric capsules. There remains one important question which the author fails to mention. As *B. Heegeri* has been widely distributed in botanical gardens, it is not improbable that it had been growing in the garden at Upsala for years. If the plant used by DAHLGREN were a derivative of an earlier, *natural* cross between *B. bursa-pastoris* and *B. Heegeri*, its possession of but one of the capsule

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[18] GWYNNE-VAUGHAN, D. T., On a "mixed pith" in an anomalous stem of *Osmunda regalis*. Ann. Botany **28**:351–354. *pl. 21*. 1914.

[19] SHULL, G. H., *Bursa bursa-pastoris* and *Bursa Heegeri:* biotypes and hybrids. Carnegie Inst. Washington Publ. no. 112. pp. 57. Washington. 1909.

———, Defective inheritance-ratios in *Bursa* hybrids. Verhandl. Naturf. Ver. Brünn **49**: 157–168. 1911.

———, Duplicate genes for capsule-form in *Bursa bursa-pastoris*. Zeitschr. Ind. Abstam. u. Vererbungs. **12**: 97–149. 1914.

[20] DAHLGREN, K. V. OSSIAN. Ein Kreuzungsversuch mit *Capsella Heegeri* Solms. Svensk Botanisk Tidskrift **9**:397–400. 1915.

factors would give no indication of the condition of any of the original Swedish biotypes, because plants having monomeric capsules occur normally just as frequently as those having dimeric capsules in the offspring of the F_1 and later generations from such a cross.—GEO. H. SHULL.

Liassic flora of Mexico.—WIELAND'S[21] superb quarto memoir of 165 pages and 50 plates has run the gauntlet of both the Mexican civil war and the world war, since the Spanish text has been printed in Mexico and the illustrations are from the famous lithographic firm of Werner and Winter of Frankfort. The only internal evidence of this situation is the rather large number of typographical errors in the Spanish text. The material was collected in the province of Oaxaca in the southwestern Pacific region of Mexico. For the most part it consists of impressions of leaves, and in a few instances fructifications of cycads or supposed Cycadophyta. Remains of Cordaitales are described also from the formation which is lowest Jurassic (Lias). One could wish, however, that the evidence in the case of this group were somewhat more definite, for it does not seem to establish definitely the presence of the Cordaitales in the middle Mesozoic any more than the results of LIGNIER have finally established the concurrent existence of Cordaitales and palms in the Lias of France. Only structural evidence of an unquestionable character could do this. One *Araucarioxylon* is described, but it differs from that genus in its typical form by the possession of rays of more than a single layer of cells in width. The memoir under review stands as one of the most important recent documents of systematic paleobotany in regard to the Cycadophyta, and takes its place with those of the same author on the extinct cycads of the United States and those of NATHORST on the Cycadophyta of Yorkshire, England.—E. C. JEFFREY.

Anatomy of Betulaceae.—HOAR[22] has investigated the anatomy of the Betulaceae with reference to the phylogenetic position of the family. In the Engler scheme, the family is placed among the most primitive Archichlamydeae, on the basis of flower structure. Since the most primitive family in the Engler scheme is the Casuarinaceae, the genus *Casuarina* was included in the investigation. The anatomy of the latter genus is either entirely primitive or so generalized as to include both primitive and advanced characters, so that its position near "the base of the dicotyledonous line" seems justified. The Betulaceae possess the aggregate condition of rays indicative of a primitive type, *Alnus* probably illustrating most completely the primitive condition of the family. The more advanced genera (*Carpinus*, *Ostrya*, and *Betula*) have

[21] WIELAND, G. R., La Flora Liasica de la Mixteca Alta. Bol. Inst. Geol. Mexico. no. 31. 1916.

[22] HOAR, CARL S., The anatomy and phylogenetic position of the Betulaceae. Amer. Jour. Bot. 3:415–435. *pls. 16–19.* 1916.

retained the aggregate condition only in conservative organs and regions, or it is recalled in them by injuries. The general conclusion, therefore, on the basis of anatomy, is that Betulaceae are rightly "ranked in a low phylogenetic position."—J. M. C.

Hawaiian bogs.—Situated at or near the summits of high volcanic mountains, at altitudes of 1000–2000 m., with a precipitation reaching the enormous proportions of 20 m. annually, the summit bogs of Hawaii are among the most inaccessible and remarkable in the world. In a general description of these areas MacCaughey[23] calls attention to the similarity of these bogs to those of other lands in general aspect and in the presence of similar mosses, sedges, and grasses. There is an absence of many familiar forms, however, such as pitcher plants, and many of the bog ericads and orchids; while other familiar genera take new and strange forms, as instanced by woody violets and lobelias. Many endemic forms occur, particularly among the dwarf trees that form clumps scattered over the tussocky surface.—Geo. D. Fuller.

Four-lobed mother cells.—Lobed spore mother cells are very conspicuous in Jungermanniales, and by most botanists are thought to be restricted to that order. The work of Allen[24] adds the Musci to the list. He finds that the spore mother cells of *Catharinea* show a distinct lobing, somewhat less than in representative Jungermanniales, but nevertheless very pronounced. Lobed mother cells are present in all of the 3 orders of the Hepaticae. Cavers reports them in *Targionia*, one of the Marchantiales; they are almost universally present in the Jungermanniales; and the reviewer finds marked lobing in the spore mother cells of species of *Anthoceros* collected by him on volcanic islets in the South Seas. The lobing of spore mother cells in bryophytes is probably of phylogenetic significance, but until much more critical work has been done it is idle to theorize.—W. J. G. Land.

Roesleria and Pilacre.—As a result of a comparison of the various forms of *Roesleria pallida* and *Pilacre Petersii*, Bayliss-Elliott and Grove[25] conclude, from the great similarity in structure and habit of these two fungi, that both are forms of the same plant, and that *Pilacre Petersii*, long regarded as a primitive basidiomycete of the auriculariaceous type, is therefore nothing more than the conidial form of the ascomycete *Roesleria pallida*.—H. Hasselbring.

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[23] MacCaughey, Vaughan, Vegetation of the Hawaiian summit bogs. Amer. Botanist **22**:45–52. 1916.

[24] Allen, Charles E., Four-lobed mother cells in *Catharinea*. Amer. Jour. Bot. **3**:456–460. *figs. 2.* 1916.

[25] Bayliss-Elliott, Jessie S., and Grove. W. B., *Roesleria pallida* Sacc. Ann. Botany **30**:407–414. *figs. 11.* 1916.

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THE
BOTANICAL GAZETTE

APRIL 1917

ENVIRONMENTAL INFLUENCES ON NECTAR SECRETION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 228

LESLIE A. KENOYER

This study was undertaken to summarize and supplement existing knowledge of the factors which stimulate or retard the secretion of nectar. The work was carried out under the direction of the botanical section of the Iowa State Experiment Station in cooperation with the chemical section, being done mostly at Ames, Iowa, from June 1914 to June 1916.

Historical

One of the most complete treatises on nectar, with quite an extensive account of some of the environmental factors in its secretion, was written by BONNIER (1). The subject of secretion has been much debated from a physical standpoint. GODLEWSKI (9) attributes it to a fluctuation in the concentration of the cell sap due to alternate splitting and recombination of complex molecules. PFEFFER (19) advances 3 possible causes for secretion: (1) an unequal permeability of the membrane of the absorbing and excreting portions of the cell; (2) an unequal distribution of solutes in the absorbing and excreting portions of the cell; (3) the transformation into sugar of the outer portion of the cell wall, and the osmotic action of this sugar upon the liquid contents of the cell. LEPESCHKIN (14), in a study of the coenocytic plant *Pilobolus*, finds

evidence that the first of PFEFFER's theories is the correct one for the excretion of water drops. WILSON (29) gives evidence in support of PFEFFER's third theory, showing that the thorough washing of a nectary stops the secretion if the nectary is past the stage of metamorphosis of the cell wall, but that secretion is resumed on the addition of sugar to the surface of the nectary. The validity of his results is called in question by LEPESCHKIN (14) and BÜSGEN (5). HAUPT (10) in a study of extrafloral nectaries finds that some nectaries become inactive after washing, while others, as those of the leaves of *Impatiens parviflora*, continue excretion of water but not sugar, thus becoming equivalent to hydathodes. LIVINGSTON (16) likens nectar secretion to guttation, accounting for the latter by a decrease in the permeability of the plasma membrane induced by an increased turgidity, and for the former by a hypothetical rapid increase in the solute content and thereby of the osmotic pressure in the cell, a change which induces a like decrease in the permeability of the membrane.

Comparatively little work has been done on the chemistry of nectar. WILSON (28), VON PLANTA (27), and BONNIER (1) have analyzed a few kinds of nectar, finding that in some cases it contains no sucrose, while in others it is almost wholly this kind of sugar. In some cases fructose and in others glucose is the dominating reducing sugar. The sucrose of nectar is almost wholly digested in honey, BROWNE (4) finding as the average composition of 138 honey samples from widely separated localities 38.65 per cent fructose, 34.48 per cent glucose, and 1.76 per cent sucrose.

Investigation

METHODS

Nectar when secreted in sufficient quantities was measured by means of a graduated capillary pipette, or weighed after absorption on strips of filter paper which had previously been weighed in small vials. Many of the most important honey plants secrete such small amounts of nectar to the individual flower that neither of these methods is practicable. In these the amount of sugar external to the nectaries was determined approximately by adding a definite

volume of water to a counted or weighed quantity of the flowers, shaking frequently for half an hour, then decanting. A similar method was employed by VON PLANTA (**27**) and BONNIER (**2**). In some of the flowers investigated, this treatment extracts some sugar from the floral tissues, as shown by the appearance in the solution of colors from the floral envelopes, hence it is of value mainly for the comparison of flowers of the same species. Buckwheat, because of its rapid maturing, its value as a honey-producing crop, as well as its comparative freedom from this source of error, was employed in many of the experiments.

Sugar determinations were made by reduction of Fehling's solution. The method found most practicable and employed for the greater part of the work was based on that described by SCHOORL (**24**). A carefully measured amount (1 cc. for minute quantities of sugar, 10 cc. of the material to be analyzed in a 150 cc. Erlenmeyer flask) was heated on an asbestos gauze over a flame so adjusted that the liquid began to boil in just 2 minutes, and then was boiled for 2 minutes longer. To the contents of the flask after cooling to 60° C. were added sulphuric acid and potassium iodide. The liberated iodine, which corresponds to the unused copper sulphate, was titrated against sodium thiosulphate. Sugar values were obtained by the careful analysis of known quantities of sugar. This method has the advantage of being both rapid and delicate enough to determine minute quantities of sugar with a probable error of not over 0.04 mg. Floral tissues, when not too bulky, could be analyzed by the same method, the reagents being added directly to the tissues after covering them with water. When tissues were more bulky or when greater accuracy was required, extractions were made with alcohol or water, and were purified by treatment with neutral lead acetate.

HUMIDITY

It is a well known fact that any watery exudation from plants accumulates when atmospheric humidity is high and evaporation is thereby retarded. This can easily be demonstrated in connection with bleeding from severed tissues or with guttation through water stomata. BONNIER (**1**) states that nectar secretion corresponds to guttation and that it varies inversely with the transpiration.

So far as the volume of nectar is concerned, I have found this to be true in all the plants experimented upon with this end in view. But there are two factors involved in nectar secretion, as shown by PFEFFER (**20**), the exudation of water and that of sugar. HAUPT (**10**) has found that extrafloral nectaries begin secreting only when humidity is relatively high, an observation which confirms the theory that secretion is due to a decreased permeability caused by increased turgor, but that after secretion begins increased air moisture increases water secretion, the secretion of sugar remaining constant. It is probable that this applies to nectaries in general. Nectar is more dilute when humidity is high, and honey that is stored at such times is likely to be high in water content.

At Ames the seasons of 1914 and 1915 represented extremes of humidity, the summer months of the former year being excessively dry and warm, while those of the latter year were excessively wet and cool. Hence comparisons of nectar washed from flowers, as given in table I, are of interest.

TABLE I

Species	1914		1915	
	Number of samples analyzed	Average mg. sugar per gm.	Number of samples analyzed	Average mg. sugar per gm.
Melilotus alba, flowers.....	6	2.13	3	0.65
Medicago sativa, flowers....	4	1.15	3	0.80
Trifolium pratense, corollas.	4	3.64	13	3.90

It is seen that the wet season yields rather less sugar than the dry. It may be stated further that bee visitors to *Melilotus* were several times as abundant in 1914 as in 1915. I have found by experiment that flowers of alfalfa grown in dry soil contain about 60 per cent more sugar than those grown in wet soil.

Buckwheat flowers kept humid under a bell jar secreted much more liquid than flowers exposed to the rather dry greenhouse air. However, 12 comparative analyses of the nectar of each show 1.04 mg. sugar per 100 blossoms in the humid, and 0.98 mg. sugar per 10 blossoms in the dry. Analysis of the flower after removal of the nectar in 6 of the above pairs of cases shows 0.74 mg. per

10 blossoms in the humid and 0.98 mg. per 10 blossoms in the dry. More sugar accumulates in a dry atmosphere and practically the same amount is excreted.

The accumulation of sugar under low moisture conditions is in line with the discovery by LUNDEGARDH (**17**) that increase of moisture favors the accumulation of starch; decrease of moisture favors its digestion.

Six plants of *Impatiens sultana* in saturated air accumulate in a day 3.26 mg. sugar each from the extrafloral nectaries (the basal teeth of the leaves), while 6 plants in greenhouse air accumulate 5.42 mg. sugar each. The excess of the latter is very likely due in part to the running away of drops under the humid conditions. The nectar averages 23.4 per cent sugar in the former and 45.3 per cent in the latter.

RAINFALL

The author has shown in a statistical study (**12**) that heavy rainfall just before the secreting season is advantageous, as it gives the plants greater vigor. But during the season of greatest secretion good years are somewhat drier than poor. Also a rainy day shows a lighter honey yield than a day before or after the rain. The deterrent effect of the rain on the honey flow is twofold: it hinders the activities of bees and it washes away the nectar. To illustrate the latter point, in 1915 on the morning following a day of continual rainfall, red clover corollas were found to contain 0.02 mg. sugar per gm., whereas a day earlier they contained 3.8 mg., a day later 0.6 mg., and 2 days later 4.4 mg. Buckwheat blossoms were subjected to an experiment to determine the extent to which rains wash away the nectar. Flowers subjected before gathering to a spray for 20 minutes, 15 mm. of water falling, were found to contain 0.12 mg. per 10 as against 1.28 mg. per 10 of untreated flowers. A 30-minute rain of 35 mm. reduced the nectar of red clover blossoms from 0.48 to 0.19 mg. per 10, and that of white clover blossoms from 0.27 to 0.07 mg. per 10.

TEMPERATURE

WILSON (**29**) states that temperature has not a marked effect upon the rate of secretion of nectaries that have commenced

secreting. He finds, however, that *Prunus laurocerasus* will not begin secretion unless the temperature is 12° C. or over. HAUPT (10) also finds that a minimum temperature is necessary to induce secretion. LEPESCHKIN (14) finds in the hyphae of *Pilobolus* a secretion steadily increasing with, and much more rapidly than, the absolute temperature. In other cases he finds an optimum above which secretion diminishes. In the case of secreting hairs of the bean leaf this optimum is 20°, in the *Abutilon* nectary it is 26°.

Experiments were carried out in uniform temperature incubators. For much of the work, to avoid light exclusion, which is detrimental to secretion, incubators were employed which were specially constructed for the purpose, being covered with two glass plates separated by an air space.

The optimum temperature for amount of secretion lies between 20° and 25° for *Cucurbita Pepo*, *Lilium speciosum*, *Canna indica*, *Euphorbia pulcherrima*, and extrafloral nectaries of *Impatiens Sultani*. For *Salvia splendens* and most of the Leguminosae tested it is about 15°. As a rule, the sugar concentration of the nectar does not differ materially for the different temperatures. Typical sugar determinations obtained from the flower of *Abutilon striatum* are given in table II, the blossoms being quartered and one piece of each placed in each incubator, thereby eliminating any error due to individual variations.

TABLE II

Time	Mg. invert sugar per flower			
	10°	16°	23°	30°
After 36 hours.....	10.20	12.00	16.00	10.32
After 16 hours (another set)	3.07	6.57	12.97	10.87

Here the optimum is clearly not far from 23°.

BONNIER and FLAHAULT (1, 3) call attention to the fact that nectar secretion is greater in the same species at high latitudes and altitudes than at low when the species grows normally in both latitudes or altitudes compared, and furthermore that species which do not secrete in France are nectariferous in Norway and in the Alps. He suggests that this fact may be due to the greater range

between maximum and minimum daily temperatures which prevails at high altitudes and latitudes, or to the greater range in the humidity of the air.

PHILLIPS (**21**) observes that alfalfa in general is valuable as a honey plant in the Great Plains region of the west and not in the eastern states; that buckwheat is of more value in New York, Pennsylvania, and Michigan, than in Indiana and Illinois; and that white clover is of greater importance in the north than in the south. Basswood is said to secrete better in the more northerly portions of its range. It seemed desirable, therefore, to investigate the hypothesis of BONNIER.

As I have shown (**12**), the study of a 30-year weight record of a hive at Clarinda, Iowa, lends strong support to this assumption. Thirty-eight periods of continual and fairly rapid gain in weight were selected, and the days of each divided about equally between days of high gain and days of low gain. In 32 cases the average diurnal temperature range for the days of high gain was greater than that for the days of low gain. In all of the 6 exceptional cases the difference between the average was small. SLADEN (**25**) states that the heaviest single day's increase in hive weight noted for two seasons in England in a record kept by EDE was on a day that began with a heavy early morning frost, the honey coming from the heather (*Calluna vulgaris*).

Table III represents the amount of reducing sugar in mg. which the author found after keeping the plants or flowering branches for a time in the incubators.

In field conditions it can readily be shown that lower temperatures increase the sugar content in dandelion and the clovers.

How does high temperature influence secretion? VAN RYSSELBERGHE (**26**) determined that with increase in temperature the permeability of the protoplast to water and solutes rapidly increases, that of *Tradescantia* epidermal cells for water being 8 times as great at 30° as at 0°, and that for solutes seeming to follow the same proportional rule. To demonstrate whether this holds for nectary cells, I determined the lowest sucrose concentration necessary to plasmolyze the multicellular secreting hairs which cover the nectary of *Abutilon*. After 4 days at 10° a 0.6 molecular solution is

sufficient; while for another portion of the same flower, after 4 days at 25° a 1.1 molecular solution is necessary.

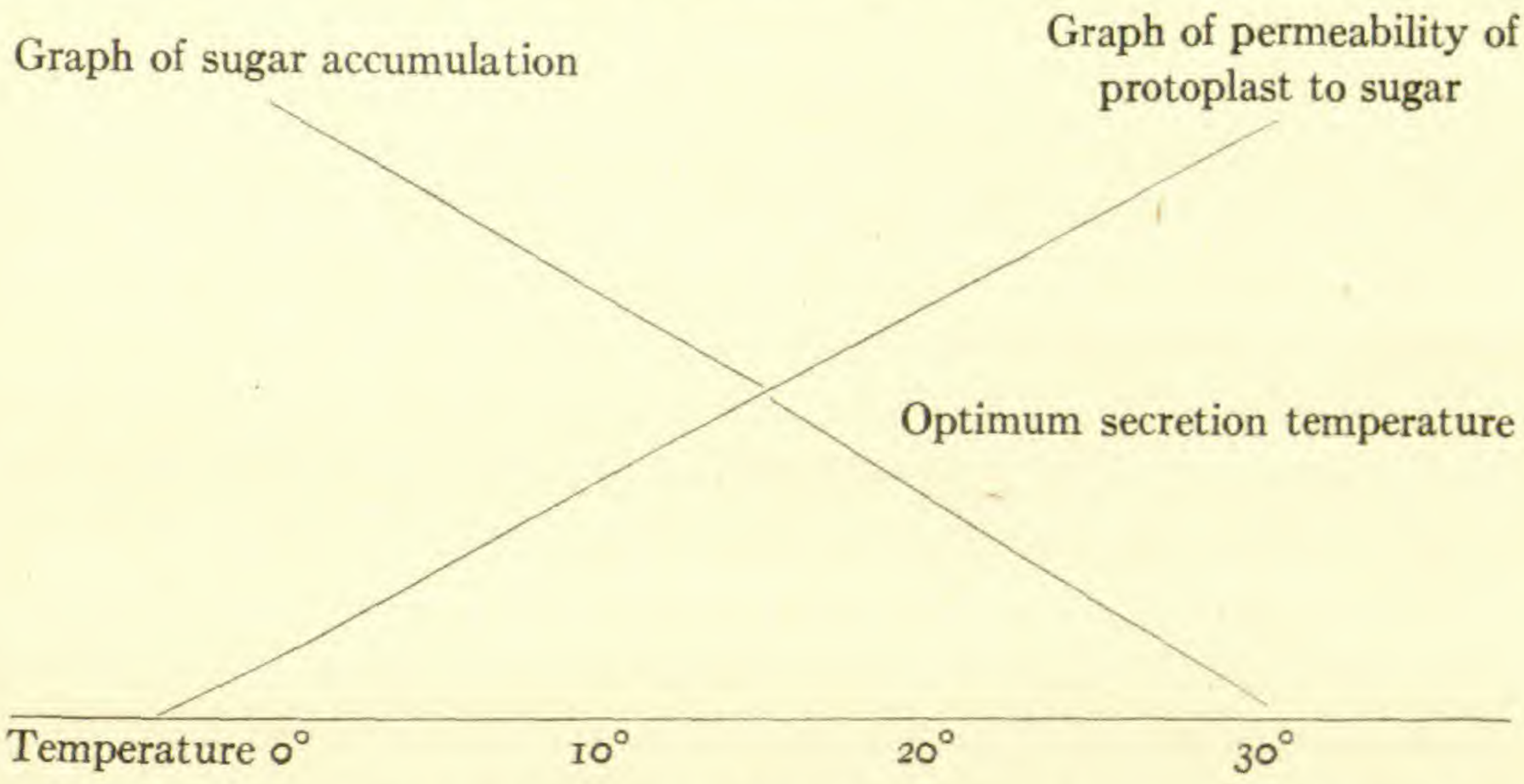
TABLE III

Species	10°	19°	25°	30°	10° (23° over night)
Trifolium incarnatum, 10 corollas, 2 days	0.56	0.63	0.43	0.38	.....
Trifolium repens, 10 flowers, 2 days	.....	1.12	0.94	0.70	.....
Nectar washed from same	.....	0.07	0.02	0.01	.....
Trifolium repens, 10 flowers, 2 days	0.93	0.63	0.47	.....	0.56
Nectar washed from same	0.0	0.0	0.0	.....	0.08
Medicago sativa, 10 flowers, 4 days	3.44	2.08	0.52	.....	.....
Caragana frutescens, 10 flowers, 2 days	37.55	22.22	11.95	.....	24.33
Nectar washed from same	0.64	4.80	2.00	.....	2.88
Fagopyrum esculentum, 10 flowers, 4 days	0.69	0.73	0.58	.....	.....
Nectar washed from same	1.36	0.80	0.0	.....	.....
Salvia splendens, 10 corollas, 4 days	.....	25.7	13.8	12.9	.....
Coleus Blumei, 10 corollas, 4 days	.....	0.86	0.78	0.62	.....
Taraxacum officinale, per gm. flowers, 1 day	38.7	45.0	19.3	13.9	.....
Taraxacum officinale, per gm. flowers, 1 day	15.6	17.1	11.9	not developing	.....

What is the influence of low temperature? MÜLLER-THURGAU (18) finds that sugar accumulates in potatoes when the surrounding temperature is below 10° C., and the same is true of hemp seedlings and various other plant organs. He advances the theory that the accumulation comes from the digestion of starch or oil more rapidly than the resulting sugar can be utilized, its respiratory destruction being retarded by the low temperature.

The accumulation of stored sugar from starch in low temperature in the twigs of woody plants is a well known phenomenon which is amply discussed by FISCHER (8). Indeed this accumulation seems to be rather common in its occurrence among plant tissues. Besides being applicable to floral tissues, as table III clearly shows, it affects the leaves and the peduncles of white clover, the former after 2 days' treatment having 30 per cent more sugar and the latter 58 per cent more at 10° than at 25°. The evidence points to the conclusion that at a uniform temperature the secretion

of nectar is a balance between two factors, namely, the accumulation of sugar in and near the flower under the influence of low temperature, and increasing permeability of the plasma membrane under the influence of high temperature. The position of the optimum, then, might be represented somewhat as follows:



The two graphs are limiting factors to nectar secretion, and the intersection, that is, the point where the effective limit stands highest, is the optimum secretion temperature. If the fact discovered by ECKERSON (7) for root cells, that above a certain point (25–35°) the permeability again decreases, applies also to nectary cells, the situation may be somewhat complicated thereby.

Better than any uniform temperature for secretion is a change from a lower to a higher temperature, as table III indicates. The influence of such a change might be graphically indicated by folding the above diagram so that two temperatures, say 10° and 30° are brought together. Both limiting factors are raised; the sugar which has accumulated at the lower temperature is secreted at the higher.

ATMOSPHERIC PRESSURES

In the study previously cited (12), I have shown that of 18 periods of continual honey production, 16 have a lower barometric pressure on the days of heavier yield than on the days of lighter yield, the two exceptional cases having very slight differences. The increased secretion already credited to high altitudes might be

attributed to the diminished pressure, but this explanation would not account of course for the similar increase at high latitudes.

In investigating experimentally the influence of pressure, I covered the plant under experiment with a tabulated bell jar waxed to fit tightly to a ground glass plate and connected by means of a stopcock with the water aspirator. A similar plant was placed under a control bell jar. In some of the experiments air was daily renewed in both low pressure and control jar; in others its continual renewal was provided for by admitting a current of air which bubbled through water, and in the case of the low pressure jar entered by means of a capillary tube with a very small aperture. This latter method is similar to one employed by SCHAIBLE (**22**). By the use of an aneroid barometer the pressure was maintained at about 50 cm., or two-thirds atmospheric pressure, the prevailing condition at altitudes of about 10,000 ft. Repeated investigations were made with the following plants: for guttation, *Tropaeolum majus* and *Avena sativa;* for nectar secretion, *Tropaeolum majus, Impatiens Sultani, Abutilon striatum, Euphorbia pulcherrima, Canna indica, Fagopyrum esculentum, Salvia splendens, Coleus Blumei, Antirrhinum majus*, and *Prunus americana.*

There were no constant differences in secretion which could be detected by either physical or chemical means. It is very doubtful, therefore, whether the much smaller variations in pressure which occur in nature could measurably affect nectar secretion. It is a matter of common knowledge among beekeepers that bees are more active when the barometer is low, the warmth and stillness of such periods favoring activity. Hence it seems very probable that any relation between atmospheric pressure and honey flow is to be attributed to the bees and not to the plants.

LIGHT

DARWIN (**6**), WILSON (**29**), and HAUPT (**10**) note the fact that the extrafloral nectaries of several species of *Vicia* are stimulated to activity by light. The first author adds *Lobelia erinus* and the last the Euphorbiaceae as plants that require the light stimulus for secretion. The two latter authors, however, state that in the greater majority of cases secretion is only indirectly related to

light. HAUPT finds that in most extrafloral nectaries even disturbances in photosynthesis by darkness show their influence on secretion only very slowly. Light in *Vicia* doubtless increases the permeability of the protoplast, as LEPESCHKIN (**15**) has found that it does in the pulvini of Leguminosae in general.

SCHIMPER (**23**) found that extrafloral nectaries on the leaves of *Cassia neglecta* cease their activity in a few days when the plant is kept in darkness or in an atmosphere deprived of carbon dioxide, but that secretion continues when the leaf is in the light and only the nectaries are darkened.

I experimented upon both the floral and the extrafloral nectaries of *Impatiens Sultani*, and it seems clear that the withdrawal of light makes its influence fairly rapidly and very decidedly felt. Table IV gives a typical study of floral nectaries, the measurements being millimeters in length of the part of the spur which contains nectar. The table includes average increases over last measurement of the spur in those flowers which were open when the last record was taken, and the average measurement for those flowers which have opened since the last record. One plant was covered by a bell jar, the other was covered by an opaque jar of about the same size.

TABLE IV

Days	Light		Dark	
	Gains	New flowers	Gains	New flowers
2	4.4	21.6	1.2	16.1
3	4.8	22.0	1.1	13.6
4	2.0	23.0	1.1	15.7
6	3.5	29.0	−1.2	17.3
7	5.0	20.0	0.1	16.0
8	4.1	21.7	0.5	14.0
9	3.3	19.0	−0.4	13.0
10	3.2	22.0	−0.5	0.0

At this time the dark plant had taken on an etiolated appearance and new flowers were scarcely developing. Secretion from extrafloral nectaries had practically stopped after 3 days in the dark.

Half the leaves of a plant were covered with black tissue paper which was fastened by means of small brass paper clips, only the

basal or nectar-secreting teeth being left uncovered. These leaves secreted very little after the third day, whereas the uncovered leaves of the same plant were uninterrupted in their secretion.

Buckwheat flowers were gathered at the same time from under light and dark jars, and it was found that after two days in the dark, although the total amount of liquid secretion was not in the least diminished, the proportion of sugar began to decrease, the secretion not tasting sweet or giving a very positive sugar test. An average of 11 such analyses of the nectar of flowers that had been covered for 2–8 days gives per 10 blossoms 1.20 mg. invert sugar in the light, and 0.41 mg. in the dark. Sugar contained in the flowers does not differ greatly, however, in the two cases, there being 0.79 mg. to 10 flowers from the light and 0.73 mg. to 10 flowers from the dark. Plants which had been left in the dark for some time continued to secrete less than normal quantities of nectar for a week or more after the removal of the cover.

That the diminution of sugar is due to the interference with photosynthesis may be shown by removing all the leaf blades from a number of plants. Eleven analyses average in milligrams invert sugar in the nectar of 10 flowers 0.36 from plants with leaves removed 3–10 days, and 0.85 from the normal plants serving as checks. Seven of the above pairs of cases give for the entire invert sugar content of the flowers 0.59 mg. from the mutilated and 0.92 mg. from the check.

The same result may be gained by covering all the leaves of the plant with black tissue paper, and comparing the nectar with that of normal plants. Here we find as an average of 6 analyses of 10 flowers 0.23 and 0.69 mg. invert sugar respectively in the nectar; 0.83 and 1.40 in the whole flowers. After 4 days in the dark there is approximately one-fourth as much sugar secreted per flower.

When the flowers only are covered from the light, they secrete fully as much sugar as those not covered; so the extrusion of sugar is clearly dependent upon the food reserves of the plant.

I found the same relation to exist in the *Canna* blossom. Darkening of the entire plant materially diminished the nectar secretion, while darkening the flower cluster alone had no influence upon it.

Other flowers analyzed, among them being *Antirrhinum majus*, *Cucumis sativus*, *Salvia splendens*, and *Coleus Blumei*, contain less sugar and secrete less sugar when kept in the dark; furthermore, the nectar is usually less in volume. *Euphorbia pulcherrima* does not begin secretion in a dark chamber, and *Abutilon striatum* secretes only very slowly and very scantily. Even plum branches, which contain supplies of stored food, when developed in the dark have little more than half as much sugar in the tissues and about one-fourth as much nectar as when developed in the light.

FERTILITY OF SOIL AND VIGOR OF PLANT

HUNTER (11) states that alfalfa yields the greatest amount of nectar under conditions that tend to give it the most vigorous growth, proper heat, and moisture upon suitable soil. All of my observations and experiments tend to confirm this for the various plants investigated. Red clovers grown on fertilized plots were found to contain slightly more sugar and to secrete slightly more as nectar than those on the unfertilized control plots adjoining.

BONNIER (2) has experimented on the secretion of several plants as influenced by different soils. He finds that *Sinapis alba*, *Isatis tinctoria*, and *Medicago sativa* yield most nectar on limy soil, while *Phacelia tanacetifolia* does better on clay and *Fagopyrum esculentum* on sand. It is probable that soils which are conducive to greater vigor and more surplus food in the plant are on the whole more favorable for nectar yield.

White clover, the leading honey plant of this section, collected the same day in the same part of the city gives the tests shown in table V.

TABLE V

CONDITION	SUGAR PER GM. OF FLOWERS IN	
	Nectar	Flowers
Stunted by rank growth of weeds...	0.2	15.9
Stunted by close mowing.....	0.6	20.8
Vigorous.....	3.7	21.6

Vigorous plants of buckwheat yield about twice as much nectar as do weak ones in the same bed. The flowers are found on analysis

to contain 20–50 per cent more sugar. Plants which had been allowed to dry to the wilting point several times in the course of their growth and were consequently stunted to about one-half normal height yielded less nectar, the average of 6 comparisons being 0.44 mg. per 10 flowers of the stunted and 1.39 mg. per 10 flowers of the normal. Plants grown in a greenhouse in which the temperature was low and which consequently were stunted to about one-third normal height, not blooming until twice the age of normal blooming plants, secreted practically no nectar.

It was noticed further that a *Salvia* deeply rooted in the soil secreted more than one which was hampered by a small pot, and that of the former plant young vigorous branches yielded more nectar than old stunted ones.

PORTION OF FLOWERING PERIOD AND AGE OF FLOWER

Table VI illustrates the relation of nectar secretion to the part of the flowering season in which the flower in question appears. In all cases the compared flowers were collected on the same day, but from patches varying in stage development.

TABLE VI

Species	Early in blooming season		Late in blooming season	
	Sugar in nectar	Sugar in flowers	Sugar in nectar	Sugar in flowers
Trifolium pratense.....	9.3	20.7	4.6	18.6
Medicago sativa per gm. of flowers.....	1.5	38.9	0.3	26.8
Buckwheat 10 flowers (average of 4 tests).....	1.62	0.72	1.09	0.60

As a rule, the younger plant is undergoing more active photosynthesis and has greater reserves of food to be secreted by the nectaries.

KURR (13) makes the statement that secretion of nectar commences very rarely before the dehiscence of the anthers; it is generally most rapid during the pollination period; and it ceases as soon as the fruit begins to develop. BONNIER (1) agrees to this proposition and asserts that nectar is simply a manifestation of the surplus of food stored in the nectariferous part corresponding to an

arrest in the development of the organ. In the case of floral nectaries, therefore, it is most pronounced after the ovary has attained maximum development and before the fruit has commenced to develop. He finds a maximum proportion of sucrose in the floral organs corresponding to this time of greatest secretion. Chemical analyses of the floral tissues show that the climax of sugar accumulation is about the time of the dehiscence of the stamens, and that as the flower withers there is a very rapid decrease in the amount of sugar. Table VII gives some examples from my work, the tissues having been extracted and purified.

TABLE VII

Species	Buds		Mature flowers		Declining flowers	
	Invert sugar	Sucrose	Invert sugar	Sucrose	Invert sugar	Sucrose
Medicago sativa per gm...	32.5	2.8	65.4	0.0	26.1	0.0
Melilotus alba per gm.....	22.2	2.6	31.1	Trace	.....	.....
Melilotus officinalis per gm.	16.0	0.0	28.8	0.0	.....	.....
Trifolium repens per gm...	8.1	0.1	8.3	1.8	7.5	0.0
Trifolium hybridum per gm.	13.8*	.....	8.6*	.....	.....	.....
Taraxacum officinale per gm.....	17.8	.....	27.2	.....	.....	.....
Impatiens Sultani per 100 flowers	47.5	0.0	248.4	0.0	190.0	48.9
Lilium speciosum rubrum per flower.....	119.5	26.7	179.3	10.9	76.0	1.0
Lilium longiflorum per flower..... younger	51.4	14.9	95.2	11.3	22.2	0.0
Lilium longiflorum per flower..... older...	95.9	21.3				

* Including sucrose.

It seems that in most cases the excess of sucrose, the storage form of sugar, is rather before the flower opens and nectar secretion begins.

Summary

1. By increasing humidity the secretion from nectaries of water but not that of sugar is increased.

2. Excessive water supply lessens the sugar surplus in the parts of the flower.

3. Dilution and washing by rain cause much of the sugar of nectar to be lost.

4. Rate of secretion for both sugar and water increases with temperature up to a certain optimum.

5. Accumulation of sugar in the flower and its vicinity varies inversely as the temperature.

6. The optimum condition for sugar secretion is an alternation of low and high temperatures.

7. Variation of atmospheric pressure has no marked influence on secretion.

8. Sugar excretion is markedly diminished in darkness on account of limitation of the food reserves of the plant. Water excretion may or may not continue, depending upon the species. Removal of the leaves has the same deterrent effect.

9. The more favorable all conditions for growth and the more vigorous the plant, the greater is the amount of sugar secreted.

10. Nectar is most abundant early in the blooming season, other things being equal.

11. Accumulation and secretion of sugar is most pronounced near the time of the opening of the flower.

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LITERATURE CITED

1. BONNIER, G., Les Nectaries. Ann. Sci. Nat. Bot. **8**:1–212. 1879.
2. ———, Influence du terrain sur la production du nectar des plantes. Ass. Fr. Av. Sci. **2**:567, 569. 1893.
3. BONNIER, G., and FLAHAULT, CH., Observations sur les modifications des végetaux suivant les conditions physiques du milieu. Ann. Sci. Nat. Bot. **7**:108–113. 1878.
4. BROWNE, C. A., Chemical analysis and composition of American honeys. U.S. Dept. Agric. Bur. Chem. Bull. no. 110.
5. BÜSGEN, M., Der Honigthau. Jenaische Zeitsch. Nat. **25**:339–428. 1891.
6. DARWIN, C., Cross and self-fertilization in the vegetable kingdom. 1877 (chapter x).
7. ECKERSON, S., Thermotropism of roots. BOT. GAZ. **58**:254–263. 1914.
8. FISCHER, A., Beiträge zur Physiologie der Holzgewächse. Jahrb. Wiss. Bot. **22**:73–160. 1886.

9. GODLEWSKI, E., Zur Theorie der Wasserbewegung in den Pflanzen. Jahrb. Wiss. Bot. **15**:602. 1884.
10. HAUPT, H., Zur Secretionsmechanik der extrafloralen Nektarien. Flora **90**:1. 1902.
11. HUNTER, S. J., Alfalfa, grasshoppers, bees. Bull. Dept. Ent. Univ. Kansas. 1899.
12. KENOYER, L. A., The weather and honey production. Iowa State Exper. Sta. Bull. 1916.
13. KURR, O. G., Bedeutung der Nektarien in den Blumen. Stuttgart. 1883.
14. LEPESCHKIN, W. W., Zur Kenntniss des Mechanismus der aktiven Wasserausscheidung der Pflanzen. Beih. Bot. Centralbl. **19**:409. 1906.
15. ———, Kenntniss des Mechanismus der Variationsbewegungen und der Einwirkung des Bedeuchtungswechsels auf die Plasmamembran. Beih. Bot. Centralbl. **24**:308–356. 1911.
16. LIVINGSTON, B. E., The rôle of diffusion and osmotic pressure in plants. 1903.
17. LUNDEGARDH, H., Einige Bedingungen der Bildung und Auflösung der Starke. Jahrb. Wiss. Bot. **53**:421. 1903.
18. MÜLLER-THURGAU, H., Über Zuckeranhäufung in Pflanzentheilen in Folge niederer Temperatur. Landw. Jahrb. **11**:751. 1882.
19. PFEFFER, W., Osmotische Untersuchungen. 1877.
20. ———, Studien zur Energetik der Pflanze. 1892 (p. 267).
21. PHILLIPS, E. F., Beekeeping. 1915 (pp. 207 and 362).
22. SCHAIBLE, F., Physiologische Experimente über das vermindertem Luftdruck. Beitr. Wiss. Bot. Fünfstück **4**:93–148. 1900.
23. SCHIMPER, A. F. W., Pflanzen und Ameisen. 1888.
24. SCHOORL, N., Zur jodometrischen Zuckerbestimmung mittels Fehling'scher Lösung. Zeit. Angew. Chem. **27**:633–635. 1899.
25. SLADEN, F. L., Secretion of nectar. Beekeeper's Review **27**:419. 1914.
26. VAN RYSSELBERGHE, F., Influence de la température sur la perméabilité du protoplasme vivant pour l'eau et les substances dussoutes. Rec. Inst. Bot. Bruxelles **5**:209–249. 1901.
27. VON PLANTA, A., Über die Zusammensetzung einiges Nektar-Arten. Zeitsch. Physiol. Chem. **10**:227–247. 1886.
28. WILSON, A. S., Chem. News **38**:93. 1878.
29. WILSON, W. P., The cause of the excretion of water on the surface of nectaries. Unters. Bot. Inst. Tübingen **1**:1–22. 1881.

DEVELOPMENT OF EMBRYO SAC AND EMBRYO IN EUPHORBIA PRESLII AND E. SPLENDENS

WANDA WENIGER

(WITH PLATES XIV–XVI)

Introduction

LINNAEUS had considered the cyathium of *Euphorbia* to be a single flower, its involucre a perianth, and the staminate flowers stamens. The inaccuracy of these assumptions was demonstrated by BROWN (**2**), who substituted for them the conceptions still held, namely, that the cyathium consists of an involucre containing a single pistillate flower and many staminate flowers.

The earliest monograph of the Euphorbiaceae was that of BAILLON (**1**), in 1858. He found that a character common to all species of the family is the uniovulate or biovulate locule. The anatropous or orthotropous ovule becomes a seed with 3 seed coats, the outer one of which usually disintegrates. The embryo is surrounded by an oily endosperm and has a rudimentary root cap. BAILLON'S figures, reproduced by STRASBURGER (**20**), show the structure of the nucellus and embryo of *E. Lathyrus*. The name "obturateur" was applied by BAILLON to the mass of cells which grows from the placenta, pushing into the micropyle, and which is thought to determine the direction of growth of, and to nourish, the pollen tube. The nucellus grows out into a beak before the time of fertilization and the cells of the obturator grow close to the nucellus.

The obturator was called by MIRBEL (**9**) a "chapeau de tissu conducteur," by PAYER (**15**) a "capuchon," and by CAPUS (**3**), who studied *E. Myrsinites* in particular, a "coussinet micropylaire." POISSON (**16**) describes the integuments and obturator of *E. Lathyrus* and *E. Peplis*. PAX'S account (**14**) of the structure of the ovule agrees with that of BAILLON. According to him, however, caruncula and obturator are one and the same structure.

Miss LYON (**8**) gives a full account of the life history of *E. corollata*. There are 3 carpels in each pistillate flower, forming a tri-

locular ovary with a single suspended anatropous ovule in each locule. The inner integument appears first, but it remains small, while the outer integument grows beyond it. The megaspore mother cell is subepidermal in origin, but the cells of the nucellus, and possibly those of the epidermis near the upper end of the embryo sac, divide with great rapidity, producing a long, slender neck, and leaving the embryo sac deeply imbedded. The embryo sac develops in the usual way from the lowest of a row of 4 axial megaspores. The synergids are extremely long and the egg is situated between them. The pollen tube passes between the synergids, and the fusion of the male nucleus with the egg nucleus was observed. The fusion of the polar nuclei takes place near the egg. The antipodal cells are ephemeral and were seen by Miss LYON but once. The neck of the nucellus and the "glandular hairs," as the cells of the obturator are called, disintegrate after the entrance of the pollen tube, and the outer integument closes the mouth of the micropyle.

HEGELMAIER (6) reports habitual polyembryony in *E. dulcis*. From 2 to 9 embryos appear at the micropylar end of the sac. One embryo, which comes from the egg and may be distinguished from the others by the presence of a suspensor, develops into the single embryo of the seed. Some of the supernumerary embryos come from the nucellus. Two of them often reach the cotyledon stage, with tissue systems differentiated; the other embryos appear as irregular masses. Since the cyathium of this species has a very small neck, HEGELMAIER thinks it improbable that the flowers are insect pollinated. Wind pollination is also improbable, because of the regularity with which seeds are formed in the locules. In a later paper HEGELMAIER (7) admits that, although fertilization in *E. dulcis* was not observed and although its possibility seems lessened by the sterility of a large proportion of the pollen grains, he cannot prove that it does not occur. Fertilization is not necessary for the production of the embryos from nucellar cells. There is a possibility that apogamy and also parthenogenesis occur.

ROEPER (17) reports observing 2 embryos in the seed of *E. platyphylla*. According to DE CANDOLLE (5), 2 embryos are also formed in *E. helioscopia*. SCHWEIGER (19) describes the obturator, nucellus,

and caruncula of many species of *Euphorbia*. The outer integument always develops before the inner. The obturator of *E. Myrsinites* begins as a small outgrowth from the placenta when the outer integument has grown almost half-way to the tip of the nucellus. The cells of the obturator increase rapidly, the outer ones becoming long and hairlike. At the time of fertilization, the obturator is mature and has the shape of a bell which fits over the micropyle. The long cells of this structure completely fill the space between the nucellus and the inner and outer integuments, but never grow into the nucellar tissue. The obturator gradually disintegrates as the embryo enlarges, until at the maturity of the seed it is represented only by a small swelling on the placenta. The form and size of the obturator differ in different species. The nucellus has a long, slender tip which is surrounded by the cells of the obturator. The caruncula is formed from the outer integument, after the embryo has developed. A row of cells differentiates it from the seed proper. This structure resembles a cap and aids in loosening the seed from the placenta at the time of dispersal. SCHMIDT (18) finds more than one megaspore mother cell in *E. palustris*. These are situated deep in the cells of the nucellus. His account of the development of the flower agrees with that of Miss LYON (8).

MODILEWSKI (11) describes an unusual development of the embryo sac in *E. procera*. The first 4 nuclei of the embryo sac are arranged in the form of a cross. Two divisions result in the formation of 4 tetrads of nuclei. One nucleus from each group migrates to the center of the sac, where the 4 unite. The mature embryo sac contains an egg apparatus, 3 antipodal cells, and 2 groups of 3 nuclei each, lying on opposite sides of the sac. In fertilization, one male nucleus fuses with the egg nucleus, and the other male nucleus fuses with the quadrivalent fusion nucleus in the center. The synergids, antipodals, and nuclear groups at the sides of the sac disintegrate. No case of polyembryony was observed. Later, MODILEWSKI (12) described the events preceding embryo sac development in *E. procera*. An archesporial row of 3 or 4 cells was found, each ultimately containing 4 nuclei. Only one of the 4-nucleate cells develops into an embryo sac. Often one or more

of the other cells of the row adheres to the developing sac for some time before it disintegrates.

According to MODILEWSKI, the embryo sacs of *E. Lathyrus*, *E. salicifolia*, *E. globosa*, *E. meloformis*, *E. Cyparissias*, *E. coralloides*, *E. variegata*, *E. helioscopia*, *E. Gerardiana*, *E. Ipecacuanhae*, and *E. heterophylla* develop normally. DESSIATOFF (4) describes the formation of 16 nuclei in *E. virgata*, in a manner similar to that described by MODILEWSKI for *E. procera* (11). In a still later study, MODILEWSKI (13) finds 16 nuclei in the embryo sac of *E. palustris*. The development proceeds exactly as in *E. procera*. On the other hand, the embryo sacs of *E. virgata* and *E. lucida* develop in the ordinary way. MODILEWSKI, whose material for the study of *E. virgata* was collected from various localities, disagrees with DESSIATOFF'S notion of the structure of the embryo sac in this species (4). He thinks that the nuclei in DESSIATOFF'S figures of the embryo sac resemble endosperm nuclei more than they do those of ordinary embryo sacs. He finds, also, that at the 2- and 4-nucleate stages the megaspore enlarges so rapidly that its wall becomes indistinct, and that the cells of the nucellus, which have been pushed aside in the growth of the spore, might easily be taken for nuclei of the developing gametophyte. DESSIATOFF, according to MODILEWSKI, mistook either endosperm or nucellar nuclei for nuclei of the mature embryo sac. If this is not the explanation of his results, MODILEWSKI thinks DESSIATOFF was mistaken in the determination of the species studied. MÖBIUS (10) has recently figured the relation of the integuments and obturator to the nucellus in *E. macrorrhiza*.

E. procera and *E. palustris*, on the present evidence, seem to be the only species of *Euphorbia* studied which deviate from the usual history of the embryo sac. In these species, MODILEWSKI found that since the endosperm nuclei are very large and usually contain 2 or 3 nucleoles, there is no danger of their being confused with the nuclei of the mature embryo sac.

Material and methods

Flowers and seeds of *Euphorbia Preslii* were collected in different stages of development during July and August 1915, along

railroad tracks in Madison, Wisconsin. They were fixed in Flemming's strong, medium, and weak fixatives, the first named giving the best results. Young buds, flowers, and seeds of *E. splendens* were fixed in various fixing solutions, including Flemming's, Carnoy's, and Juel's, and acetic alcohol fixatives. The best results in this case were obtained with the latter, the Flemming solutions failing to penetrate soon enough, due to the great amount of latex in all portions of the plants. The material was obtained during March and April 1916, from plants grown in the greenhouse. Sections were cut 5 or 6 μ in thickness. Some sections of embryos 10 μ thick were made. Flemming's triple stain was used with good results.

Observations

Euphorbia Preslii

Cyathium

The first evidence of the formation of the cyathium in this species is the appearance of a papilla (fig. 1, *p*) between 2 bracts (*b*) at the end of a peduncle. At the base of this papilla, staminate flowers (fig. 2, s^1) and the bracts of the involucre (*in*) soon appear. The outer bracts (*b*) continue to grow up about the developing cyathium. Ovules (fig. 3, *o*) next arise as small swellings on the central papilla (*p*). The carpels (*c*) of the pistillate flower appear at the base of the papilla and gradually grow up about it, forming a short style and 3 deeply 2-lobed stigmas (fig. 4, *sg*). The involucre now grows up about the staminate and pistillate flowers. The staminate flowers never extend above the neck of the involucre; in the early stages of the history of the pistillate flower only the stigmas project beyond the neck of the involucre. The pistillate flower consists of a single pistil, whose trilocular ovary terminates a stalklike structure which is jointed below to the pedicel (fig. 32). Soon after fertilization the stalk of the pistillate flower elongates, causing the pistil to project from the cyathium and nearly to close the opening of the involucre. When a stamen is nearly mature a depression appears, marking the point of juncture of the pedicel and the filament. Secondary staminate flowers arise as branches from the older ones (fig. 3, s^2). This description of the develop-

ment of the cyathium agrees with that given by Miss LYON (8) for *E. corollata*.

Embryo sac

A single ovule (fig. 4, *o*), which soon becomes anatropous, is formed in each locule of the ovary. Before the integuments begin to appear, the megaspore mother cell can be distinguished by the size of its nucleus (figs. 5, 11). It is subepidermal in origin and larger than the surrounding cells of the nucellus. After increasing considerably in size (fig. 12), 2 divisions occur which result in the formation of a typical row of 4 megaspores (fig. 13), of which the innermost is the largest and the one destined to develop into the embryo sac.

The inner integument begins to develop first (fig. 7, *ii*), but the outer (*oi*), which appears a little later, grows the more rapidly. It has been stated by POISSON (16), working on *E. Lathyrus* and *E. Peplis*, and by SCHWEIGER (20), investigating many species of *Euphorbia*, that the outer integument develops before the inner. It would be easy to arrive at a similar conclusion in the case of *E. Preslii*, since one rarely obtains a preparation showing the stage at which the inner integument is appearing at the base of the nucellus before any trace of the outer is to be seen, and since the outer integument grows so rapidly that it very early extends beyond the inner. In all probability, closer study of this species would show that in these also the inner integument begins its development first, as is the case in *E. corollata*, *E. Preslii*, and *E. splendens*.

At the time of the first nuclear division in the functional megaspore, the outer integument reaches about half-way to the tip of the nucellus; the inner integument is still extremely small (fig. 8). As the inner integument begins to grow more rapidly, the obturator first appears as a small swelling on the placenta (fig. 8, *ob*). Its cells increase in number (fig. 10, *ob*), the outer ones becoming long and slender and giving to the structure a very irregular outline. The nucellus grows out into a long beak (fig. 10, *n*) which extends beyond the integuments. At this time, the developing embryo sac has reached the 8-nucleate stage, and the obturator completely fills the space between the beaklike prolongation of the nucellus,

the placenta, and the ovary wall. The outer integument always extends considerably beyond the inner, even at the maturity of the embryo sac (fig. 10). The sac becomes deeply imbedded in the cells of the nucellus (figs. 9, 10). It is very long, and averages about 5 or 6 μ in thickness.

The functional megaspore grows considerably (fig. 14) before the first division of its nucleus. The other 3 megaspores disintegrate, but are visible at least as late as the 4-nucleate stage of the embryo sac as small, dark-staining cells at the micropylar end of the sac (fig. 17). The 2 nuclei resulting from the first division are usually to be found near the respective ends of the sac (fig. 16). In one case observed (fig. 17), however, one nucleus of each pair had moved nearer the center of the sac. That the latter case is exceptional is indicated by the fact that the 8 nuclei formed by the third division lie in 2 groups of 4 each at the respective ends of the sac. Cell division now occurs in the typical way (fig. 15). The synergids are oval in shape and each has a characteristic vacuole below the nucleus. The egg extends farther toward the center of the sac than the synergids. The 3 antipodals are well defined, angular cells, each with a conspicuous vacuole. After cell division is completed, the polar nuclei remain for a time in what seem to be their original positions near the respective ends of the sac (fig. 18). In one case they were found to have moved nearer the center of the sac (fig. 19), but no case was observed in which they had come in contact with each other previous to fertilization. MODILEWSKI (13) found no evidence of a fusion of the polar nuclei in *E. virgata*, which has a typical embryo sac of 8 nuclei. HEGELMAIER (6) found no fusion of either male or female nuclei, or of polar nuclei, in *E. dulcis*. I found no case showing actual fertilization. Fig. 19 shows the antipodal cells and the synergids apparently disintegrating, but the polar nuclei have not yet fused, and the egg nucleus shows no evidence of fusion with a male nucleus. This history differs from that observed by Miss LYON in *E. corollata*, where the antipodal nuclei are ephemeral and were observed but once, and where the polar nuclei fuse soon after their formation.

It is difficult to trace the course of the pollen tubes, should they be present, because of the long cells of the obturator. There seems

to be little possibility for self-pollination, for the neck of the cyathium is very small and the staminate flowers do not extend above the surface of the cyathium. When the embryo sac is mature, the staminate flowers are still rudimentary. Insect pollination is improbable because of the smallness of the cyathium and the smallness of the opening. Seeds are formed with marked regularity, which would hardly be the case if wind pollination occurred. HEGELMAIER (**6, 7**) found the same conditions with regard to pollination in *E. dulcis*.

Embryo

The fertilized egg divides in a plane parallel with the long axis of the sac (fig. 20). The second division occurs at right angles to the plane of the first (fig. 21). Further divisions result in the formation of a globular mass of cells (figs. 22, 23, 24). In all cases observed, the embryo formed no suspensor. The beak of the nucellus and the obturator gradually disintegrate as the embryo is formed, and the inner and outer integuments grow so as nearly to fill the large opening originally constituting the micropyle, but still leaving a small opening (fig. 25).

As early as the 2-celled stage of the embryo, endosperm nuclei appear at either side of the embryo. In the case represented in fig. 20, one endosperm nucleus (*en*) lies between the embryo and the micropylar end of the sac. The endosperm nuclei increase rapidly in number and are distributed quite uniformly throughout the peripheral region of the sac (fig. 24). Cell division does not occur in the endosperm until the embryo has come to consist of several hundred cells. The endosperm gradually fills the space originally occupied by the nucellar tissue (fig. 25, *n*).

The embryo changes as it grows from a globular (fig. 26) to an elongated form (figs. 28, 29). Fig. 30 shows the earliest stage at which cotyledons were observed. A well developed root cap is present in the mature embryo (figs. 27, 31, *rc*). When mature, the embryo (figs. 27, 31) is straight and its length nearly equals that of the seed, the root cap (*rc*) being pressed closely against the micropyle. Surrounding the embryo, except at the tip of the root cap, is the endosperm (fig. 27, *end*), whose cells contain a large amount of reserve food material in the form of starch, fat, and aleurone grains.

EUPHORBIA SPLENDENS

Embryo sac

The flowers of this species develop just as do those of *E. Preslii*, and there is also a similarity in the general structure of the obturator and the ovule. As in *E. Preslii*, the megaspore mother cell may be distinguished before the integuments have begun to develop (fig. 33). The mother cell is easily recognizable by its size, being several times as large as the surrounding cells of the nucellus, and by the fact that it contains a very large nucleus. It is situated 3 layers beneath the epidermis, and although in numerous cases I have found it only in this position, it is probable that in this species also it originates as a subepidermal cell and that the cells of the nucellus above it divide so that it comes to lie more deeply in the nucellus. After the mother cell (fig. 34) grows and elongates, and its nucleus also grows considerably, the latter prepares for division. At the time of the synaptic stage (figs. 35, 36), the nucleus has moved to the micropylar end of the cell. One unusually favorable preparation showed a late anaphase of the heterotypic division (fig. 37). The spindle in this figure occupies a central position and its long axis lies in the plane of the long axis of the nucellus. There are 12 small, nearly spherical daughter chromosomes in each of the 2 groups on the spindle.

Although the formation of the row of 4 megaspores was not observed, it is certain that 4 are formed, for when the functional megaspore has increased in size (fig. 38), 3 dark-staining masses can be distinguished at its micropylar end. This stage agrees with the corresponding one in *E. Preslii*, in which it is plainly the innermost of the 4 megaspores that develops into the embryo sac. This megaspore (fig. 38), even at the division of its nucleus, is not as large as the megaspore mother cell. In one case (fig. 39) 2 developing megaspores were found lying parallel to each other with their long axes parallel to the long axis of the nucellus. Each has a dark-staining mass of apparently 3 disintegrating cells at the micropylar end, but the number of these cannot be distinguished with certainty. This occurrence of 2 functional megaspores is doubtless very unusual, for it was observed in but one ovule.

The developing embryo sac becomes deeply imbedded in the nucellar tissue. After some growth of the functional megaspore, its nucleus divides (fig. 40) and one daughter nucleus moves to each end of the cell. At this stage further growth takes place, and while the first and second divisions of the nucleus (figs. 40, 41) are taking place, a large central vacuole is formed which persists for some time. An 8-nucleate stage was not found. In two cases 4 nuclei were found at the micropylar end (fig. 42), but only 3 at the antipodal end of the sac. In most cases in which the egg apparatus had been differentiated, no nuclei were to be found at the antipodal end of the embryo sac, indicating that the antipodal nuclei (and cells, if formed) must be ephemeral, unlike the antipodal cells of *E. Preslii*. Fig. 43 shows a sac with the egg apparatus fully formed and the polar nuclei apparently about to fuse near the egg, while the antipodal end of the sac shows 2 daughter nuclei of a recent division, with a cell plate between them. It is possible that after the second nuclear division in the developing megaspore, one of the two nuclei at the antipodal end divides some time before the other. One of the daughter nuclei of this (the third) division might then move to the micropylar end and function as a polar nucleus, its sister nucleus disintegrating before the remaining nucleus in the antipodal end (a daughter nucleus of the second division) finally divides. This explanation would also fit in with the condition found in the sac shown in fig. 42, which had only 3 nuclei at the antipodal end.

Fig. 44 shows another peculiar embryo sac in which there are plainly 8 nuclei at the antipodal end of the sac, at least 3 of which are surrounded by cell membranes. In this case, it is conceivable that each of the 4 nuclei at the antipodal end of the sac has divided and that none has as yet disintegrated. In all but these cases, the antipodal nuclei (or antipodal cells) had disintegrated. The egg apparatus seems to be quite typical (figs. 43, 45). At first the nucleus of the egg occupies the center of the cell, but later, as the egg grows, the nucleus moves to the side of the cell farthest from the micropyle. The egg is spherical, and its nucleus is not, in general, larger then the nuclei of the synergids. In the sac shown in fig. 45 one of the polar nuclei lies close to the egg, while the other seems

to be moving along the side of the sac toward the egg. The polar nuclei are at first not as large as the other nuclei of the sac, but before their fusion (fig. 46) they increase in size, each becoming larger than the egg nucleus. Fusion takes place below the egg in a plane either at right angles to (fig. 46) or parallel with (fig. 47) the long axis of the sac. The 2 nucleoles persist in the fusion nucleus until fertilization takes place.

The embryo sac of *E. splendens* differs from that of *E. Preslii* chiefly in the history of the antipodal cells, which in the latter species persist for some time after their formation; another difference is that in *E..Preslii* the polar nuclei remain in their original positions until after cell division occurs.

If fertilization does not occur immediately after the fusion of the polar nuclei, the synergids disintegrate, leaving the egg and the fusion nucleus close together at the micropylar end of the sac. In 2 embryo sacs in which fertilization was observed, the synergids were disintegrating, but their nuclei were still recognizable as dark-staining masses. In the sac shown in fig. 48, the pollen tube has destroyed one of the synergids and discharged one male nucleus, which may be seen in contact with the nucleus formed by the fusion of the polar nuclei. The latter nucleus still shows 2 nucleoles and is considerably larger than the egg nucleus. It has already moved a little way toward the antipodal end of the sac. The male nucleus also possesses 2 nucleoles and is crescent-shaped. The second male nucleus is a dark-staining mass still in the pollen tube and little more than its nucleole can be distinguished. It is about to pass down to the egg nucleus, the tip of the tube being within the cell membrane of the egg. Fig. 49 shows another pollen tube which has not yet discharged its male nuclei. It contains densely staining material which seems to be aggregated into several masses, but no nuclei are distinguishable. The polar nuclei in this case have not completely fused and the egg nucleus is in a resting stage. The pollen tube could not be traced back into the micropyle in either case, for only its tip seems to contain material that stains densely. With the triple stain, the contents of the tip of the tube always take up the safranin. Fertilization does not take place at the same time in the 3 ovules within the same pistil. A pollen

tube may be seen in each of them, but in one case it may be discharging its nuclei, while in another case the endosperm nucleus may have undergone several divisions. Figs. 48 and 50 were drawn from 2 embryo sacs within the same ovary.

Embryo

The fertilized egg does not divide immediately after the fusion of the nuclei within it. Usually there are 5 or 6 nuclear divisions in the endosperm before division of the egg occurs. In the sac shown in fig. 50, there are 4 endosperm nuclei, the egg still being undivided. The first division of the egg (fig. 51) is at right angles to the long axis of the nucellus. The developing embryo forms a short suspensor which is several cells in diameter. The terminal cell divides by a longitudinal wall after the embryo is about 4 cells in length (fig. 52). There seems to be nothing definite about the planes in which later walls are formed (figs. 53, 54), but a more or less globular mass of cells is formed at the end of the short suspensor (figs. 55, 56). In the embryo represented in fig. 56 the suspensor seems to be disintegrating. The mature embryo has a structure similar to that of *E. Preslii*, there being a well differentiated root cap and epicotyl.

Summary

1. The cyathium of both species studied begins as a papilla which arises between two bracts. The order of appearance of the parts of the cyathium is as follows: staminate flowers, involucre, ovules, carpels, and secondary staminate flowers which arise as branches of the first set.

2. The megaspore mother cell is subepidermal in origin in *E. Preslii*, and probably also in *E. splendens*.

3. An axial row of 4 megaspores is formed, the lowest of which develops into the embryo sac; the other 3 spores disintegrate.

4. The inner integument begins to develop before the outer, but the latter grows rapidly and soon overtops the inner.

5. The mature embryo sac is long and narrow, and is deeply imbedded in the tissue of the nucellus. In *E. Preslii* it has the

structure usual in angiosperms. In *E. splendens* there are peculiarities in the history of the antipodal nuclei which require further study to make definite conclusions possible. It seems probable that each of the 4 antipodal nuclei may undergo a second division.

7. The obturator arises as an outgrowth of the placenta. It fills the space between the beaklike prolongation of the nucellus, the placenta, and the ovary wall. Its cells disintegrate after the embryo begins its development.

8. At about the time of the first division of the egg of *E. Preslii*, endosperm nuclei come to lie between it and the micropylar end of the embryo sac.

9. The embryo becomes a round mass of cells; this mass elongates and later 2 cotyledons and a well developed root cap are formed. The mature embryo is straight, and, except at the tip of the root cap, is surrounded by the endosperm. In *E. Preslii* no suspensor was observed; in E. *splendens* there is a short suspensor.

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LITERATURE CITED

1. BAILLON, E. H., Étude général du group des Euphorbiacées. Paris, 1858; rev. in Bull. Soc. Bot. France **5**:776–780. 1859.
2. BROWN, R., Miscellaneous works. London. 1866 (vol. I, p. 28).
3. CAPUS, G., Anatomie du tissu conducteur. Ann. Sci. Nat. Bot. VI. **7**:209–291. 1878.
4. DESSIATOFF, N., Zur Entwicklung des Embryosacks von *Euphorbia virgata*. Ber. Deutsch. Bot. Gesells. **29**:33–39. 1911.
5. DE CANDOLLE, A. P., Organographie végétale. **3**:71. 1827; cited by HEGELMAIER (6).
6. HEGELMAIER, F., Über einen neuen Fall von habitueller Polyembryonie. Ber. Deutsch. Bot. Gesells. **19**:488–499. 1911.
7. ———, Zur Kenntnis der Polyembryonie von *Euphorbia dulcis*. Ber. Deutsch. Bot. Gesells. **21**:6–19. 1913.

8. LYON, FLORENCE, Contribution to the life history of *Euphorbia corollata*. BOT. GAZ. **25**:418–426. 1898.
9. MIRBEL, C. F., Histoire naturelle générale et particulière des plantes. Paris. 1800–1809; cited by HEGELMAIER (**6**).
10. MÖBIUS, M., Microscopisches Practicum für systematische Botanik. Berlin. 1912 (pp. 107–109).
11. MODILEWSKI, J., Zur Embryoentwicklung von *Euphorbia procera*. Ber. Deutsch. Bot. Gesells. **27**:21–26. 1909.
12. ———, Weitere Beiträge zur Embryoentwicklung einiger Euphorbiaceen. Ber. Deutsch. Bot. Gesells. **28**:413–418. 1910.
13. ———, Die anomale Embryosackentwicklung bei *Euphorbia palustris*. Ber. Deutsch. Bot. Gesells. **29**:430–436. 1911.
14. PAX, F., Euphorbiaceae. ENGLER und PRANTL, Die natürlichen Pflanzenfamilien. **5**:1–119. Leipzig. 1887.
15. PAYER, J. B., Traité d'organogénie comparée de le fleur. Paris. 1857; cited by SCHWEIGER (**20**).
16. POISSON, J., Du siège des matières colorées dans la graine. Bull. Soc. Bot France **25**:47, 60. 1878.
17. ROEPER, J. A. C., Enumeratio Euphorbiarum. 1824 (*pl. 1. fig. 67*); cited by HEGELMAIER (**6**).
18. SCHMIDT, H., Über die Entwicklung der Blüten und Blütenstände von *Euphorbia*. Beih. Bot. Centralbl. **22**:21–69. 1907.
19. SCHWEIGER, J., Beiträge zur Kenntnis der Samenentwicklung der Euphorbiaceen. Flora **94**:339–382. 1905.
20. STRASBURGER, JOST, SCHENK, und KARSTEN, Lehrbuch der Botanik. Jena. 1910 (pp. 473–477).

EXPLANATION OF PLATES XIV–XVI

All drawings were made with an Abbé camera lucida at table level, and Leitz oculars and objectives. The following combinations were used: ocular 4, objective 3, tube length 170 mm. (×200), figs. 1–10, 24, 28–31; ocular 4, objective 6, tube length 170 mm. (×800), figs. 11, 23; ocular 4, oil immersion 1/16, tube length 170 mm. (×2000), figs. 12, 13, 22, 33, 41, 45–47; ocular 4, oil immersion 1/16, tube length 212 mm. (×2600), figs. 14–21, 34–40; ocular 1, objective 3, tube length 140 mm. (×100), figs. 25, 27, 32; ocular 4, oil immersion 1/16, tube length 140 mm. (×1700), figs. 42–44, 48–56.

The following abbreviations are used: *b*, bract; *c*, carpel; *cot*, cotyledon; *en*, endosperm nucleus; *end*, endosperm; *i*, integument; *ii*, inner integument; *in*, involucre; *n*, nucellus; *o*, ovule; *ob*, obturator; *oi*, outer integument; *p*, papilla; *rc*, root cap; s^1, staminate flower; s^2, secondary staminate flower; *sg*, stigma.

In all figures the micropylar end is at the top of the drawing; figs. 18, 19, 21, 42–50 are reconstructed from 2 or 3 sections each.

PLATE XIV

Euphorbia Preslii.—FIG. 1.—Appearance of a papilla which will develop into a cyathium.

Fig. 2.—Staminate flowers and involucre developing at base of papilla.

FIG. 3.—Ovules appearing at top of papilla, and carpels, staminate flowers, and involucre at base; at the left, a secondary staminate flower.

FIG. 4.—Longitudinal section of cyathium, showing 2 ovules within the ovary, and developing staminate flowers at base of pistillate flower; integuments have not yet appeared.

FIG. 5.—Nucellus, containing megaspore mother cell, but with no integuments as yet.

FIG. 6.—Inner integument just appearing at base of nucellus.

FIG. 7.—Inner and outer integuments at base of nucellus; embryo sac is developing.

FIG. 8.—Obturator appearing on placenta, and outer integument overtopping inner.

FIG. 9.—Obturator pushing up to nucellus.

FIG. 10.—Mature embryo sac deeply imbedded in tissue of nucellus, which has developed a beak; the long cells of obturator have filled space between nucellus and placenta.

FIG. 11.—Nucellus, showing megaspore mother cell.

FIG. 12.—Megaspore mother cell before division.

FIG. 13.—Axial row of 4 megaspores.

FIG. 14.—Functional megaspore, now deeply imbedded in nucellar tissue.

FIG. 15.—Binucleate embryo sac.

FIG. 16.—Four-nucleate embryo sac.

FIG. 17.—Four-nucleate embryo sac, with 2 of nuclei near center of sac; an unusual condition.

FIG. 18.—Mature embryo sac.

FIG. 19.—Polar nuclei have moved nearer center of sac; synergids and antipodal cells seem to have disintegrated.

FIG. 20.—Two-celled embryo, with endosperm nuclei; one of latter between embryo and micropylar end of sac.

FIG. 21.—Four-celled embryo; several endosperm nuclei between embryo and micropylar end of sac.

FIG. 22.—Embryo a globular mass of cells.

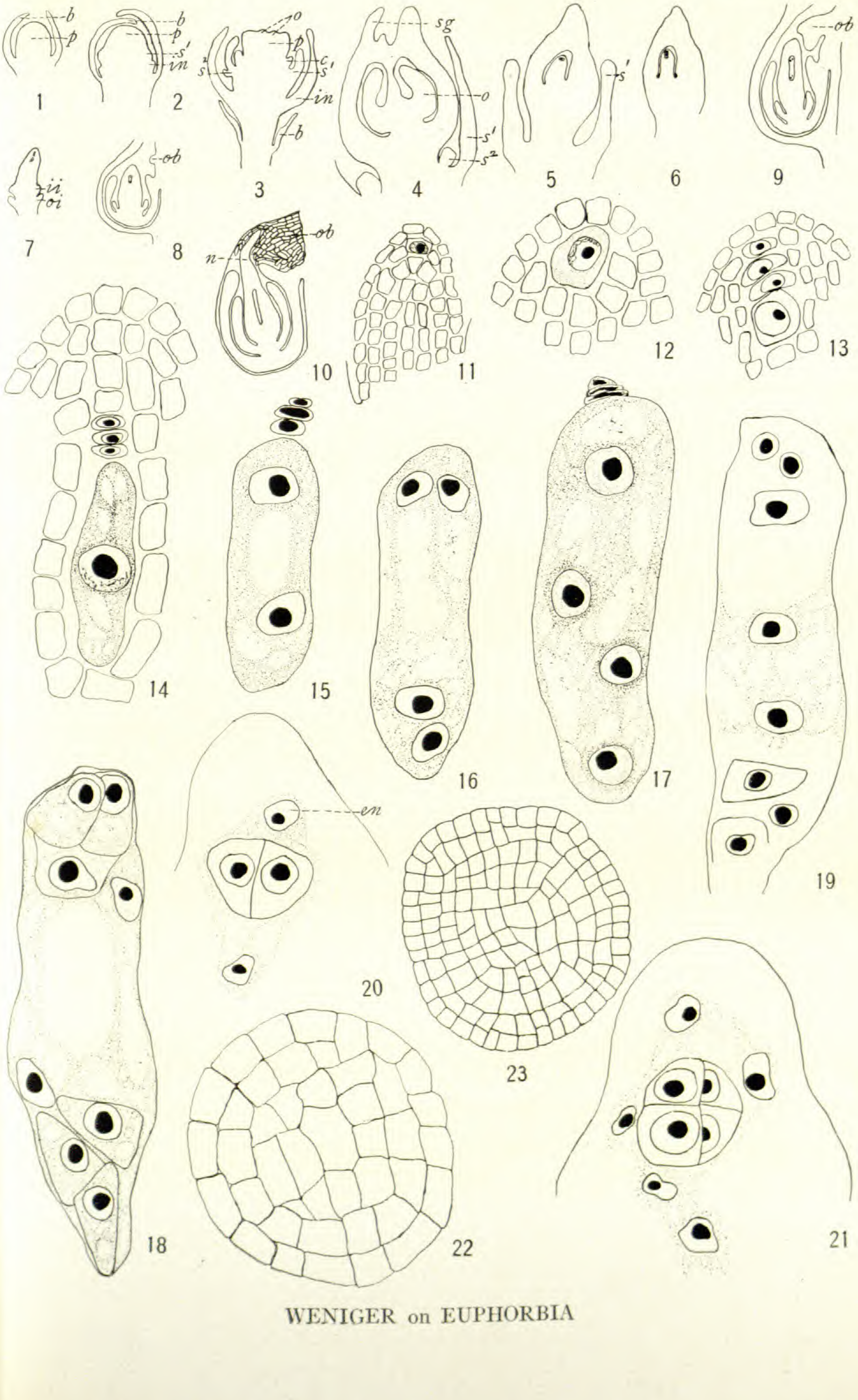
FIG. 23.—Still later stage in development of embryo.

PLATE XV

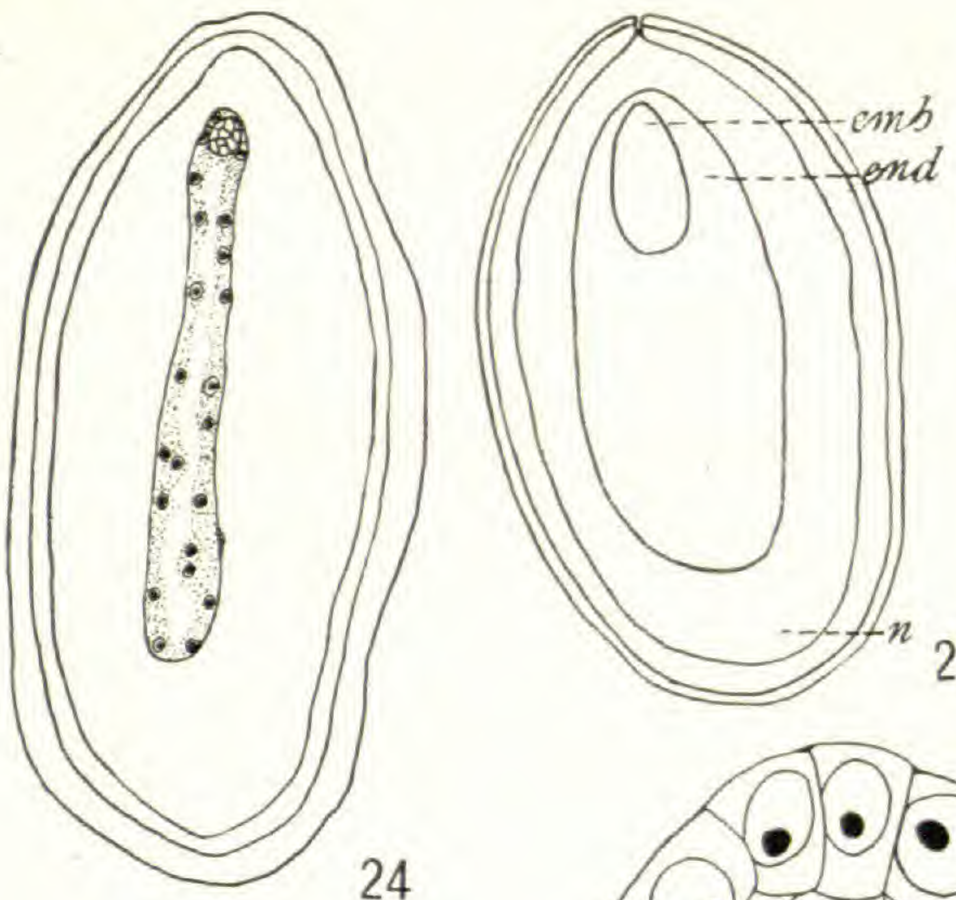
E. Preslii.—FIG. 24.—Longitudinal section of seed, with embryo at micropylar end of embryo sac, and endosperm nuclei distributed in peripheral region of sac.

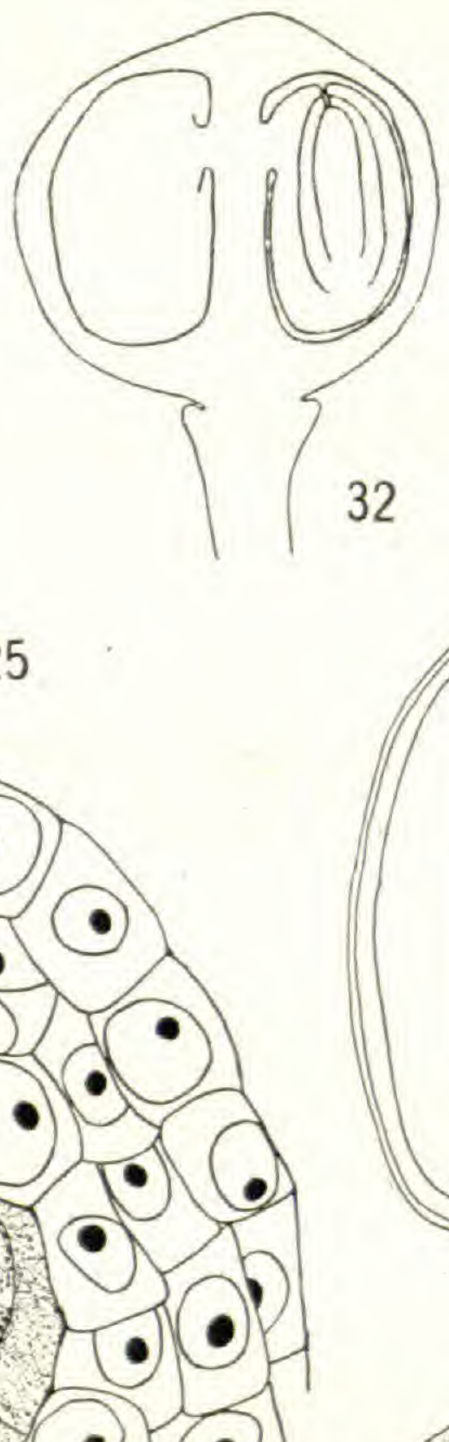
FIG. 25.—Elongated embryo imbedded in endosperm.

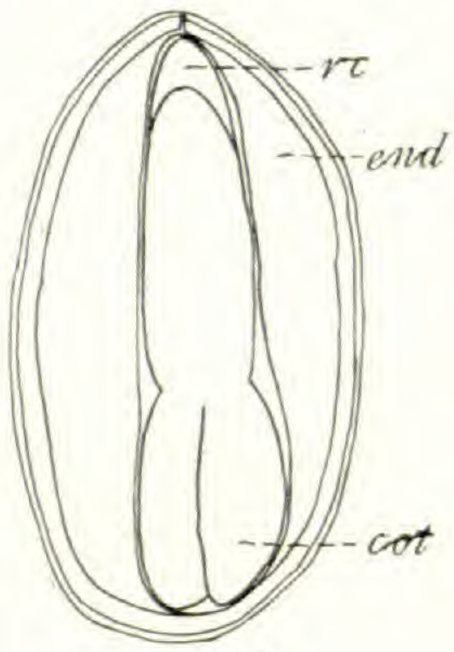
FIGS. 26, 28, 29, 30.—Elongation of embryo and appearance of cotyledons.

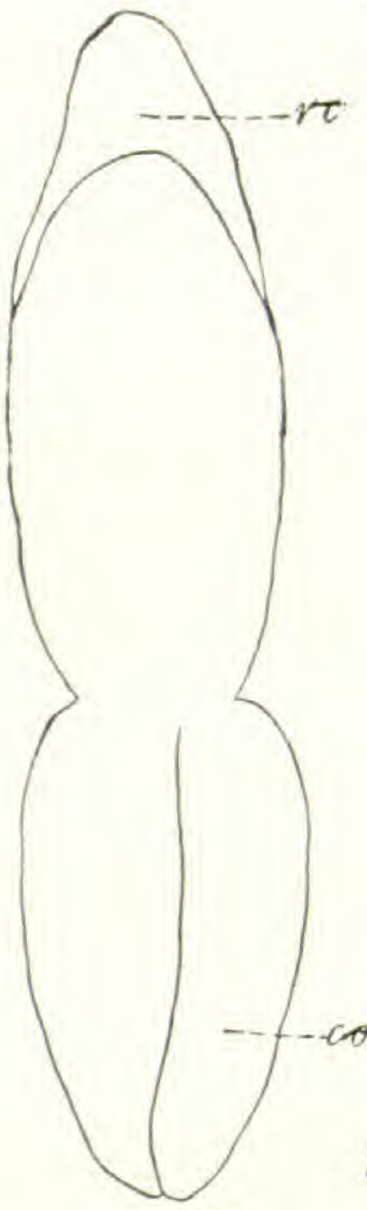


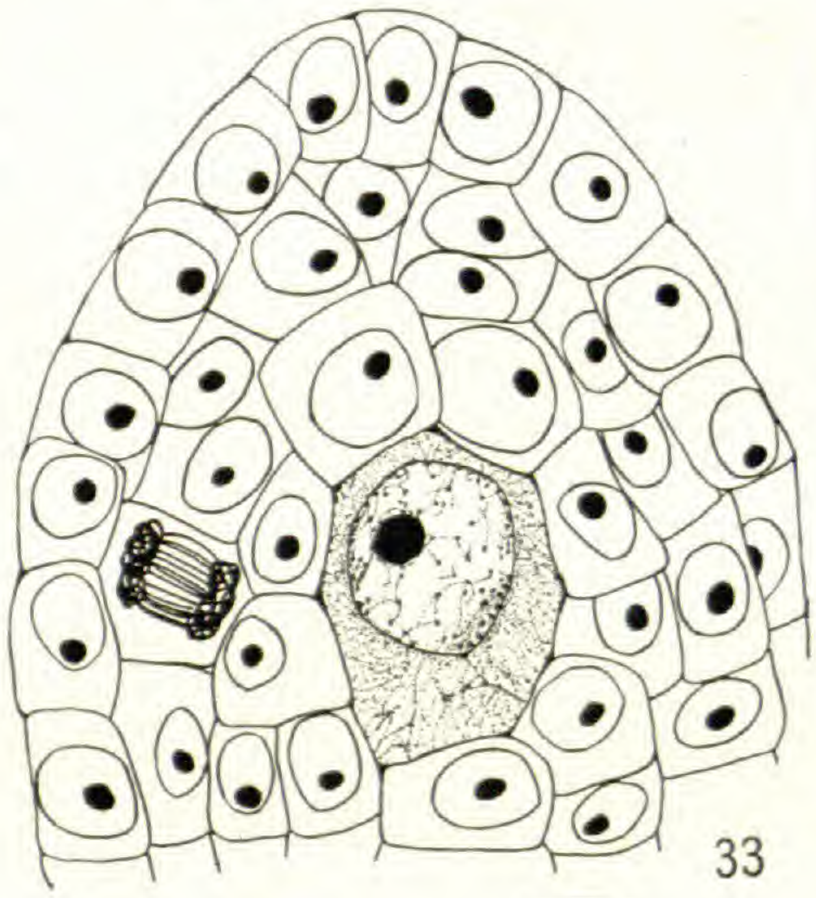
WENIGER on EUPHORBIA

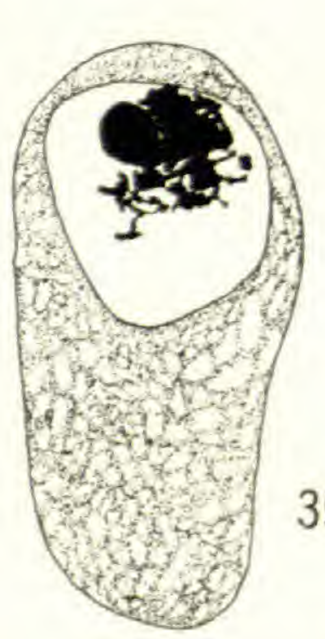


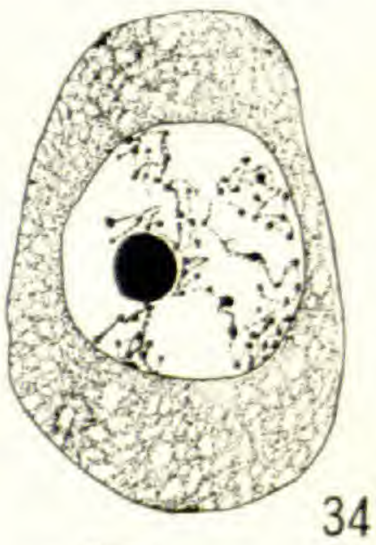


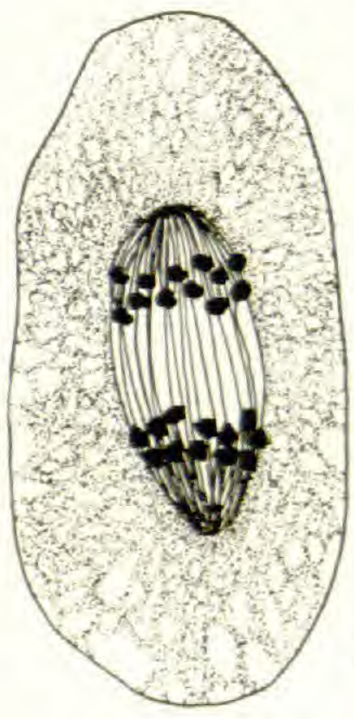


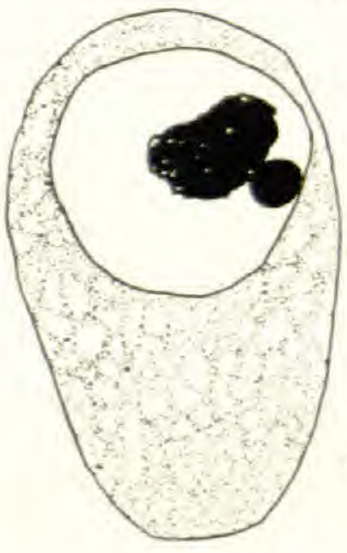


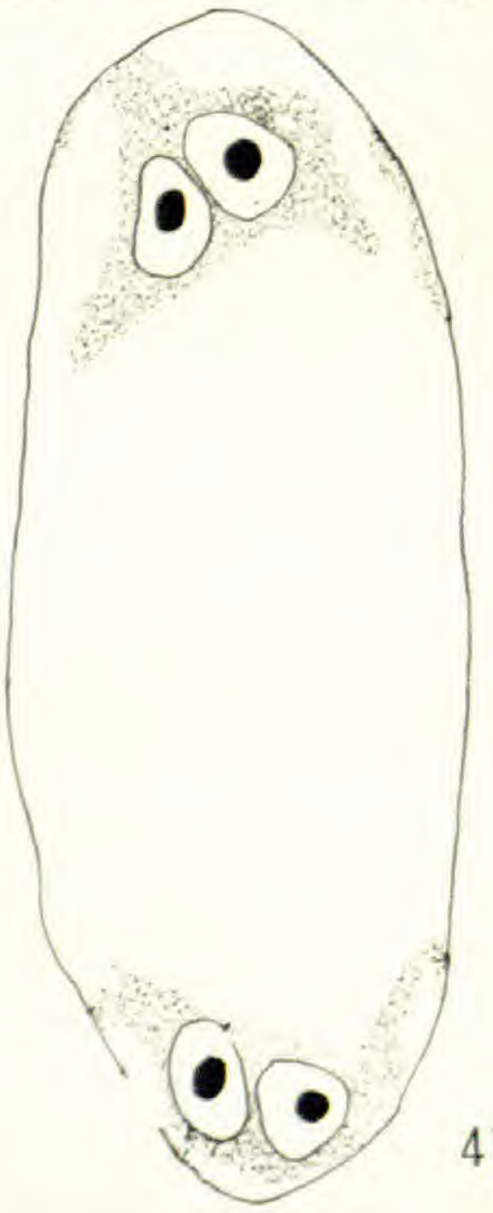


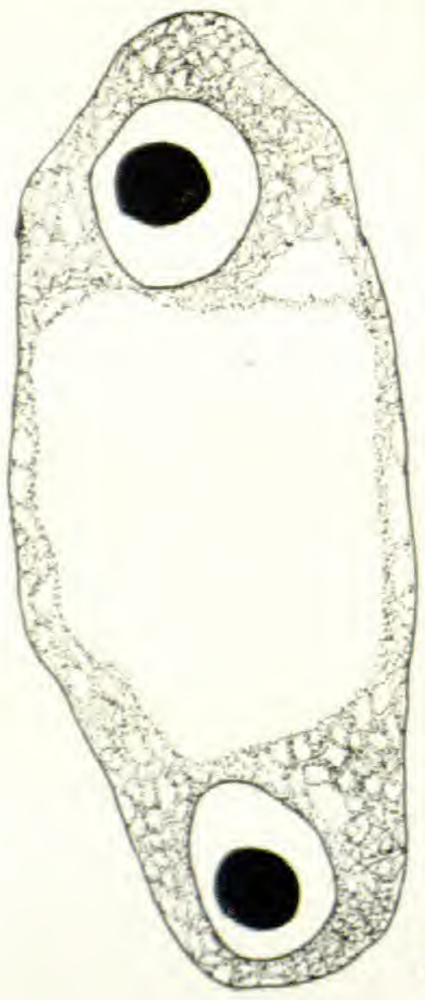


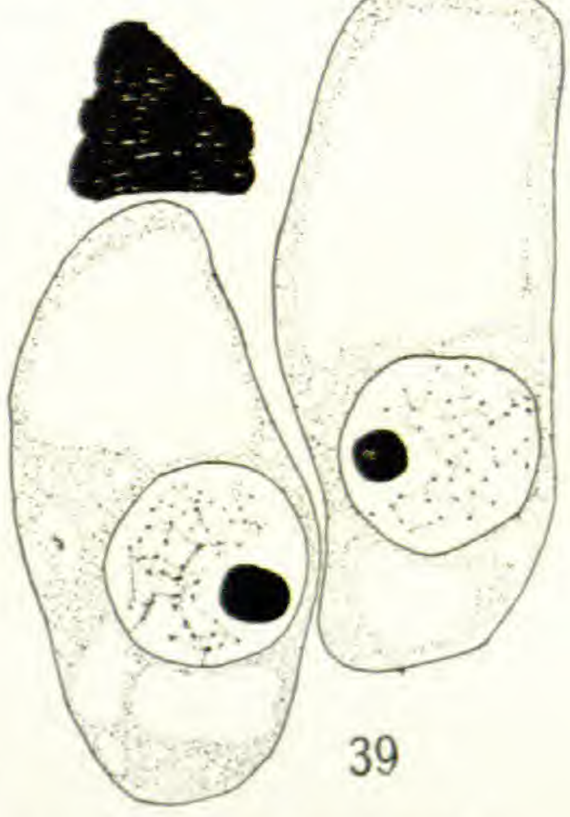












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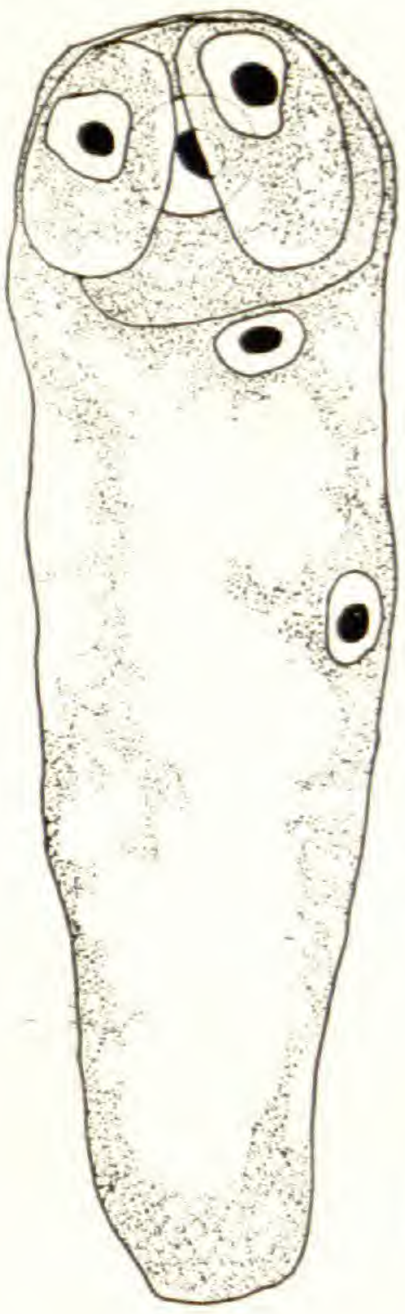

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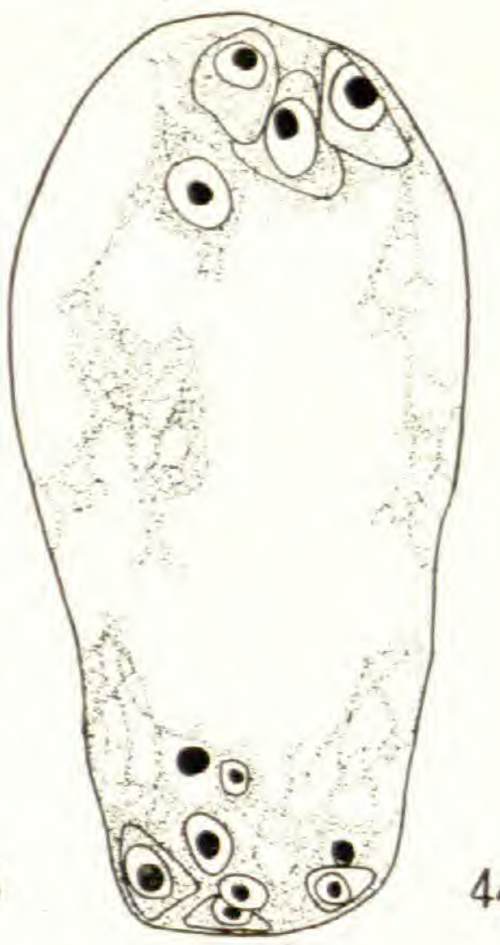

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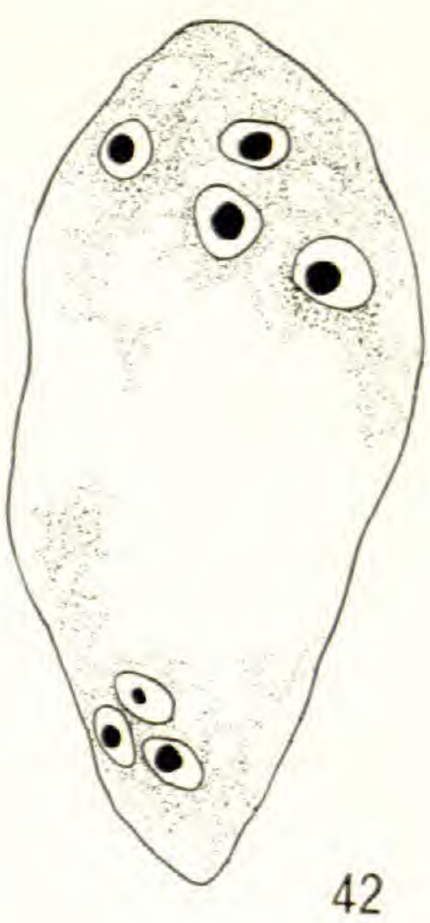

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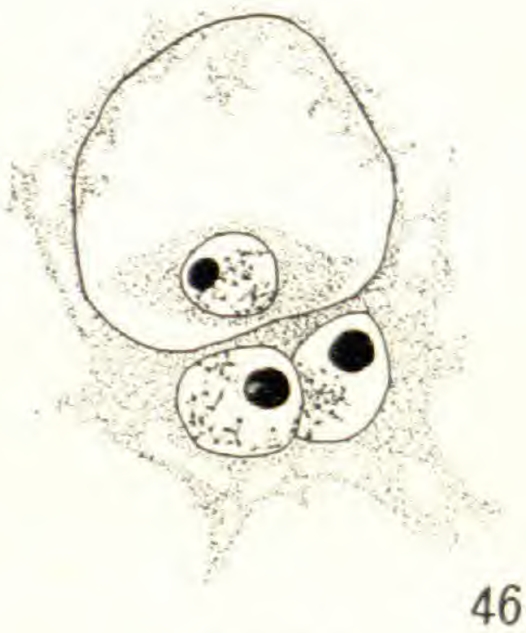

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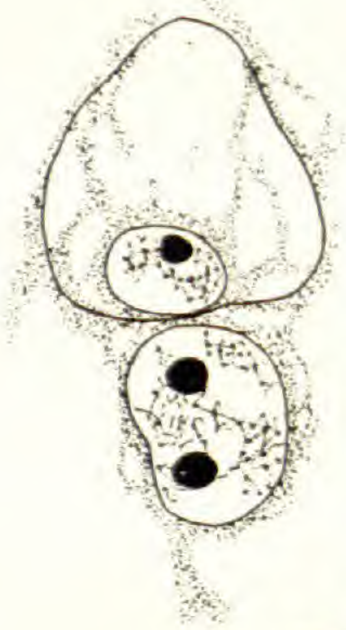

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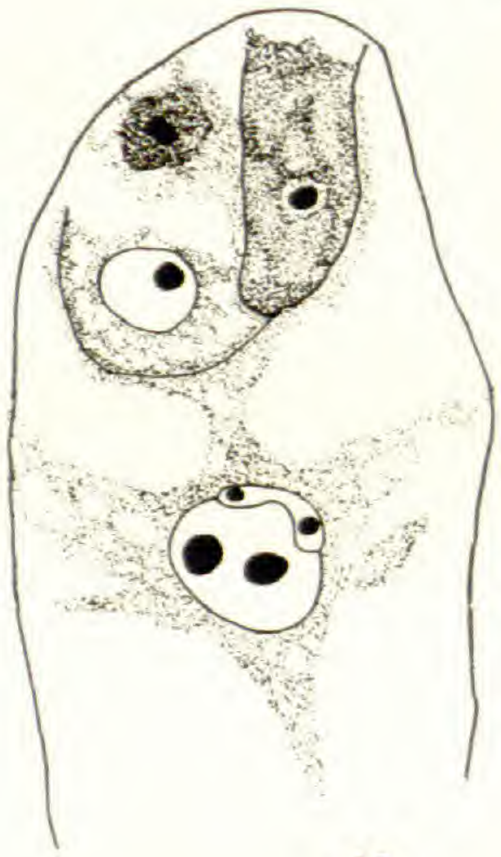

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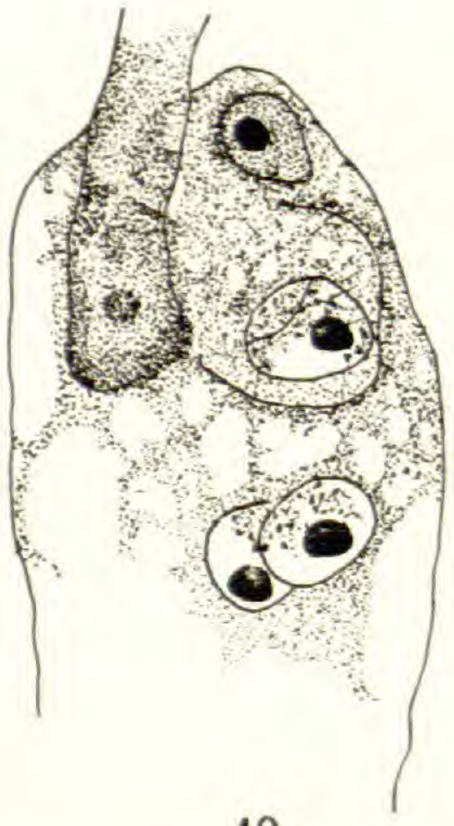

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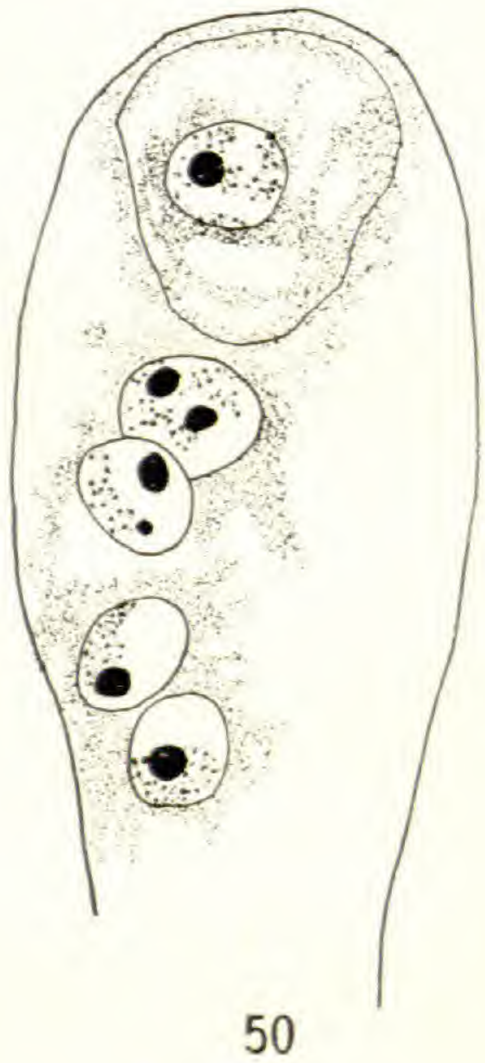

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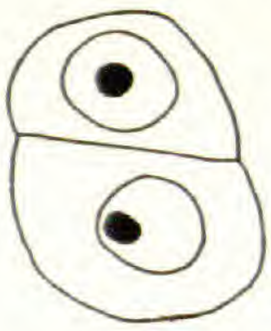

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FIG. 27.—Mature seed in longitudinal section.

FIG. 31.—Mature embryo.

FIG. 32.—Pistillate flower, with ovary jointed to pedicel.

E. splendens.—FIG. 33.—Nucellus with megaspore mother cell 2 layers below epidermis.

FIG. 34.—Megaspore mother cell with resting nucleus.

FIGS. 35, 36.—Synapsis in megaspore mother cell.

FIG. 37.—Anaphase of heterotypic division in megaspore mother cell.

FIG. 38.—Lowest of the 4 megaspores, which is to develop into embryo sac; other 3 megaspores have disintegrated.

FIG. 39.—Two functional megaspores side by side, each accompanied by what appear to be 3 disintegrating megaspores.

FIG. 40.—Binucleate embryo sac.

FIG. 41.—Four-nucleate embryo sac.

PLATE XVI

FIG. 42.—Embryo sac with 4 nuclei at micropylar end, and only 3 at antipodal end.

FIG. 43.—Embryo sac showing egg apparatus fully formed; 2 polar nuclei about to fuse near micropylar end; 2 daughter nuclei with cell plate near antipodal end.

FIG. 44.—Embryo sac with egg apparatus and 1 polar nucleus at micropylar end; 8 nuclei at antipodal end, 3 of which are inclosed by cell membranes.

FIG. 45.—Polar nucleus from antipodal end approaching other polar nucleus which lies close to egg apparatus.

FIG. 46.—Polar nuclei about to fuse near egg.

FIG. 47.—Polar nuclei fused, but 2 nucleoles still persisting; egg nucleus at one end of cell.

FIG. 48.—Fertilization: one male nucleus in contact with nucleus formed by fusion of polar nuclei; other male nucleus is still in pollen tube.

FIG. 49.—Pollen tube within embryo sac; no evidence of fertilization, and male nuclei not distinguishable within pollen tube; polar nuclei about to fuse.

FIG. 50.—Fertilized egg still undivided; 2 nuclear divisions have occurred in endosperm.

FIG. 51.—First division of egg.

FIG. 52.—Young embryo with terminal cell divided by longitudinal wall.

FIGS. 53, 54.—Later stage in embryo development.

FIG. 55.—Embryo consists of rounded mass of cells at end of a short suspensor.

FIG. 56.—Embryo has increased in size, and suspensor is beginning to disintegrate.

THE DEVELOPMENT OF THE ASCOCARP OF RHIZINA UNDULATA FR.

HARRY M. FITZPATRICK

(WITH PLATES XVII AND XVIII)

Our knowledge of the earliest stages in the development of the fruit body in the Helvellales is restricted to a limited number of species of the Geoglossaceae and one species of the Helvellaceae. Practically nothing is known of the ontogeny of any member of the Rhizinaceae. The question of the origin of the hymenium in this family, therefore, is of considerable interest.

Until recently it was believed that the ascocarp in members of the Helvellales, even in the youngest stages, is not covered by an inclosing membrane. In the system of classification employed by SCHRÖTER (**27**) the Helvellales are separated from the other orders of the Discomycetes on the basis of the gymnocarpous origin of the fruit body. The statement that members of this group are never angiocarpous, however, was evidently based upon general observations rather than upon careful study of young ascocarps, and subsequent investigations have demonstrated its falsity. The first evidence of the presence of a veil in this group was presented by DITTRICH (**10**) in connection with investigations on the Geoglossaceae. He discovered that in the youngest stages the fruit body of *Leotia lubrica* is inclosed by an envelope comparable to the volva of the Agaricaceae. This membrane later gelatinizes and is ruptured by the expansion of the ascoma within. Observations made by him on *Mitrula phalloides* disclosed a similar condition in that species. His collections of fruit bodies of representatives of the Helvellaceae, including species of *Helvella* and *Gyromitra*, revealed, however, no stages young enough to shed light on the question of the presence or absence of a veil in the beginning.

DURAND (**14**) in his monograph of the Geoglossaceae of North America reviews the work of DITTRICH on the development of *Leotia* and *Mitrula* and states that observations of his own on several different species point unmistakably to the same conclusion.

He has found a veil in *Spathularia velutipes*, *Mitrula phalloides*, *Microglossum viride*, and *Cudonia lutea*. The most conspicuous veil seen is present in *Cudonia lutea* and *Spathularia velutipes*. It persists in both species until the plants are one-third or even one-half grown, when it fragments into irregular pieces and falls away. DURAND publishes photographs showing very clearly the dehiscing membrane on the hymenium of maturing plants. He examined, however, very young ascomata of *Geoglossum glabrum*, *G. difforme*, and *Trichoglossum velutipes* without finding any trace of such a membrane. Finally, he expresses the opinion that "when the development of the Discomycetes shall be better understood it will be found that in none of them, not even in the Helvellaceae, is the hymenium exposed from the first."

More recently MCCUBBIN (**25**) has studied the development of the fruit body in *Helvella elastica*, and states that, in the earliest stages, the young ascocarp is inclosed by an envelope which later dehisces and completely disappears. He presents photomicrographs showing in median longitudinal section a single young fruit body bearing at the periphery an adhering bit of tissue, and says that this is a fragment of the transitory veil which earlier enveloped the ascocarp. Although his discussion covers stages earlier than that figured, his photomicrographs are unconvincing.

BROWN (**4**) has studied the development of *Leotia lubrica* and *L. chlorocephala*, but his account contains no information bearing on the question of the presence or absence of a veil in the early stages. CARRUTHERS (**7**) describes at considerable length the cytology of *Helvella crispa*, but does not attempt to study the earliest stages in the development of the fruit body. MASSEE (**24**) in his monograph of the Geoglossaceae makes no mention of the occurrence of a veil on any of the species in this family. So far as the writer is aware, no other investigation on the development of the fruit body in this order has been undertaken. No representative of the Rhizinaceae has been studied in the young condition.

Considerable difficulty is experienced in obtaining the youngest stages of the fruit bodies of members of the Helvellales, either by collection or culture, and it is not surprising therefore that students of the fungi have given little attention to developmental

studies in this group. Moreover, the number of species included in the Rhizinaceae is not large, and collections of some of these are rarely made (UNDERWOOD **29**, HONE **22**, BURT **6**).

During the summer of 1914 the writer discovered an abundant supply of the apothecia of *Rhizina undulata*, and was able to collect numerous very young fruit bodies in addition to the older stages. These have furnished all the necessary material for a thorough study of the development of the fruit body in this species. *Rhizina undulata* is particularly suitable for investigation, since it is the type of the genus and family, and probably the best known member of the group.

SCHRÖTER (**27**) separates the Rhizinaceae from the Geoglossaceae and Helvellaceae on the basis of the sessile fruit body. BOUDIER (**3**), attempting to arrange the Discomycetes in a natural classification, has developed a system very different from that of SCHRÖTER. He makes his primary separation on the basis of the method of rupture of the ascus. He places in one large group (Operculés) those forms whose asci open by an apical lid, and in the other group (Inoperculés) those whose asci open merely by a pore. By this separation the Helvellaceae and Rhizinaceae fall in the first group and the Geoglossaceae in the second. BOUDIER regards the Rhizinaceae as more closely related to such genera as *Peziza*, *Aleuria*, and *Sarcoscypha* of the Pezizales than to either the Helvellaceae or Geoglossaceae. LAGARDE (**23**) makes the primary separation also on the method of rupture of the ascus.

The facts brought out in the study of the development of the fruit body in various genera of the Discomycetes are especially interesting for the bearing they have on the questions involved in these two opposing systems of classification. The present investigation is undertaken with the hope that more complete information with reference to ontogeny will render less difficult the consideration of the phylogeny of the group.

Rhizina undulata Fr.

HISTORICAL.—The genus *Rhizina* was founded by FRIES (**16**) in 1815. It is characterized by the possession of prominent rope-like strands of mycelium, termed rhizoids. These are developed

in considerable numbers on the lower surface of the fruit body, and serve to attach it to the substratum. Representatives of this genus, therefore, are not easily mistaken.

Rhizina undulata was apparently first described by SCHAEFFER (**26**) under the name *Elvela inflata*. This writer published a colored figure of the plant which illustrates well the more evident characters of the species. FRIES (**16**) later described the fungus as *Rhizina undulata*, and discusses it under this name in *Systema mycologicum* (**17**). In accordance with the international rules of botanical nomenclature the writer designates the species by this name, but it has more commonly been referred to in recent literature as *Rhizina inflata*. The plant has been described by many writers and has frequently been figured. Excellent colored plates are given by BOUDIER (**2**). On account of the fact that the fungus is parasitic on the roots of certain trees its morphology and life history have received considerable attention (TUBEUF **28**).

HARTIG (**19, 20, 21**) discusses at some length the structure of the mature fruit body. He made no attempt to study its development. More recently WEIR (**30**) has published photographs of apothecia with notes on the parasitism of the fungus. None of these workers describes other than the mature condition.

MATERIAL AND METHODS.—The apothecia used by the writer in these investigations were collected in July 1914 in a small pine wood north of Beebe Lake near the Cornell University campus at Ithaca, New York. Due to favorable weather conditions the fruit bodies were developing in great profusion, and dotted the ground throughout a considerable portion of the wood. Although no attempt was made to obtain corroborative evidence as to the parasitism of the species, it was noted that the fruit bodies in many cases were firmly attached to the roots of living pines. Transverse sections through pine rootlets will be noted in the accompanying plates. In fact, the youngest fruit bodies were obtained more easily by tearing up a superficial root invested with wefts of mycelium on which the young fruit bodies were being differentiated. In this manner immature fruit bodies in all stages of development were obtained easily. Mature apothecia were available in such abundance that several quart jars of material were preserved for class

use. The writer's determination of the fungus was confirmed independently by E. J. DURAND and F. J. SEAVER, and his thanks are due both of these gentlemen.

The young apothecia collected for the study of the development of the fruit body were immediately placed in medium strength chromo-acetic acid fixer. They were carried into paraffin, and were studied in serial sections of 4–7μ thickness. The material was stained chiefly with Heidenhain's iron alum-haematoxylin, no counter stain being used. For certain of the more mature stages the shortened Flemming's triple stain proved more useful. The material was sectioned and stained in the laboratories of the Brooklyn Botanic Garden in the summer of 1915, while the writer held a visiting fellowship at that institution. He wishes to take this opportunity to express his appreciation of the courtesy of Director C. S. GAGER in extending to him the facilities of the laboratories, and to acknowledge his indebtedness to Dr. E. W. OLIVE for many kindnesses, including several helpful suggestions concerning technique. The investigation was carried to completion in the laboratories of the Department of Plant Pathology at Cornell University.

THE MATURE FRUIT BODY.—The mature apothecia exhibit great variation in size and shape. Considerable irregularity of contour is characteristic, and the early fusion of several fruit bodies results at maturity in large unsymmetrical structures. The apothecia shown natural size in fig. 1 illustrate well the extent of variation. The two fruit bodies in the lower left hand corner of the figure were inverted to reveal the clusters of ropelike rhizoids which give to the genus its name. The fruiting surface varies from a rich chestnut to a dark brown, and when moistened is peculiarly sticky and glutinous. Around the margin of the apothecium a sterile zone is indicated by a narrow, white, encircling band which contrasts sharply with the brown hymenium. This white margin is very evident in all stages. In the youngest fruit bodies the entire surface is white, the brown fruiting layer later making its appearance at the center and increasing rapidly in extent. The smaller of the fruit bodies pictured in fig. 1 show this condition clearly.

THE HYMENIUM.—The hymenium at maturity contains 3 types of structures: asci, paraphyses, and paraphysis-like structures which the writer will designate as setae, since they arise far below the hymenium, and are dark colored and thick-walled. The asci are narrow, cylindrical to clavate, and 8-spored. The spores are uniseriate, fusiform, hyaline, unicellular, and at maturity bigutulate. The paraphyses are filamentous, unbranched, multiseptate, hyaline, and at the apex distinctly clavate. The setae are heavy-walled, brown, non-septate, unbranched tubes originating far below the hymenium (fig. 11) and discharging a brown sticky secretion at their tips. This secretion flows over the surface of the hymenium made up of the swollen tips of the paraphyses, and gives a condition superficially resembling an epithecium. HARTIG (**20, 21**) states that it is impossible to procure a pure culture from the spores of this fungus on account of the bacteria which swarm in myriads in this glutinous secretion and find their way down between the paraphyses. These bacteria induce a rapid decay of the entire apothecium, and give to it in age a peculiar water-soaked, brittle consistency. In fig. 13 a portion of the hymenium is shown at a stage approximating maturity. The broad, deep-staining tubes are the setae. Surrounding these are the paraphyses, and pushing up from below may be seen the young, uninucleate asci. The swollen tips of the paraphyses are obscured by the layer of deep-staining glutinous material.

MYCELIUM.—The mycelium of *Rhizina undulata* possesses more than ordinary interest for the systematist. It is described by HARTIG (**21**) as bearing clamp connections. He says: "Although I have much diffidence in maintaining that this feature, which otherwise is peculiar to the Hymenomycetes, is characteristic of this parasite, still I cannot doubt that these filaments with clamp cells belong to it." The writer has given the mycelium careful examination, and has been unable in his collections to find clamp connections on hyphae certainly belonging to the fruit bodies of *Rhizina*. He does not feel, however, that sufficient investigation of this point has been carried on to enable him to state definitely that they never occur. The mycelium develops profusely, and covers the soil particles and small rootlets as a whitish, moldlike

growth. Upon this subiculum compact masses of hyphae develop as minute, snow white knobs. These represent the primordia of fruit bodies.

DEVELOPMENT OF THE ASCOCARP.—The youngest fruit body sectioned measures slightly less than 0.3 mm. in lateral diameter. A considerable number of others possess a maximum diameter of 1 mm. or less. The youngest fruit body studied (fig. 2) is a wholly undifferentiated "button" of mycelium. The hyphae making up the primordium arise in this case from about a small rootlet, and pushing upward between other rootlets run more or less distinctly parallel toward the surface of the ground, where they radiate in every direction, giving the primordium its rounded form. At this early stage there is no indication of sexual cells, and no evidence other than shape that this "button" of mycelium is to develop into a fruit body.

The hyphae at the surface of the primordium form a more or less definite palisade layer, although at this early period they are sufficiently flexuous to destroy the very definite palisade effect evident later. These hyphae in many cases can be traced backward with ease to the point of origin of the fruit body. No structure of the nature of an enveloping veil is present, and it is incredible that one could have existed at an earlier period. Neither in this nor in any later stage has the writer been able to find remnants of a ruptured envelope such as that figured by MCCUBBIN (**25**) for *Helvella elastica*. He has searched for these in sections of many very young fruit bodies and is absolutely convinced that in *Rhizina undulata* the ascocarp is at no stage provided with a veil. The fruit body is therefore gymnocarpous and the hymenium is "exposed from the first." Fig. 2 shows in median longitudinal section a fruit body of *Rhizina undulata* considerably younger than the youngest stage photographed by MCCUBBIN in *Helvella*. The deep-staining spots at the side and base of the primordium are transverse sections of pine rootlets.

MCCUBBIN states that in *Helvella elastica* "the envelope which covers the fruiting body in its early stages arises from the palisade layer. Many of the club-shaped hyphae of the latter continue to grow out beyond the general surface, then turn at right angles,

and interlacing in every direction along the surface form a matted web 2–8 threads in thickness. This membrane is very transitory, however, and undergoes degeneration at an early period. Its protoplasm takes on a granular appearance, the cell outlines become indistinct, and finally the whole disintegrates into a deeply staining mass in which the nuclei are the most prominent feature. Long before the process is complete, however, the rapid growth of the underlying tissue bursts the envelope so that it adheres in flakes (figs. 57, 58). Then the paraphyses and intercalary palisade hyphae pushing out to the surface complete the separation and all traces of it are cast off."

It is to be regretted that McCubbin did not publish photomicrographs of stages younger than that shown in his fig. 57. If in *Helvella*, as he states, the envelope, which incloses the fruit body in the early stages, arises from the palisade layer, it might be concluded that the section of *Rhizina undulata* shown in fig. 2 is too young to possess the envelope, and that it might logically be expected to develop later on older fruit bodies. That it does not do so, however, is certain. The writer has had available a sufficiently large number of fruit bodies in all stages of development to preclude any misinterpretation with reference to this point. No veil or fragment of a veil has been found on any of the fruit bodies sectioned.

Figs. 3–6 show median longitudinal sections through primordia somewhat older than that pictured in fig. 2. The magnification in the 5 cases is the same, being 40 diameters. Other fruit bodies sectioned, of intermediate sizes, bring out no additional facts. In fig. 3 the palisade nature of the hyphal arrangement at the periphery is evident. The deep-staining area on the upper surface to the left of the center is a fragment of a sectioned rootlet other portions of which were cut away in trimming the print. Other sections of similar rootlets appear at different places in the interior of the fruit body. At the base of the ascocarp can be noted the tendency of the mycelium to form thick rhizoids. These young rhizoids appear in section in figs. 3, 5, and 6. Fig. 5 shows the palisade layer of hyphae very clearly. In fig. 10 a portion of the palisade layer of a fruit body approximately the same age as that

in fig. 5 is shown much enlarged. It will be noticed here that the tips of the hyphae at the periphery stain very deeply. This is probably due to the fact that, since growth is taking place much more rapidly at the tips of the hyphae, the protoplasm at this point contains as the result of metabolism more deeply staining contents.

In certain young fruit bodies (fig. 4) the setae are developed much earlier than in others. The reason for this is not known. They are prominent organs, originate from the deeper lying tissue of the fruit body, and protrude beyond the palisade layer as deep-staining spines. These are shown much enlarged in fig. 14. It will be noted that they are of much greater diameter than the other hyphae of the ascocarp. They arise as differentiations of ordinary vegetative hyphae.

SEXUALITY.—Near the center of the sections shown in figs. 3 and 5 are to be seen deep-staining elements. These bodies constitute the sexual apparatus of the fungus, and at a somewhat later stage (fig. 9) give rise to the ascogenous hyphae. Since the writer is engaged in the preparation of another paper dealing with the details of the sexual process in *Rhizina undulata*, he will refrain from further comment on these structures at this point.

PARAPHYSES.—The layer of paraphyses is developed comparatively early in the history of the fruit body and constitutes a well defined zone long before the asci are produced. Fig. 7 shows a median longitudinal section through a young apothecium on the upper surface of which the layer of paraphyses is being differentiated. In fig. 8 this same layer is shown more highly magnified. The paraphyses arise from the ordinary hyphae in the interior of the fruit body, and are in reality a specialized portion of the palisade layer. As the fruit body enlarges by the elongation and branching of the hyphae at the periphery, those palisade hyphae which lie on the upper surface increase in number, run more nearly parallel, and come to stand very close together. They soon constitute a well defined zone, the individual units of which appear straighter, slightly narrower, and many times more abundantly septate than the palisade hyphae covering the remainder of the fruit body. This layer of paraphyses continues to develop at the margin as the fruit body increases in diameter, the line of demarcation between

paraphyses and palisade hyphae at the point of contact never being very sharp. Fig. 9 pictures approximately one-half of a median longitudinal section through an older fruit body in which the layer of paraphyses has become sharply differentiated from the tissue of the fruit body below. The rounded sterile margin of the apothecium is here evident.

ASCOGENOUS HYPHAE.—Immediately beneath the paraphyses is a deeper-staining zone filled with the ultimate tips of the profusely branching ascogenous hyphae. These hyphae have their origin near the base of the fruit body in the sexual apparatus previously mentioned, and may be seen ramifying throughout the interior of the ascocarp as they branch and rebranch on their upward journey toward the hymenium. At this stage these threads have not yet undergone crozier formation at their tips, and no young asci are present. Fig. 12 shows a section through the hymenium of a more mature apothecium in which the young asci are pushing up among the paraphyses. The septate paraphyses, the tubular setae, and the young, deep-staining, clavate asci show here to good advantage. In fig. 13 the asci are shown at about one-half their mature size, and the fusion nucleus may clearly be seen in each. In this and other sections the deep-staining glutinous secretion previously discussed forms a well defined layer above the clavate tips of the paraphyses.

General considerations

The results of the present investigation on the origin and development of the ascocarp in *Rhizina undulata* are particularly interesting in the light of the facts disclosed by various workers on other allied forms. Before the publication of the work of DITTRICH (10) on the development of *Leotia lubrica* and *Mitrula phalloides*, it was generally assumed that in the 3 families of the Helvellales the fruit body is gymnocarpous. After the appearance of DITTRICH'S paper the pendulum of opinion swung to the other extreme, and we find the statement made by DURAND (14) that in his opinion "when the development of the Discomycetes shall be better understood it will be found that in none of them, not even in the Helvellaceae, is the hymenium 'exposed from the first.'" It is evident now from the results of researches on various

Geoglossaceae that certain members of this family are at first provided with a veil. It is equally certain that in *Rhizina undulata* no enveloping membrane is ever present. Both conditions occur therefore within the order. Whether it will prove possible to separate the families of the orders on the basis of the presence or absence of a veil is doubtful, but additional investigations on members of the 3 families will be necessary to determine this point. Since the work of McCUBBIN (**25**) on *Helvella elastica* is the only contribution of any importance to our knowledge of the development of the fruit body in the Helvellaceae, it is desirable that other representatives of this family be studied. Also, since McCUBBIN has stated definitely "from observations on a very complete series of stages that *Geoglossum hirsutum* shows no trace whatever" of a veil, it is desirable that photographs be published demonstrating the gymnocarpous nature of the ascocarp in this or other members of the Geoglossaceae in which a veil is absent at all stages. Finally, the development of the fruit body in additional species of the Rhizinaceae should be studied to determine whether the conditions described for *Rhizina undulata* are typical of the entire family.

It has become increasingly evident since the publication by SCHRÖTER (**27**) of his system of classification of the Discomycetes that his basis for the separation of the Helvellales from the other orders of the group is untenable. Not only has it been demonstrated that in certain of the Helvellales the fruit body is angiocarpous, but also in the Pezizales it has been shown that certain species possess a fruit body which is clearly gymnocarpous. As representatives of this latter group may be enumerated *Ascodesmis* (CLAUSSEN **8**), *Pyronema confluens* (HARPER **18**, CLAUSSEN **9**, et al.), *Lachnea stercorea* (FRASER **15**), *L. scutellata* (BROWN **5**), and *Ascobolus magnificus* (DODGE **11, 12, 13**). The presence or absence of a hyphal envelope, therefore, cannot be used to separate the Helvellales and Pezizales as constituted by SCHRÖTER, and some other system of classification of the Discomycetes must be employed. That of BOUDIER (**3**) has met with considerable favor.

As pointed out by DODGE (**13**) and ATKINSON (**1**), several well defined types of ascocarps are present in the Ascomycetes, and these

should be considered in any system of classification. The somewhat loose use of the terms "angiocarpous" and "gymnocarpous" and of the phrase "hymenium exposed from the first" has resulted, however, in some confusion. In some species (for example *Leotia lubrica*) the ascocarp is at the beginning inclosed by an envelope which is transitory and disappears before the hymenium is formed, while in others (for example, *Rhizina undulata*) it lacks at all stages any indication of a veil. In both cases the hymenium is "exposed from the first," but the development of the fruit body is essentially different, and if the veil has any phylogenetic significance the two forms cannot be regarded as closely related. DODGE (13) states that "the real question as to whether an ascocarp is to be classed as open or closed in its early stages depends upon whether the young hymenial layer arises endogenously, as in *Ascobolus furfuraceus*, or is from the first free and exposed, as in *Pyronema*." It seems to the writer of greater significance to determine whether the ascocarp is itself at any stage inclosed by an envelope. This is certainly true from the standpoint of phylogeny.

Summary

1. The mycelium of *Rhizina undulata* Fr. spreads among the soil particles, and covers the smaller rootlets of pines and other trees, forming a whitish moldlike growth. Upon this subiculum compact masses of hyphae develop as minute, snow white, rounded knobs. These constitute primordia of ascocarps.

2. The ascocarp primordium in the youngest stages shows no evidence of a sexual apparatus. It is made up of undifferentiated hyphae, which at its surface form a palisade layer.

3. The ascocarp is neither at the beginning nor at any subsequent period provided with a hyphal envelope. The fruit body is therefore gymnocarpous and the hymenium is "exposed from the first."

4. There is developed in the interior of the young ascocarp a well defined sexual apparatus from which the ascogenous hyphae arise. The details of the sexual process have been studied and will be described in a later paper.

5. The ascogenous hyphae branch repeatedly and undergo crozier formation in the development of the young asci.

6. The paraphyses are a differentiation of the palisade layer which covers the fruit body at all stages.

7. In the ascocarp of this species there are present paraphysis-like structures which arise early in the history of the fruit body. They are non-septate, thick-walled tubes which originate far down in the hypothecium, traverse the hymenium, and discharge a brown, glutinous secretion at their tips. The writer has applied to these the term "setae."

8. At maturity the ascocarp is variable in size and shape. The brown hymenium is bordered by a sterile white margin.

9. There are present on the lower surface of the ascocarp numerous prominent rhizoids.

DEPARTMENT OF PLANT PATHOLOGY
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LITERATURE CITED

1. ATKINSON, G. F., Phylogeny and relationships in the Ascomycetes. Ann. Mo. Bot. Gard. **2**:315–376. 1915.
2. BOUDIER, ÉMILE, Icones mycologicae **2**: *fig. 251*. 1904.
3. ———, Histoire et classification des Discomycètes d'Europe. Paris. 1907.
4. BROWN, W. H., The development of the ascocarp of *Leotia*. BOT. GAZ. **50**:443–459. *figs. 47*. 1910.
5. ———, The development of the ascocarp of *Lachnea scutellata*. BOT. GAZ. **52**:273–305. *pl. 9. figs. 51*. 1911.
6. BURT, E. A., A list of Vermont Helvelleae with descriptive notes. Rhodora **1**:59–67. *pl. 4*. 1899.
7. CARRUTHERS, D., Contributions to the cytology of *Helvella crispa* Fries. Ann. Botany **25**:243–253. *pls. 18, 19*. 1911.
8. CLAUSSEN, P., Zur Entwickelungsgeschichte der Ascomyceten. *Boudiera*. Bot. Zeit. **63**:1–28. *pls. 1–3. figs. 6*. 1905.
9. ———, Zur Entwickelungsgeschichte der Ascomyceten. *Pyronema confluens*. Zeitschr. Bot. **4**:1–64. *pls. 1–6. figs. 13*. 1912.
10. DITTRICH, G., Zur Entwickelungsgeschichte der Helvellineen. Cohn's Beiträge zur Biologie der Pflanzen **8**:17–52. *pls. 4,5*. 1898.
11. DODGE, B. O., Artificial cultures of *Ascobolus* and *Aleuria*. Mycologia **4**:218–222. *pls. 72–73*. 1912.
12. ———, Methods of culture and the morphology of the archicarp in certain species of the Ascobolaceae. Bull. Torr. Bot. Club **39**:139–197. *pls. 10–15. figs. 2*. 1912.
13. ———, The morphological relationships of the Florideae and the Ascomycetes. Bull. Torr. Bot. Club **41**:157–202. *figs. 13*. 1914.

14. DURAND, E. J., The Geoglossaceae of North America. Annales Mycologici 6:387–477. *pls. 5–22*. 1908.
15. FRASER, H. C. I., On the sexuality and development of the ascocarp in *Lachnea stercorea*. Ann. Botany 21:349–360. 1907.
16. FRIES, ELIAS, Observationes mycologicae 1:161–162. 1815.
17. ———, Systema mycologicum 2:33. 1822.
18. HARPER, R. A., Sexual reproduction in *Pyronema confluens* and the morphology of the ascocarp. Ann. Botany 14:321–400. *pls. 19–21*. 1900.
19. HARTIG, R., Untersuchungen über *Rhizina undulata*. Bot. Centralbl. 45:237–238. 1891.
20. ———, *Rhizina undulata* Fr. Der Wurzelschwamm. Forst. Naturw. Zeitschr. 1:291–297. 1892.
21. ———, Text-book of the diseases of trees. Transl. by W. SOMERVILLE. Rev. and edit. by H. MARSHALL WARD 123–129. *figs. 61–70*. 1894.
22. HONE, DAISY S., Minnesota Helvellineae. Minn. Bot. Studies 3:309–321. *pls. 48–52*. 1904.
23. LAGARDE, J., Contribution à l'étude des Discomycètes charnus. Annales Mycologici 4:125–256. *figs. 58*. 1906.
24. MASSEE, G., A monograph of the Geoglossaceae. Ann. Botany 11:225–306. *pls. 12, 13*. 1897.
25. MCCUBBIN, W. A., Development of the Helvellineae. I. *Helvella elastica*. BOT. GAZ. 49:195–206. *pls. 14–16*. 1910.
26. SCHAEFFER, I. CH., Fungorum Bavariae et Palatinatus Icones. *pl. 153*. 1800.
27. SCHRÖTER, J., Helvellineae, Pezizineae; in ENGLER and PRANTL'S Die natürlichen Pflanzenfamilien 1': 162–243. 1894.
28. TUBEUF, KARL VON, Diseases of plants induced by crytogamic parasites. Eng. ed. by W. G. SMITH. London. 1897 (pp. 272–274. *figs. 144–147*).
29. UNDERWOOD, L. M., On the distribution of the North American Helvellales. Minn. Bot. Studies 1:483–500. 1896.
30. WEIR, J. R., Observations on *Rhizina inflata*. Jour. Agric. Research 4:93–96. *pl. 8*. 1915.

EXPLANATION OF PLATES XVII AND XVIII

FIG. 1. *Rhizina undulata* Fr., natural size, Ithaca, New York, July 1914; group of apothecia selected to show variation in size and shape; note sterile white margin on both young and old plants, and tendency for adjacent fruit bodies to fuse; at lower left hand corner of figure 2 apothecia are inverted to show lighter colored, lower surface and dense clusters of stout rhizoids which serve to attach the fruit body to substratum.

FIG. 2.—Median longitudinal section through a very young ascocarp primordium, ×40; note pine rootlets in section at side and base.

FIG. 3.—Median longitudinal section through a somewhat older fruit body, ×40; deep-staining body at periphery above is fragment of section

through a pine root such as those shown in the lower half of fruit body; deep-staining structures near center of section are sexual cells which later give rise to ascogenous hyphae; at base young rhizoids are shown in section.

FIG. 4.—Median longitudinal section through a young fruit body in which setae have developed early, ×40; these may be seen projecting above layer of palisade hyphae.

FIG. 5.—Median longitudinal section through a young fruit body, ×40; palisade layer of hyphae at periphery shows plainly; note sexual cells at center of section.

FIG. 6.—Median longitudinal section through a slightly older fruit body, ×40.

FIG. 7.—Median longitudinal section through a somewhat older fruit body in which the layer of paraphyses is being differentiated from palisade layer, ×29.

FIG. 8.—Layer of paraphyses shown in fig. 7 enlarged to show structure more clearly, ×40; note indefinite line of demarcation between layer of paraphyses and palisade layer of sterile margin.

FIG. 9.—Approximately one-half of a median longitudinal section through considerably older fruit body, ×32; note well defined layer of paraphyses, sterile margin, and definite, deep-staining zone below the paraphyses made up of tips of ascogenous hyphae; ascogenous hyphae can be seen originating near base of apothecium and branching profusely as they ramify throughout the fruit body and approach hymenium.

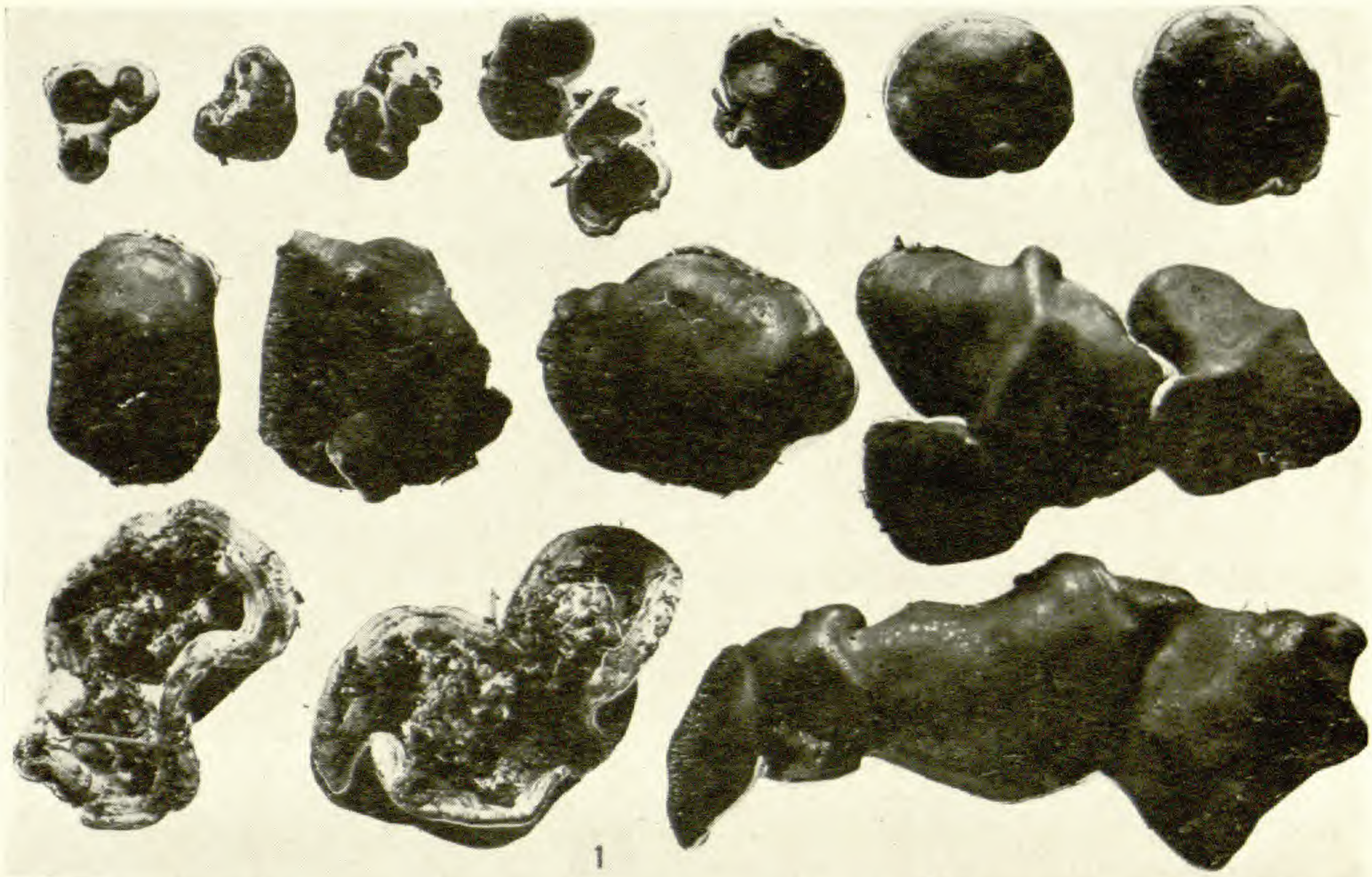
FIG. 10.—Portion of section such as presented in fig. 5 enlarged to show structure of palisade layer, ×192; note deep-staining tips of hyphae.

FIG. 11.—Longitudinal section through young hymenium of fruit body of about the same age as that shown in fig. 9, ×192; note numerous prominent setae originating below hymenium; note also deep-staining layer at tips of paraphyses, resulting from glutinous secretion poured over hymenium by setae.

FIG. 12.—Longitudinal section through immature hymenium of fruit body somewhat larger than that shown in fig. 9, ×192; note slender, septate paraphyses, prominent tubular setae, and young deep-staining asci.

FIG. 13.—Longitudinal section through hymenium of fruit body approaching maturity, ×192; asci have not yet formed spores; fusion nucleus is visible in some cases.

FIG. 14.—Portion of section given in fig. 4 enlarged to show setae at margin of fruit body, ×192; note that they are much larger in diameter and more deeply staining than the hyphae of palisade layer.



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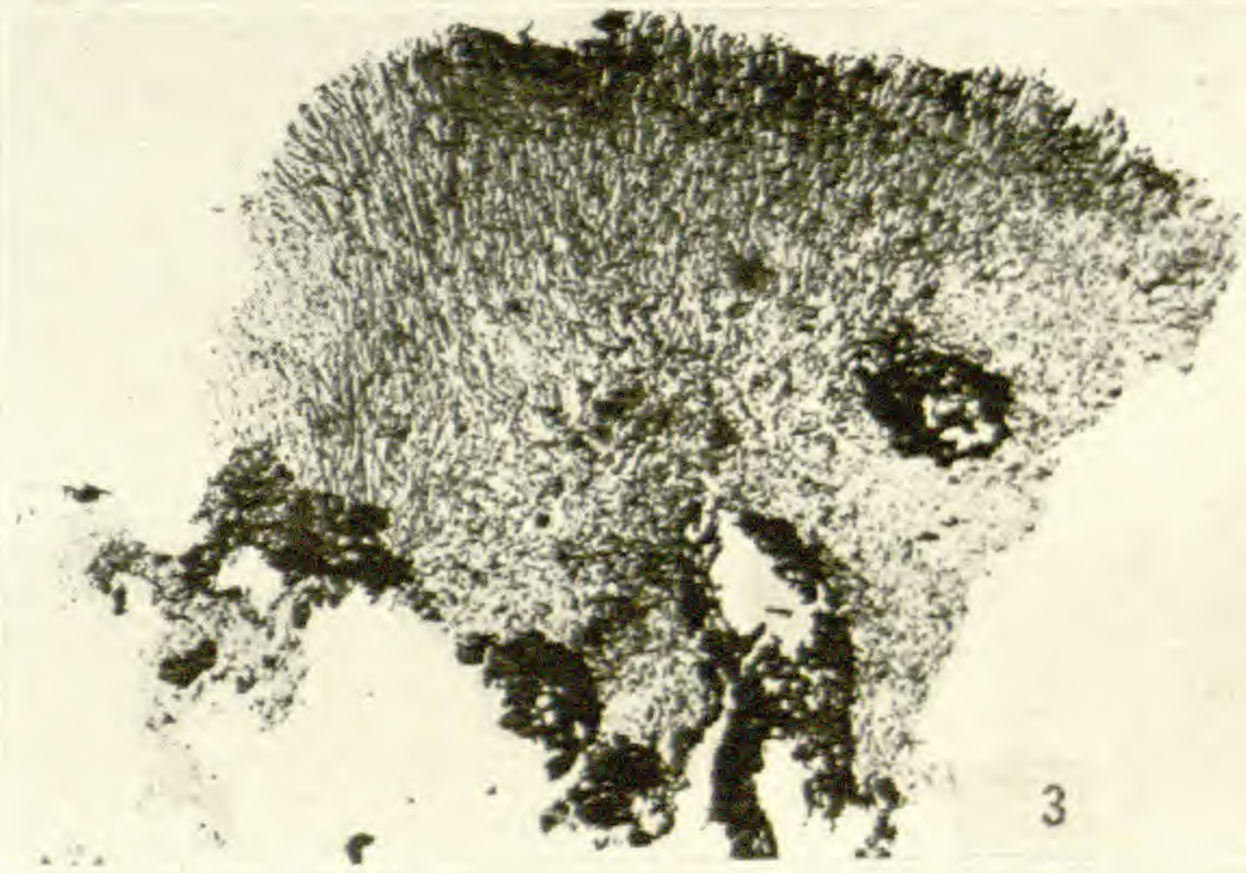

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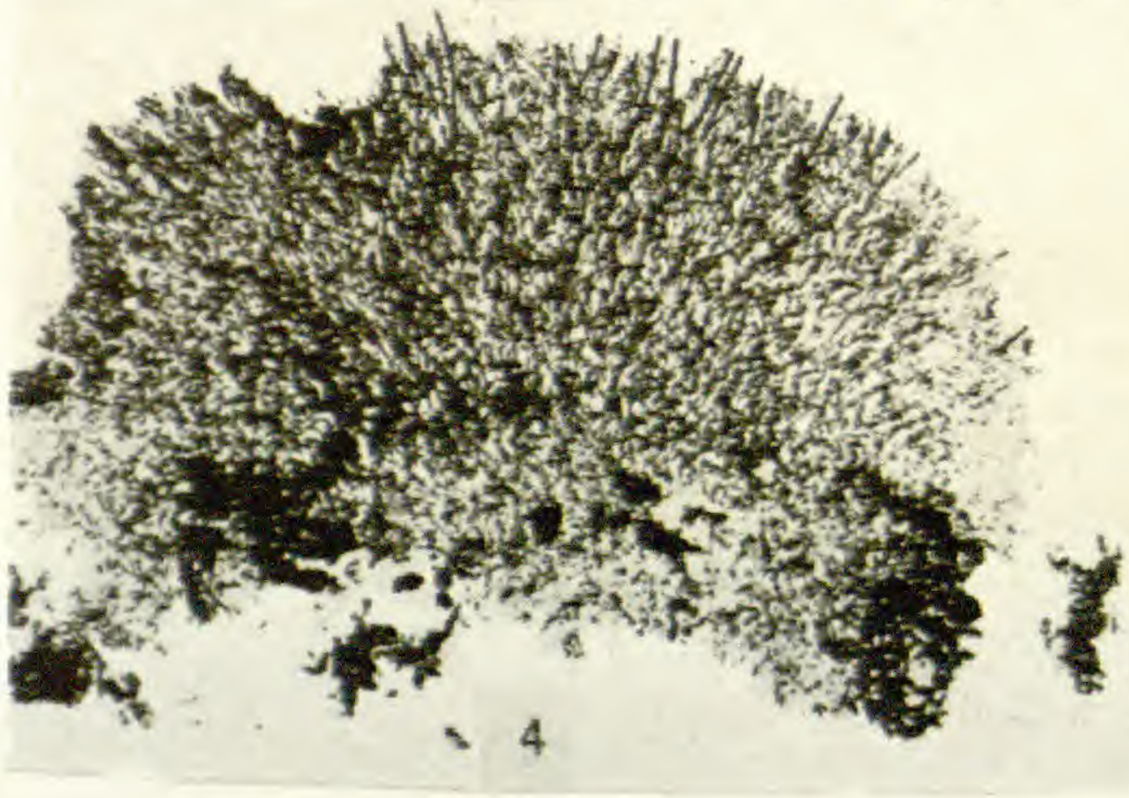

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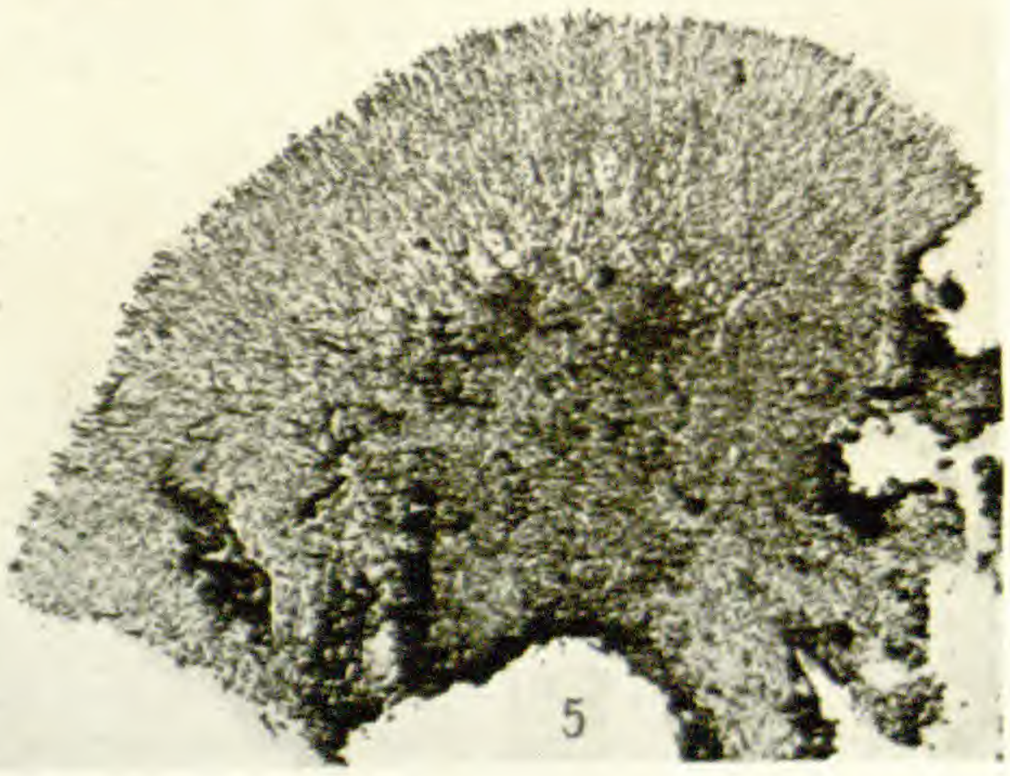

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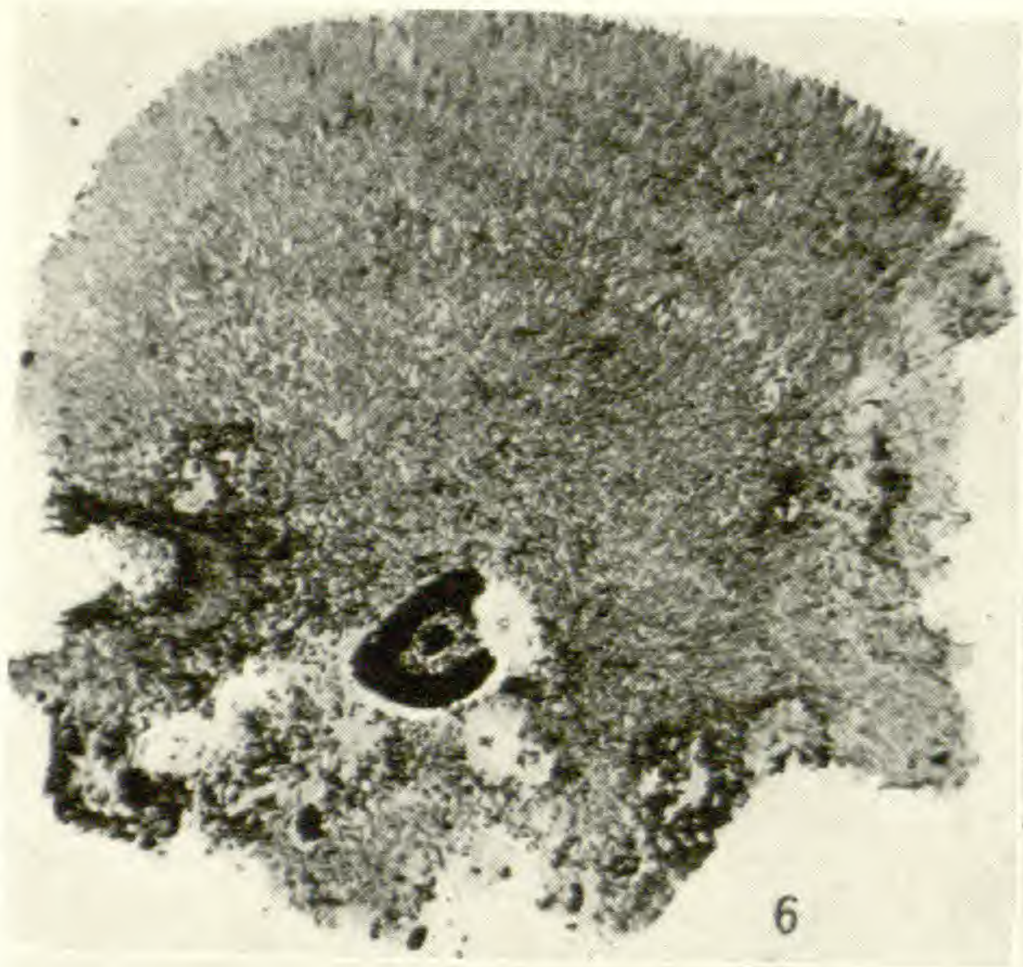
FITZPATRICK on RHIZINA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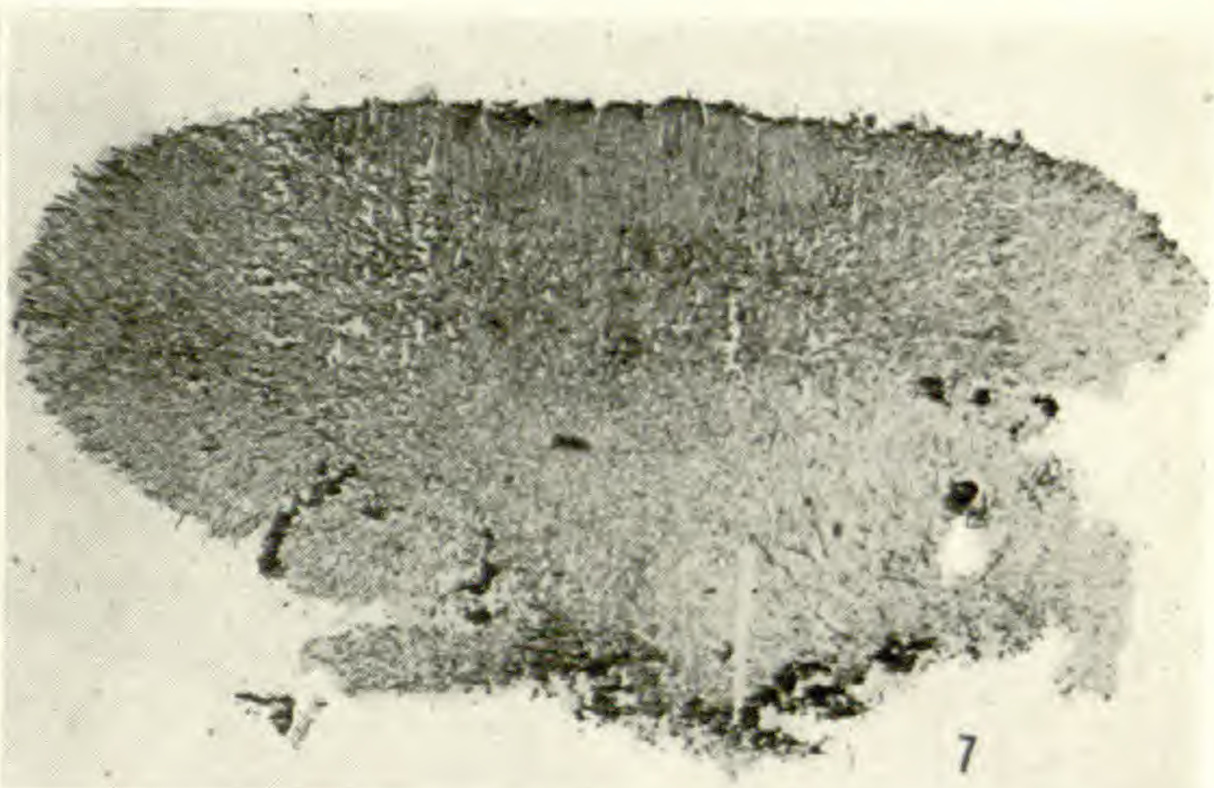
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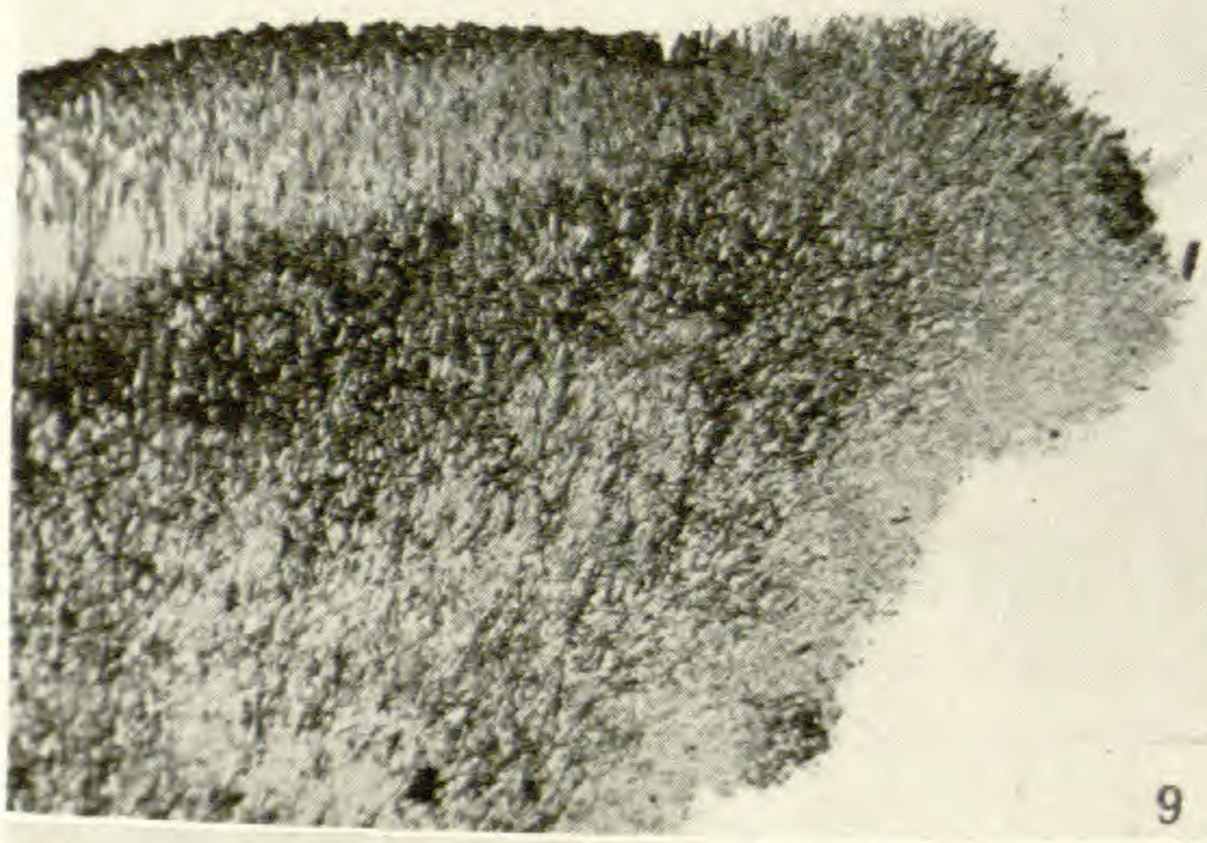
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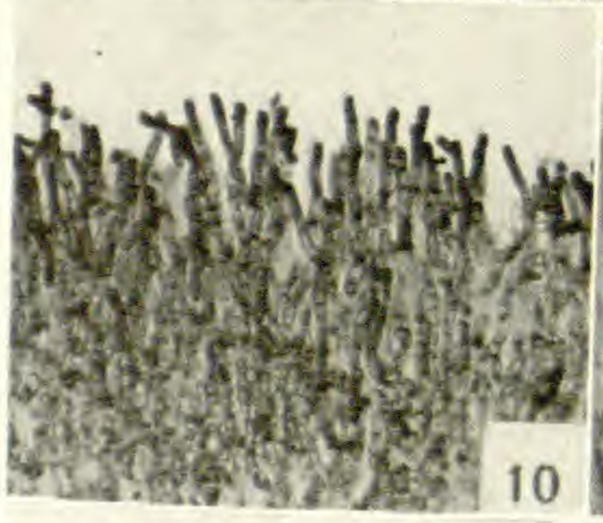
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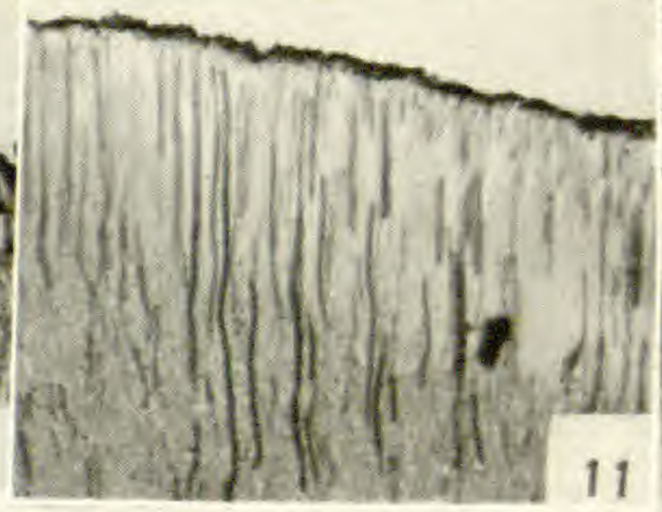
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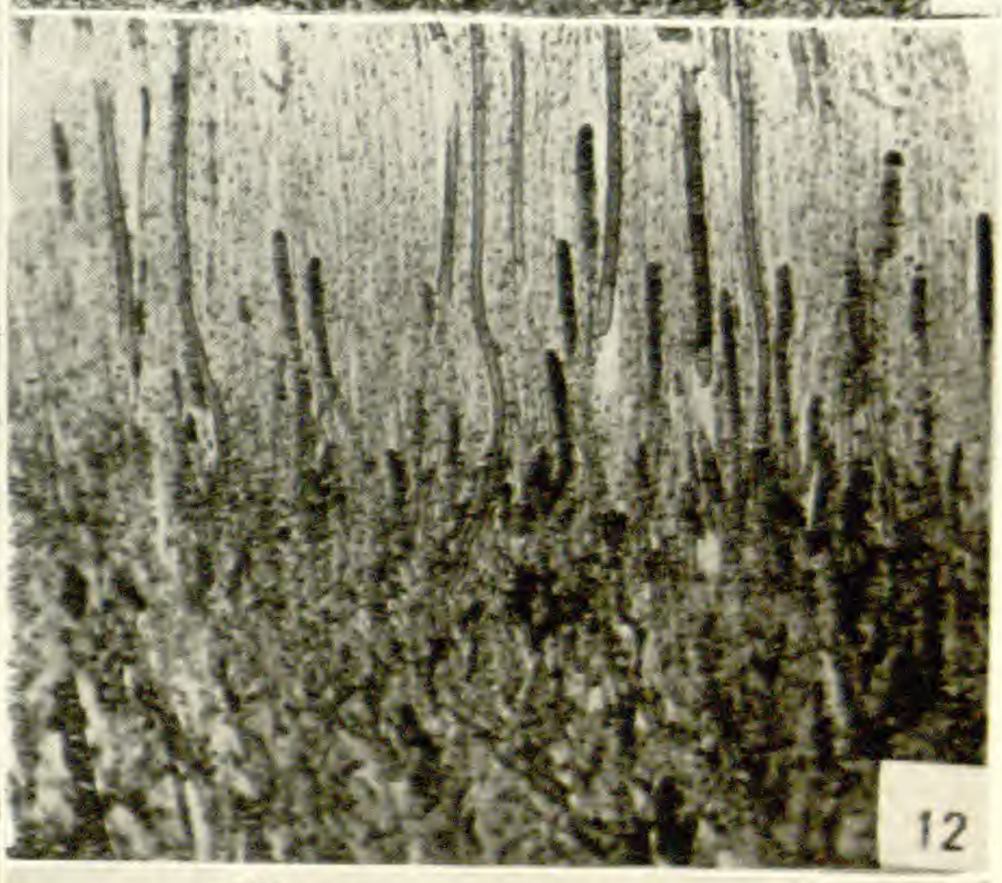
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FITZPATRICK on RHIZINA

PROBLEMS OF PLANT PATHOLOGY[1]

F. L. Stevens

Plant pathology is primarily and essentially an economic subject, and it is mainly from this viewpoint that it will be considered in this paper, attention being called also to the relation which the practice of pathology bears to science. The chief application of plant pathology is to agriculture, and as so applied the main, practical achievements may be summarized briefly as follows: (1) the control or partial control of various fungi, notably of orchard, vineyard, truck, and floral crops by sprays of copper compounds; (2) the substitution in many instances, notably on drupaceous hosts, of lime-sulphur compounds; (3) treatment by excision and the introduction of so-called tree-surgery; (4) the avoidance of susceptible varieties, for example, carnations, pears, strawberries, chrysanthemums, cowpeas, asparagus, and cantaloupes; (5) the development or utilization of disease-resistant or partially resistant strains, for example, asparagus, pears, watermelons, cowpeas, oats, wheat, flax, and cabbage; (6) the prevention of disease through knowledge of necessary alternate hosts, for example, pomaceous rusts; (7) the prevention of disease introduction by quarantine and inspection; (8) prevention through knowledge of mode or time of infection, or mode of transference, for example, certain cereal smuts, bean and cotton anthracnose, cabbage blackrot, and potato scab.

In this enumeration, the first 5 captions cover the major part of the early fruits of pathology, the easily gathered fruits, first ripe, and which could be harvested without deep, scientific knowledge. Such practices do not necessarily rest upon subtle principles, but are rather the outcome of cut-and-try methods of experience.

The accident which led to the experiments which in turn brought into prominence the use of copper sprays and thence led to much that has been done to perfect sprays and dust applications

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is well known; also the taking over of the lime-sulphur mixture from the entomologist. The susceptibility of certain varieties of crop plants has led the farmer in the south to reject special types; for example, the Bartlett and Seckel pears, and some of the strawberries. The florist, likewise, has been obliged to eliminate certain varieties of carnation. They would have done so had no science of pathology existed. Similarly, many of our so-called disease-resistant varieties were discovered by the farmer or are either not highly resistant or are of such poor quality that they are not used extensively.

Lest I may be regarded as pessimistic concerning the relation of science to plant pathology, I shall say at once that though these large advances were made in the main empirically, their progress was in reality hastened and made easier by the faith and confidence fostered by basic knowledge in mycology, bacteriology, and physiology; and definitely aided by advances in chemistry and special technique. With the remaining categories the dependence upon science is direct and evident. For example, knowledge of the alternate host relation in pomaceous rust and currant rust could not have been attained without the upbuilding of a broad knowledge of the rusts in general, indeed of the fungi as a whole; nor could the canker relation in apples (*Glomerella*, *Sphaeropsis*, *Bacillus*) have been found without knowledge of the nature and morphology of the causal fungi; and the same is true of the present troublesome citrus canker. Adequate quarantine inspection measures cannot be taken without definite knowledge of the identity, nature, and causes of disease. The quarantine question looms into particular prominence when we regard the subject of disease migration both interstate and international. The following are examples of diseases, the migration of which is more or less definitely known: pear blight, asparagus rust, grape anthracnose, cabbage club root, potato wart, potato blight, grape blackrot and downy mildew, chestnut bark disease.

Seed transference of disease is exemplified in the cabbage blackrot, celery leaf spot, and bean and cotton anthracnose. It is barely possible, but not at all probable, that this relation could have been discovered without intimate knowledge of the causal

agents, but it really was a rigid, scientific method which gave us our present knowledge of these diseases. The valuable results of the work on cereal smut infection furnish a fine example of achievement in disease-prevention that could not have been attained without both basic knowledge in mycology and a technique enabling trustworthy experimentation.

Upon entering a new biological territory, the first work is to collect and to classify, to know the material. So in the new field of plant pathology much of the early work was descriptive. The number of important plant diseases that are reasonably well described in two volumes of the Report of the United States Department of Agriculture for the years 1887 and 1888 is remarkable.

While the descriptive period in plant pathology is not entirely past, trivial diseases of cultivated plants, weeds, and wild plants still remaining undescribed, there have been very few really important diseases of general interest recently discovered in this country, few which compare in importance with the apple bitter rot, tomato leaf spot, onion smut, potato blight and scab, the cereal rusts or smuts. Many of the diseases recently described are of minor importance or are at present of very narrow geographic range; some have never been noted except by those who described them.

With the general principles of treatment established and the field for discovery of new diseases dwindling in importance, the time has now come when further progress, with rare exception, must be the outcome of fundamental, special knowledge and crucial experiments. It is evident that the easy crop from the virgin soil has been harvested, and that now we are entering upon the era of intensive cultivation.

The conquests of the future will be mainly the result of intensive study of the diseases and disease agents now known. Compare the degree of thoroughness of our knowledge of any one plant disease with any one disease in medicine. For example, compare from the research viewpoint our knowledge of *Pseudomonas campestris* with that of *Bacillus typhosis;* of the morbid histology of wheat rust with that of diphtheria; of the "epidemiology" of any plant disease with that of any human disease. Of course, the

parallel is not fair, since the values are not commensurate, but it serves to make the point that if such knowledge in medicine is probably contributory to prevention, probably it is also contributory in our science.

Thoroughness such as is attained in human pathology is in reality manifestly impossible for several reasons, one being the large number of plant diseases. Each plant species, to an extent, has its own fungous parasites; there are more than 40 listed for the apple alone. There are, perhaps, between 300 and 400 really significant, economic plant diseases, and to master knowledge of these is a great undertaking which is far from realization as yet.

Parasitic diseases present two chief elements, the host plant and the parasite. There is also, what is perhaps more important, the interrelation between these two, and what is also very important, the relation between these two and the factors of environment. It is with the study of these 5 elementary factors that pathology has to do. Large attention in the past has been given to the parasite, and in many cases it is the parasite alone which has been studied. Proportionately little study has been given to the relations existing between the host and the parasite, while the relations existing between environment and host, and environment and parasite, unquestionably of great significance, present a comparatively unworked field.

I wish to call attention briefly to the types of problems that exist under the above analysis. Perfecting and stabilizing of the taxonomy and nomenclature of the parasites are of course of fundamental value to pathology. The limitation of the families and orders of the fungi is, in the main, confessedly artificial; the boundaries of the genera are poor, and within the genera but little is really satisfying. To illustrate, the form genera *Penicillium* and *Coremium* are separated by ordinal rank, yet a single culture, dependent upon conditions, may give the characters of one or the other. Ordinal questions occur regarding *Meliola*, *Thielavia*, *Fusarium*, *Actinonema*, *Helminthosporium*, and many other genera.

Examples of problems in generic limitation are the *Phoma-Phyllosticta*, the *Septoria-Rhabdospora-Cylindrosporium*, the *Meliola-Capnodium-Apiosporium-Antennaria* questions. Within the

genus a good example is *Septoria* with 1200 species, or *Phyllosticta* with 1150 species. The former has nearly 700 species between 20 and 50 μ in spore length. The latter has 128 species with spores measuring 5–6 μ long. *Septoria* has 115 species on Compositae, and 77 species on the Gramineae (26 of these are within the limits of 20–40 μ in spore length).

Our present knowledge of such genera, as given by SACCARDO, is essentially that of a preliminary cataloguing of these forms by their hosts, the necessary first step. And we may add, as examples, the species of such genera as *Phoma*, *Rhabdospora*, *Cercospora*, *Nectria*, *Sclerotinia*, *Guignardia*, *Physalospora*, and *Phyllachora*. The bearing of this condition upon practice is evident, since numerous forms described as separate species upon the same economic host plant in reality may be identical or may be co-specific with forms described as distinct species or as belonging to other genera, families, or orders on the same or other hosts. The next step, well exemplified by such work as the monographs of THEISSEN, will consist in morphological comparison and readjustment of the species. This raises the question of life histories, of course, and shows the need of much such work as that of SHEAR and WOOD on *Glomerella*, HIGGINS on *Cylindrosporium* (*Coccomyces*), CLINTON on *Venturia*, WOLF on rose black spot (*Diplocarpon*), etc.

In connection with these problems arises the question of host relation and of biological specialization, as best exemplified, perhaps, in the rusts and the powdery mildews. What is the status of such specialization in the Fungi Imperfecti, in *Phyllosticta*, *Septoria*, *Cercospora*, etc., in the Ascomycetes, *Nectria*, *Sclerotinia*, *Phyllachora*, and many other genera? This forms a large and enticing field, in which much good work has been done, but a vast amount remains still to be done.

Coupled with these problems, come of necessity physiological, morphological, and cytological studies. The *Oospora-Actinomyces-Streptothrix* problem will require, apparently, all the possible sidelights before solution. This illustrates admirably the dependence of practice upon science, since fundamental questions of practice must rest their answer upon the degree of biological specialization

and variation of this organism, which causes potato scab, and concerning which we cannot decide as yet whether it belongs to the Eumycetes or to the bacteria.

Morbid histology of the various diseases presents a large field for activity. Concerning many diseases our knowledge in this regard is as yet really nil. It may be in many cases that such knowledge will not affect practice, but in many cases it surely has done so. Its utility appears clearly in relation to cereal smuts, tree surgery, etc.

The whole question of disease-resistance and susceptibility is fundamental and practical, as the age incidence of disease; the causes of resistance, whether mechanical, chemical, or physiological; the factors of air, soil, or heredity causing variation in resistance, and the possibility of artificially changing these factors. Breeding for disease-resistance is a special problem of extreme importance, involving knowledge of the factors of resistance and susceptibility, the needs of cultural conditions, and laws of breeding. Notable progress has been made with many crop plants, as oats, cabbage, asparagus, cantaloupes, carnations, flax, melons, and cowpeas.

Hibernation of the parasite has been the subject of much study. In some cases it offers the key to prophylaxis. It is, of course, inseparably linked in many cases with life history studies, and seems also sadly in need of study by those with sound ecological training. Indeed, an ecological study of certain plant parasites, with analysis of the environmental factors and with environments under experimental control, touching also upon seasonal relations, should be very productive. Problems abound on the border fields between mycology, physiology, ecology, and pathology relating to the age relation to disease, to mode of infection, to the climatic and seasonal relations of the parasite, to increase and decrease of susceptibility with changes of environment, to the results of varying the mass of the inoculum, and to change of the virulence of the pathogen with environment.

Epidemiology (to borrow the term from medical usage) is clearly linked with these topics. There is a vast amount of uncorrelated information in the literature concerning the relation between temperature, rainfall, etc., and various diseases, but there is ample

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room for a complete "epidemiological" study of any one of many really serious diseases.

A large field involving knowledge of extreme value and demanding ingenuity of experiment is that of pathogen transference. We know a little about transference by insects, but very little about wind and other agents.

Fungicides and their action are in need of more study. It is remarkable if accident has really given us the best fungicides in copper sulphate and lime sulphur. Our knowledge of their action and of their composition can be increased; so, too, the time to apply them and the strength to use. The exact time of application is undoubtedly of much importance; in some diseases, notably apple rust, the variable results are presumably linked with the time relation. Exact knowledge of such relation is needed in many cases. The subject of fungicide injury to fruits or foliage also arises here.

There are many diseases which have been described in a preliminary way, the causes of which are not yet known. Some of these are of great injury, notably the various so-called "mosaics," peach yellows and rosette. Their list is essentially included in the compilation of LANTZ (Ill. Agric. Exp. Sta., Circ. no. 183). It is not too much to hope that some of these will give up their secret under proper attack; some seem to have done so recently; for example, beet curly-top and the crown-gall. The status of others, such as Jonathan spot, tomato blossom-end-rot, tobacco mosaic, and numerous other mosaics, is not so clear. There is here opportunity for good descriptive work that we may know definitely with what conditions we have to deal. When the anatomical, histological relations are definitely recorded, we shall at least be able to classify these various types, and to know, for example, whether in reality the various things that we now call mosaic are of similar or different nature. Abnormal enzyme relations have been invoked to explain some disease conditions, but in general such explanations lack conclusiveness, and certainly lack practical application.

In these remarks I have not aimed at completeness. I have desired rather to indicate the need of intensive, thorough study of the problems before us, and to give a suggestive general view

of the field and of the diversity of research material. It is evident that no one person, either by temperament, inclination, or equipment, is fitted to investigate in all of these fields. The range is broad, and with a veritable wealth of research material, and a survey of the past shows that the well worked subject often is just as productive of results as an apparently much fresher subject. For years the powdery mildews have been introductory subjects in mycology. The group has been thoroughly monographed, collected, listed, years devoted to their biological specialization, treatments devised, etc. The field appeared too thoroughly worked to be promising of large results; yet recent studies have revealed the bud-scale hibernation habit of certain of these, and thus added fundamental knowledge useful in prophylaxis.

Finally, the diseases themselves, not the fungi, need classification. Various classifications have been used, as to cause, as to host, etc., but these do not serve to emphasize relationships of conditions which it is of service to know.

Aside from the non-parasitic diseases, those caused by improper environment of soil, air, light, by abnormal hereditary tendencies, by unknown factors and by predatory animals (including insects), and considering only those known to be caused by parasitic fungi, there are certain groups of conditions which stand out strongly marked as being similar. It is of distinct advantage in studying, in teaching, and in devising and promulgating remedies, to recognize and define these categories. By their very similarity, certain diseases have gravitated together; for example, the vascular diseases, fungous or bacterial diseases, with plugging of the bundles, popularly and very properly call the "wilts."

It is interesting to note that one of the most significant contributions along this line appeared in one of our elementary texts; significant, too, that this contribution should come from one not primarily interested in pathology. COULTER, in his *Elementary studies in botany*, gives us the conception of three general categories of plant diseases: (1) those in which the parasites kill the living cells; as pear blight, spot diseases, downy mildew, potato blight; (2) those in which the parasite does not kill the living cells, but lives in association with them, feeding upon their products; as rust, crown-gall, curl, black knot; (3) those in which the parasites

invade the vessels and live in the sap; as cabbage, cucurbit, and mushroom wilts and wood rot.

I have given some thought to this subject, and, as the only part of this paper which may lay any claim to originality, would present the following suggestions as a step toward a classification of plant diseases caused by fungi, separating them into the following categories:

1. Wilt diseases due to mechanical stoppage of the vascular bundles by parasites. These may be called cases of *embolism;* for example, vascular diseases due to *Ps. campestris*, *B. solanacearum*, and *Acrostilagmus*.

2. Disintegration of the xylem structures; for example, the various wood rots due to *Thelephora*, *Hydnum*, *Poria*, *Polyporus*, *Fomes*, *Trametes*, *Dedalea*, *Schizophyllum*, etc.

3. Diseases due to parasites wholly contained within the living protoplasm of the host cell. This is the strictest type of parasitism, and may appropriately be known as *endocellular parasitism;* for example, diseases due to *Synchytrium*.

4. Diseases due to parasites which draw their nutriment from living cells by haustoria, which may be called *endocellular haustorial parasitism;* for example, diseases due to *Phyllactinia*, *Peronospora*, *Albugo*, and *Plasmopara*. In this group the conspicuous feature is the relatively large development of the haustorial surface as compared with the remainder of the internal mycelium.

5. Diseases in which the live epidermal cells only are directly parasitized. These may be called cases of *epidermitis*, for example, diseases due to the Erysiphales (exclusive of *Phyllactinia*), *Meliola*.

6. Diseases in which the parasite grows between the living host cells. Haustoria may be present, but if so they are not prominent, and the apparently dominant part of the absorptive system is the intercellular mycelium. This may be called *intercellular mycosis;* for example, rusts, Exoascales, Exobasidiales, and *Cephaleurus*.

7. Diseases in which the host tissue is displaced or replaced by fungous masses. This may be called *mycosclerosis;* for example, diseases caused by *Claviceps*, *Phyllachora*, *Rhytisma*, and the smuts.

8. Diseases of the type produced by *Pseudomonas tumefaciens*, which may be called *tumor*.

9. Diseases in which the dominant feature is death of the host cells before they are actually invaded by the parasite. To these may be applied the term *necrosis*. Subdivision may be made on the basis of the part involved, as:

9*a*. *Cortical necrosis*, in which the cortex chiefly is involved; for example, cankers caused by *Sphaeropsis*, *B. amylovorus*, and *Endothia*.

9*b*. *Parenchymal necrosis*, in which chiefly the parenchyma is affected, including the greater number of the soft rots; for example, soft rots caused by *B. carotovorus*, *Rhizopus*, *Penicillium*, *Phythiacysitis*, *Rhizoctonia*, *Pythium*, *Phytophthora*, *Sclerotinia*, *Botrytis*, *Colletotrichum*, and *Gleosporium*.

9*c*. *Macular necrosis*, in which necrosis is limited to spots, chiefly occurring on leaves. This is divided into (1) macular necrosis with abscission (the "shothole" diseases caused, for example, by *Cylindrosporium* and *Marssonia*); (2) macular necrosis without abscission (chiefly the leaf spots, caused, for example, by *Pseudopeziza*, *Entomosporium*, *Macrosporium*, *Lophioderma*, *Guignardia* [*Phyllosticta*], *Ascochyta*, *Ramularia*, *Septoria*, *Diplodia*, *Cercospora*, *Colletotrichum*, *Gleosporium*, *Fusicladium*, *Cladosporium*, and *Alternaria*.

The following synopsis may make these categories and their interrelations clear.

I. The parasite living in the sap or in cavities or parts devoid of living protoplasm: (1) embolism; (2) wood rots.

II. The parasite for the major part of its life drawing its nutriment from host cells that are still living: (3) endocellular parasitism; (4) endocellular haustorial parasitism; (5) epidermitis; (6) intercellular mycosis; (7) mycosclerosis; (8) tumor.

III. The parasite living within host cells or tissues which have recently been killed or partially disorganized by it: (9) necrosis; (9*a*) cortical necrosis; (9*b*) parenchymal necrosis; (9*c*) macular necrosis; (9*c*′) macular necrosis with abscission; (9*c*″) macular necrosis without abscission.

There is an apparent omission of hypertrophy and hyperplasia, but I regard these two manifestations as symptoms rather than as definite diseases.

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FLOWERS AND INSECTS. XX

EVOLUTION OF ENTOMOPHILOUS FLOWERS

CHARLES ROBERTSON

In his *Fertilisation of flowers* (pp. 594, 595) MÜLLER arrives at the following conclusions with regard to the development of flowers:

The transition from wind fertilization to insect fertilization, and the first traces of adaptation to insects, could only be due to the influence of quite short-lipped insects with feebly developed color-sense. The most primitive flowers are therefore for the most part (except, for instance, *Salix*) simple, widely open, regular, devoid of honey or with their honey unconcealed and easily accessible, and white or yellow in color (for example, most Umbelliferae and Alsineae, many Ranunculaceae and Rosaceae).

Gradually, from the miscellaneous lot of flower-visiting insects, all much alike in their tastes, there arose others more skilful and intelligent, with longer tongues and acuter color-sense; and they gradually caused the production of flowers with more varied colors, honey invisible to or beyond the reach of the less intelligent short-tongued guests, and various contrivances for lodging, protecting, and pointing out the honey.

The Ichneumonidae at first surpassed all other visitors in observation and discernment, and they were thus able to produce inconspicuous flowers which escaped the notice of other visitors. On the appearance of sand wasps and bees these inconspicuous flowers were banished by competition to the less frequented localities (for example, *Listera* to shady woods).

The sand wasps (Sphegidae) apparently took the place to a great extent of the ichneumons, and produced flowers where organs had to be thrust apart (Papilionaceae), or where a narrow cavity had to be entered (Labiatae), or where some other action similar to the act of digging had to be performed. Subsequently bees seem to have entered on joint possession of most of these flowers, and to have added special adaptations of their own.

The true wasps (Vespidae) could establish themselves by the fear of their sting (and of their jaws) in sole possession of certain flowers with wide open mouths and abundant honey. These they developed further in relation to their wants (*Scrophularia*, *Symphoricarpos*, *Epipactis latifolia*, *Lonicera alpigena*); but where wasps are scarce the flowers are utilized by other insects.

Bees (Apidae), as the most skilful and diligent visitors, have played the chief part in the evolution of flowers; we owe to them the most numerous, most varied, and most specialized forms.

Whether the primitive flowers were pollinated by wind or by insects is uncertain. The forms of flowers which preceded the angiosperms were probably entomophilous. The carpels closed over the ovules to form an ovary and the stigma was developed to receive the pollen. The stigma and closed ovary are regarded as entomophilous characters and as having been developed after the visits of insects were established. The origin and development of entomophilous flowers, no doubt, were connected with the origin and specialization of the bees, Hymenoptera which adopted the habit of provisioning their nests with nectar and pollen. Along with the acquisition of this habit, the bees developed a coat of feathery hairs to which the pollen might cling, these hairs on certain parts of their bodies, as the hind legs and the ventral surface of the abdomen, being greatly modified to form a special pollen-carrying apparatus. Thus the pollen became absolutely essential in the economy of the bees. To the flowers, on the other hand, the bees became important visitors, because they had to resort to flowers frequently and because they were provided with a coat specially fitted to retain the pollen, and at the same time exerted themselves to get the coat as full of pollen as possible.

Bees, as we know them, visit flowers both for nectar and for pollen, but it is possible that the primitive bees visited flowers only for pollen and that the secretion of nectar came after.

The view has been expressed[1] that the ordinary short-tongued bees can collect only viscid pollen, and that therefore they could have begun to use pollen to provision their nests only after pollen had become sticky in adaptation to insect pollination. Species of *Chloralictus* collect the dry pollen of grasses and of *Plantago*, however, and ordinary bees collect from a considerable number of flowers pollen which is so dry that it pours out as soon as it is released from the anthers. So bees may have commenced to collect pollen when only dry pollen existed. The fact that bees are the most highly specialized of Hymenoptera, and the latest developed, does not prove, and does not seem to establish a reasonable presumption, that any considerable evolution of entomophilous flowers preceded their advent.

[1] ROBERTSON, CHARLES, Flowers and insects. XIX. BOT. GAZ. **28**:39. 1899.

Putting speculation aside, the further consideration of this subject will be limited to the structure and affinities of the flowers, and the behavior of the insects which we know. Social flowers are those which are so closely approximated that the visitors may readily pass from one to another without taking wing or climbing. They are usually found in heads, spikes, or close umbels. The simplest flowers which we know are non-social flowers of class *AB*, flowers with partly concealed nectar. Insect visits to them show:

	Species	Bees	Diptera	Other Hymenoptera	Lepidoptera	Coleoptera Hemiptera	Total
Class *AB*.....	41	56.8	31.2	4.7	4.8	2.3	866
Visits.....	14	43.7	32.8	19.5	2.7	1.2	405
Individuals.....	0	70.2	20.4	8.0	0.7	0.5	2438

These are evidently bee flowers, although they are not exclusively visited by bees. No insects except bees prefer flowers of this kind. There are no non-social flowers of class *AB* which are adapted to miscellaneous insects or to particular kinds of visitors except bees. On 14 species of class *AB* bees showed 43.7 per cent of the visits and 70.2 per cent of the individuals. Of course it is possible that the primitive non-social flowers of class *AB* were visited by a miscellaneous set of the least specialized anthophilous insects. If so, the short-tongued bees must have tended early to monopolize them, while the other insects paid more attention to the forms which became social.

Observations of 221 visits to 17 non-social flowers of class *A*, flowers with exposed nectar, show: bees 33.4, Diptera 45.7, other Hymenoptera 14.4, Coleoptera and Hemiptera 6.3. Here the Diptera predominate, and the group is rather miscellaneous. Some of the group are distinct fly-flowers (*Asimina triloba*); some are quite simple (*Asimina*, *Myosurus*, and *Caulophyllum*). The dark color and pendulous position of *Asimina* are hardly typical, and *Myosurus* and *Caulophyllum* have peculiar petals. None of these are simple like ordinary non-social flowers of class *AB*. Most non-social *A* have epigynous nectaries (*Hypoxis*, *Circaea*,

Galium). A characteristic flower is *Circaea lutetiana*. Its visitors are:

	Bees	Diptera	Total
Species	81.8	18.1	11
Individuals	94.0	5.9	84

Class *A* is a poor place to look for simple flowers. The majority are social and have epigynous nectaries, both forms of specialization. Except class *B*,[1] this class is the only one in which the majority of the species are social. The visits to 23 social *A* are as follows: bees 21.9, Diptera 38.3, other Hymenoptera 27.3, Lepidoptera 2.6, Coleoptera and Hemiptera 9.6, making a total of 2335.

Table I, based on 10,041 visits, shows the percentages of visits of all classes to flowers adapted to short-tongued insects, usually small flowers with nectar exposed, partly or wholly concealed, but never deep seated.

TABLE I

	Bees	Diptera	Lepidoptera	Coleoptera	Hemiptera	Lower Hymenoptera
PERCENTAGE OF VISITS						
To non-social flowers	18.1	12.4	11.2	8.6	6.1	4.1
To social flowers	41.8	73.5	34.8	83.3	78.7	84.9
PERCENTAGE OF TOTAL VISITS						
To non-social flowers	59.5	25.3	7.2	2.0	0.3	5.3
To social flowers	31.0	33.8	5.0	4.4	1.0	24.4

Of the visits of bees, 18.1 per cent are to non-social small flowers, and these form 59.5 per cent of the total insect visits to such flowers. Sixteen non-social small flowers, on which the individual insects were taken as they came and counted, showed 335 visits and 1520 individuals. The percentage of bee visits was 59.1, but of bee individuals 74.2, showing that bees are more important than the percentage of visits indicates.

The relations of bees and other insects to non-social and social flowers in general (based upon 13,942 visits of 1287 insects to 437 flowers) are shown in table II.

TABLE II

	Lower Hymenoptera	Hemiptera	Coleoptera	Diptera	Lepidoptera	Bees	All Except Bees	Total
PERCENTAGE OF VISITS								
To non-social flowers.....	4.4	6.1	9.3	13.6	22.6	32.1	11.9	20.7
To social flowers.....	95.5	93.8	90.6	86.3	77.3	67.8	88.0	79.2
PERCENTAGE OF TOTAL VISITS								
To non-social flowers.....	3.6	0.2	1.4	17.8	9.3	67.4	32.5	99.9
To social flowers.....	20.4	0.9	3.5	29.4	8.3	37.2	62.7	99.9

Of the total visits of bees, 32.1 per cent are to non-social flowers, and these form 67.4 per cent of the total insect visits to such flowers. Of the total visits of other insects to non-social flowers, the percentage is 4.4 for lower Hymenoptera, 6.1 for Hemiptera, 9.3 for Coleoptera, 13.6 for Diptera, and 22.6 for Lepidoptera; or a general percentage of 11.9. Since bees make over two-thirds of the insect visits to non-social flowers, it is evident that they have been chiefly instrumental in the origination of such flowers.

Of the total visits of bees, 67.8 per cent are to social flowers, so that bees show a strong preference for these flowers also, although not as strong a preference as the Lepidoptera with 77.3, the Diptera with 86.3, the Coleoptera with 90.6, the Hemiptera with 93.8, and the lower Hymenoptera with 95.5 per cent.

One might suppose, with MÜLLER, that the non-aculeate Hymenoptera have had an influence in the development of some primitive flowers, and that these flowers were further modified by the aculeate Hymenoptera, and finally became highly specialized in connection with the development and specialization of the bees. When, however, we look for such flowers, we find only the so-called ichneumon flowers, *Listera ovata* and *Chamaerorchis alpina*, belonging to the most highly specialized of monocotyledons. In the

case of the Ichneumonidae only 2.5 per cent of the visits are to non-social flowers.

The only flowers supposed to have been modified by the Vespidae are the so-called wasp flowers, *Epipactis latifolia* (Orchidaceae) belonging to the most highly specialized group of non-social monocotyledons, *Scrophularia nodosa* (Scrophulariaceae) belonging to a distinctly melittophilous family, *Lonicera alpigena* belonging to a melittophilous genus, and *Symphoricarpos racemosus* belonging to the epigynous Caprifoliaceae. None of these belong to primitive forms of flowers which might have preceded the advent of the bees. Only 8.7 per cent of the visits of Vespidae are to non-social flowers.

With the exception of *Symphoricarpos*, all of the flowers mentioned by MÜLLER as having been modified in adaptation to the lower Hymenoptera are zygomorphous: Orchidaceae, Papilionaceae, Labiatae, *Scrophularia*, and *Lonicera alpigena*. Zygomorphous flowers, except such forms as *Aristolochia*, with *siphonate* zygomorphy, and the outer flowers of the umbels of *Heracleum*, with *radiate* zygomorphy, are typically non-social and adapted to bees which visit each flower separately. They have a landing either above or below the stamens and pistils and usually dust the visitor on the lower or upper side. It is fairly inconceivable that zygomorphy should have originated in crowded inflorescences where the flowers might be approached from any side. Excluding such flowers as *Heracleum*, *Aristolochia; Amorpha*, *Petalostemon*, and *Melilotus* in Papilionaceae; and *Pycnanthemum*, *Lycopus*, and *Mentha* in Labiatae, 100 zygomorphous flowers show: bees 74.3, Diptera 8.5, other Hymenoptera 9.1, Lepidoptera 7.1, Coleoptera and Hemiptera 0.7, making a total of 1117 visits.

Visits to the Papilionaceae show:

	Bees	Diptera	Other Hymenoptera	Lepidoptera	Coleoptera, Hemiptera	Total
Non-social (24)	97.5	1.6	0.8	0.0	0.0	123
Social (9)	56.1	16.3	23.0	1.5	2.9	447
Total (33)	65.0	13.1	18.2	1.2	2.2	570
Amorpha, etc. (4)	45.3	18.5	29.4	2.3	4.3	302

When the lower Hymenoptera together make only 0.8 per cent of the visits to the non-social Papilionaceae, it is evident that they have had little to do with the evolution of the Papilionaceae, even if they were instrumental in their origin. To support the latter condition it would be necessary to show that the non-social forms were developed from the social forms.

Visits to Labiatae show:

	Bees	Diptera	Other Hymenoptera	Lepidoptera	Coleoptera, Hemiptera	Total
Non-social (13)	83.1	5.8	0.8	10.0	0.0	119
Social (12)	39.5	20.8	24.6	12.7	2.2	897
Total (25)	44.6	19.0	21.8	12.4	1.9	1016
Lycopus, etc. (5)	29.6	24.6	34.5	7.8	3.2	576

When the lower Hymenoptera show 24.6 per cent of the visits to social Labiatae and only 0.8 per cent to non-social Labiatae, it is hard to connect them with the origin of the Labiatae unless we suppose that the non-social developed from the social. MÜLLER (*Fertilisation of flowers*, p. 471) says: "DELPINO considers *Mentha* and *Coleus* degraded forms of the labiate type; he, however, gives no reason for thinking them to be such, and not rather less specialized forms, differing less from the common ancestors of the Labiatae." If there are non-social zygomorphous wasp flowers or ichneumon flowers, no doubt they should be regarded as modified from bee flowers.

The view held here, that the early flowers were non-social and were modified in connection with the visits of bees, and that the flowers mainly visited by other insects are later, is supported by what is known of the behavior of insects and by inferences from the affinities of the flowers. Of course, if it can be shown that the primitive flowers were social and that the non-social flowers were developed from them, this view will have to be abandoned for that of MÜLLER.

Of the total visits of the lower insects, 88.0 per cent are to social flowers, and of the total insect visits to social flowers the lower insects make 62.7 per cent. Now the flowers which these insects

prefer are not the simple ones, but the majority are social and have epigynous nectaries.

The original or normal bees are polylectic. They have a general relation to the flora and more special relations to certain flower classes. From these have originated the oligolectic bees and inquilines. The oligoleges collect pollen exclusively from flowers belonging to particular natural groups. They do not prefer flower classes except in so far as their particular flowers happen to belong to those classes. The inquiline bees live in the nests and at the expense of the other bees. They get only nectar from the flowers which happen to be the most convenient and easiest for them to visit. The importance to the flora of these 3 sets of bees is partly indicated in table III.

TABLE III

	Species	Visits	Average
Normal, polyleges....	132	4448	33.6
Inquilines.....	72	781	10.8
Oligoleges.....	83	668	8.0
Prosopis.....	9	166	18.4
Total.....	296	6063	20.4

In a considerable number of polyleges the flight of the males is quite different from that of the females. The males do not make half as many visits as the females, and the flowers which they visit are so different that their visits to flowers should be considered separately. Table IV shows the differences.

TABLE IV

	Females (♀ excluded)			Males		
	Number	Visits	Average	Number	Visits	Average
Large polyleges.....	50	1239	24.6	50	806	16.1
Small polyleges.....	72	2098	29.1	64	722	11.2
Total.....	122	3337	27.3	114	1528	13.3

The groups of visitors preferring certain classes of non-social flowers are separated as shown in table V. Usually large flowers

with deep seated nectar are referred to as *Ma*, usually small flowers with nectar not deep seated are referred to as *Mi*, while *Pol* indicates the extreme social forms.

TABLE V

	Non-social			Social				Total
	Ma	Mi	Total	Ma	Mi	Pol	Total	
Flora	30.2	24.2	54.4	18.7	21.5	5.2	45.5	437
Small bees, polyleges ♀	5.0	30.4	35.5	11.7	43.6	9.0	64.4	2098
Large bees, polyleges ♀	35.9	13.4	49.4	30.9	15.8	3.7	50.5	1239
Sphingidae	54.5	0.0	54.5	40.9	4.5	0.0	45.4	22
Humming-bird (*Trochilus*)	82.7	3.4	62.2	13.7	0.0	0.0	13.7	29

The females of the short-tongued polylectic bees form the only group of insects preferring non-social *Mi*. They are credited with the origin of such flowers. The females of the long-tongued polylectic bees, the Sphingidae, and *Trochilus* are the only groups preferring non-social *Ma*. This is the largest flower class, originally modified by long-tongued bees. The Sphingidae and *Trochilus* prefer such flowers and in some cases have entirely appropriated them.

As flowers have become social they have been preferred in the order shown in table VI.

TABLE VI

Groups of insects	Non-social	Social
Large bees, polyleges ♂	37.8	62.1
Lepidoptera (ex. Sphingidae)	22.0	77.9
Large bees, inquilines	20.6	79.3
Small bees, polyleges ♂	19.5	80.4
Prosopis	13.8	86.1
Diptera	13.6	86.3
Large bees, oligoleges	13.4	86.5
Small bees, inquilines ♀	12.7	87.2
Small bees, oligoleges	11.3	88.6
Coleoptera	9.3	90.6
Hemiptera	6.1	93.8
Lower Hymenoptera	4.4	95.5
Small bees, inquilines ♂	4.0	96.0

Some female bees on their pollen visits show a preference for social flowers. Eighty-five species of long-tongued bees, with

806 pollen visits, and 1155 nectar visits of females and workers, show the following percentages of visits to social flowers with exposed pollen: for nectar 41.1; for pollen 49.2. Compared with the visits of the females for nectar, the females when collecting pollen make 8.1 per cent more visits to social flowers.

There are some large social inflorescences composed of flowers with exposed or only slightly concealed nectar. Long-tongued bees practically avoid them on their nectar visits, but often visit them for pollen. Such are *Cornus*, *Hydrangea*, and *Viburnum*. *Vitis*, with exposed nectar, seems to be an important source of pollen for female bumblebees. The aggregation of flowers in social clusters has been interpreted as an adaptation for gitonogamy, but it occurs about as often in cases where gitonogamy is impossible.

Finally, the evolution of entomophilous flowers is held to have proceeded in the following manner. The primitive flowers were non-social flowers of class *AB*, with partly concealed nectar, adapted to short-tongued bees. These have produced flowers with exposed nectar more favorable to flies, and flowers with more concealed nectar still more favorable to bees. A few have become adapted to flesh flies (*Asimina*), and others to minute flies (*Aristolochia*).

The non-social small bee flowers have produced social forms still favoring small bees, but admitting other short-tongued insects. These finally pass into the extreme social forms which have become modified to suit miscellaneous short-tongued insects.

The non-social small bee flowers have been modified further and developed into non-social long-tongued bee flowers. Some of these have been appropriated by birds and others by Sphingidae, and perhaps still others by butterflies.

The non-social long-tongued bee flowers have also been modified into social forms attracting Lepidoptera and long-tongued Diptera. These are still considered as bee flowers, but some of them may more properly be regarded as adapted to miscellaneous long-tongued insects. The social long-tongued bee flowers also pass into social short-tongued bee flowers, and finally into social flowers adapted to miscellaneous short-tongued insects.

CARLINVILLE, ILL.

DOES THE TEMPERATURE COEFFICIENT OF PERMEABILITY INDICATE THAT IT IS CHEMICAL IN NATURE?

W. J. V. OSTERHOUT

In a recent paper STILES and JORGENSEN[1] state that the absorption of hydrogen ions by tissues of the potato has the temperature coefficient of a chemical reaction (2.18–2.22). They apparently reach the conclusion that "the substance with which the acid reacts" is "presumably the plasma membrane or some part of it," and that the facts suggest the view "held by PAULI and SZÜCS, who regard the entrance of ions into the cell as due to the reversibility of such a reaction between ions and the plasma membrane." These statements, together with the title of their paper, "The effect of temperature on the permeability of plant cells to the hydrogen ion," indicate that they regard the temperature coefficient found by them as the temperature coefficient of permeability to hydrogen ions.

This view, if well founded, is of considerable interest, as it indicates that permeability is chemical[2] rather than physical in nature, since (unless vapor tension is a determining factor) no physical processes are apt to be involved in this case which have a temperature coefficient as high as 2.[3] In view of this the statements of STILES and JORGENSEN deserve careful examination.

It should be noted that the only criterion of permeability employed by them was absorption from a solution. Their method consisted in placing slices of potato in a solution of HCl and

[1] Ann. Botany **29**:611. 1915.

[2] BROWN and WORLEY (Proc. Roy. Soc. London B. **85**:546. 1912) have shown that the temperature coefficient of absorption of water by seeds is 2, but it is not clear whether this applies to imbibition (or other processes) taking place inside the cells, or to the permeability of the protoplasm. If it is really the coefficient of permeability to water, it is by no means necessary to extend this conception to permeability to substances other than water.

[3] Cf. KANITZ, A., Temperatur und Lebensvorgänge. Berlin. 1915 (p. 165).

determining the loss of hydrogen ions from the solution by means of a hydrogen electrode.

It may be observed in this connection that the absorption of dissolved substances by living cells has been employed extensively as a criterion of permeability. The amount of absorption is usually determined by analysis (of the solution or of the tissue) before and after the organism is placed in the solution. A more convenient method, which suffices in some cases, is to determine the conductivity of the solution. Nephelometry is also useful. Such methods may also be used to determine the excretion of substances by the organism.

The results obtained by these methods have been so largely misinterpreted that there is widespread confusion in regard to their significance. This confusion is due in part to uncritical technique and in part to overlooking some of the many variables involved in such experiments; but the principal difficulty lies in confusing permeability with absorption.

The nature of this difficulty is evident from the following illustration. Suppose a glass tube closed at one end by a membrane in contact with a solution to which it is freely permeable. The solution will pass through the membrane into the tube until equilibrium is established. If, however, we place in the tube something which precipitates the dissolved substance, more of the latter will diffuse in, and this will go on as long as the precipitation continues. It is not even necessary that the precipitation should occur, since the result can be obtained by causing the dissolved substance to unite with something within the tube so as to form a compound which cannot pass out through the membrane.[4] The dissolved substance will then continue to pass into the tube.

It is evident that the permeability of the membrane remains the same whether precipitation or other chemical action occurs or not. But while the permeability remains the same, the amount of adsorption will vary enormously.

This may be observed with the living cell. When a cell is placed in a dye which is precipitated within the cell (giving a visible precipitate), the absorption of the dye goes on as long as

[4] Cf. LOEB, J., Dynamics of living matter. 1906 (p. 72).

the precipitate continues to form, while in the case of a dye which is not precipitated (and which does not form a compound incapable of passing out), the absorption ceases as soon as the concentration within the cell equals that of the solution.

It is evident, therefore, that the temperature coefficient observed by STILES and JORGENSEN may be that of a chemical process[5] involving the union of hydrogen ions with some constituent of the cell other than the plasma membrane (or other surface), in which case it would have no bearing upon the problem of the nature of permeability.[6]

Some time ago the writer sought to throw some light on this problem in ascertaining the temperature coefficient of permeability by a method which is free from the objections just discussed. By this method[7] the electrical conductivity of living tissue was determined in such a way that it may be regarded as a measure of the permeability of the protoplasm.

In these experiments a series of disks of *Laminaria* were packed together (like a roll of coins) so as to form a solid cylinder about 2 inches in length. The electrical conductivity was then measured at various temperatures. The temperature coefficient obtained in in this way was 1.33. The tissue was subsequently killed, whereby the conductivity was increased to practically that of sea water. The temperature coefficient of the dead tissue proved to be 1.26, which is practically the same as that of sea water.

If most of the resistance were due to apparatus, cell walls (intercellular substance), and sea water, and these had low temperature coefficients (for example, 1.26), that part of the resistance which is due to living protoplasm might have a high temperature coefficient (for example, 2) without much raising the temperature coefficient of the total resistance. The resistance of the apparatus (and the sea water contained in it) was determined at each temperature and subtracted from the total (giving what is called the

[5] Absorption may also play a part in this connection.

[6] Cf. Biochem. Zeitschr. **67**:272. 1914.

[7] Even if the hydrogen ion unites "with the plasma membrane or some part of it," the temperature coefficient of this process would not necessarily be the temperature coefficient of permeability.

net resistance), so that we need consider only the resistance of the protoplasm, of the cell walls imbibed with sea water, and of the capillary films of sea water between the disks.

When the net resistance is 1200 ohms, we find that on killing the tissue the resistance drops to about 100 ohms. Since this represents the resistance of the cell walls and of the sea water plus the resistance of the dead protoplasm, it is evident that the resistance of the cell walls and of the sea water together must be less than 100 ohms. So far as can be judged from microscopic measurements, the fraction of the cross-section of the conducting column occupied by the cell walls is less than half, and it is probable that when the net resistance is 1000 ohms, not more than 50 ohms are due to cell walls and to the sea water adhering to the tissues.[8]

It is evident, therefore, that if the resistance of the living protoplasm had a temperature coefficient of 2, the temperature coefficient of the total resistance would be only a little less than 2.

We may conclude, therefore, that the temperature coefficient of permeability is not far above 1.33. This indicates that permeability is not chemical in nature, although it is not absolute proof, as some chemical reactions have low temperature coefficients.

It would seem, therefore, that we cannot accept the idea that permeability is chemical in nature without much more conclusive evidence than we possess at present.

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[8] The problem is complicated by the arrangement of the protoplasmic masses.

BRIEFER ARTICLES

A METHOD FOR PRODUCING CONDUCTIVITY WATER SUITABLE FOR WATER CULTURE EXPERIMENTS[1]

(WITH ONE FIGURE)

An examination of one of the most recent types of water stills has convinced the author that there is abundant opportunity for the presence of copper and other metals in the distillate. The boiler is made of copper, not completely covered by tin; consequently, a coating of copper carbonate forms which may be conducted over in the spray during rapid distillation. Although the condenser itself may be of block tin, there is ample opportunity for contact of the distillate with brass connections.

On account of the reputed great physiological activity of copper, it was deemed advisable to use water free from the suspicion of contamination. This led the author to devise a method for producing water of high resistance in sufficiently large quantity for water culture experiments. The apparatus here described has been used for some time and has been found entirely satisfactory.

The level of water in a tubulatured retort is regulated automatically by a siphon (1) which discharges to a constant level in receiver (3). The end of the water tube (2) of the regulator is so adjusted that air can rise into the reservoir above only when the level in the retort is lowered slightly by distillation. Then the vacuum pressure is relieved slightly so as to allow water to flow into the siphon.

It was found necessary to provide the water seal siphon with an upright tube to prevent the stoppage of the siphon by gases expelled from the water when heated.

The constant level of the water in the retort offers the advantage of greater space above the boiling liquid to minimize the danger of spray being carried over. A pledget of glass wool is placed in the bent neck of the retort to remove spray and return it to the retort. Condensation of the distillate is affected by a glass water jacket fitted to the neck of the retort by rubber stoppers.

[1] Published by permission of the Secretary of Agriculture.

The system can be isolated almost entirely from the atmosphere by the protecting tubes and the water seals as shown in the diagram. The principal advantages of the apparatus are that it requires little attention

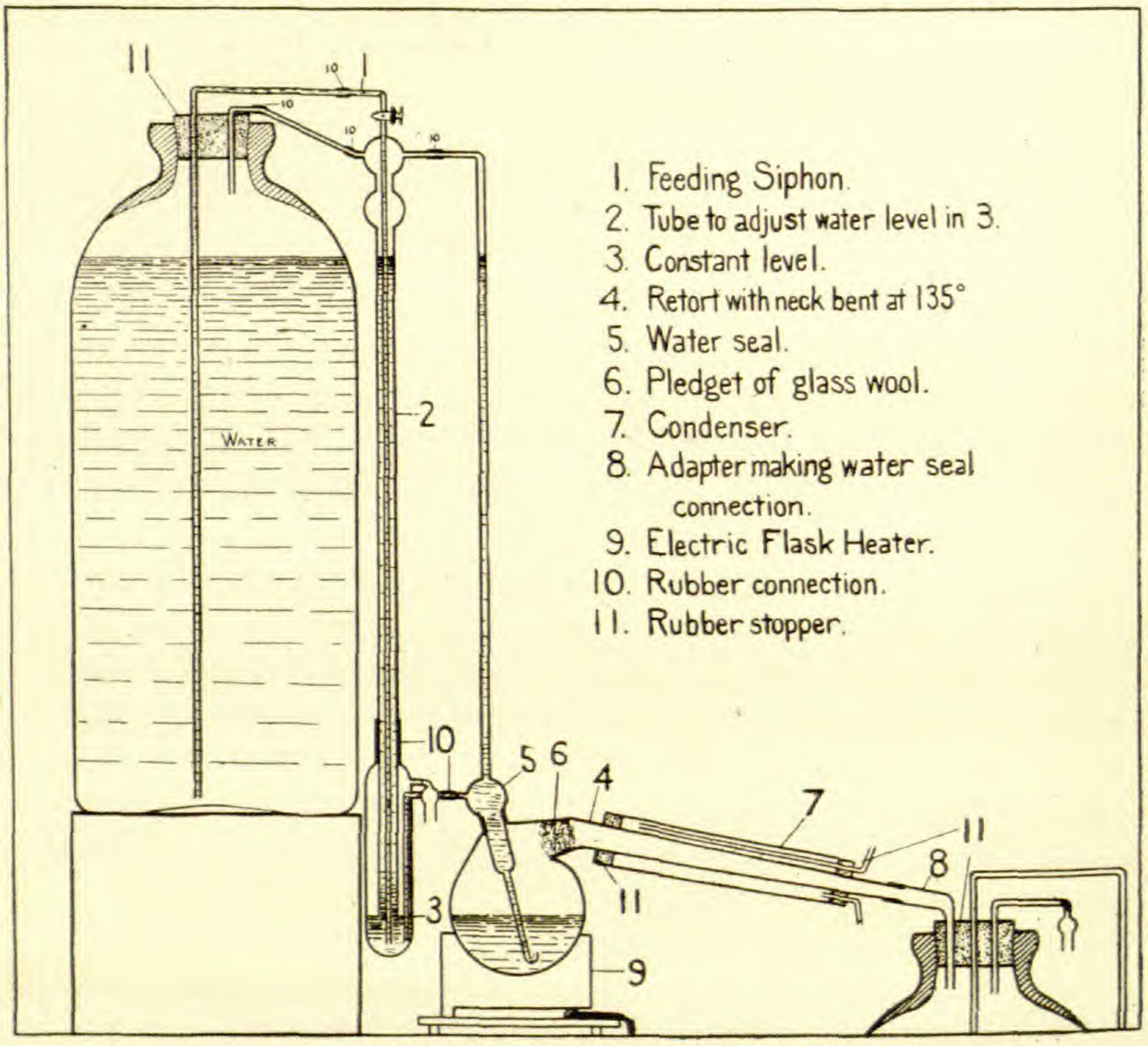

FIG. 1

beyond occasional cleaning, and can be relied upon to produce a constant supply of high resistance water containing only materials dissolved from slightly soluble glass.—RODNEY B. HARVEY, *U.S. Department of Agriculture, Washington, D.C.*

CURRENT LITERATURE

BOOK REVIEWS

Two new college texts

The two recent texts by GANONG and GAGER agree in the modern spirit evident in each, but they differ distinctly in the selection and arrangement of material. In GANONG'S text[1] the arrangement of topics is primarily morphological. This arrangement will undoubtedly prove here, as it has in many an earlier text, its peculiar fitness for an introductory textbook, because of simplicity and its ready intelligibility to the beginner, who already knows the primary organs of the plant by sight and by name. In the 6 chapters of the body of the book the discussion in succession of leaf, stem, root, flower, fruit, and seed is carried out in each case with that close interrelating of structure and function which is essential to the clear comprehension of the relative significance and of the intimate interdependence of these two phases of plant organization. Each of these chapters includes a section on the economics and cultivation of the structure under consideration. The chapter on the flower contains concise but clear discussions of the significance of sex, of heredity, of evolution, and of plant breeding, while the following chapter has a brief section on plant diseases. Ecology and paleobotany are not discussed in this part.

The 274 illustrations include a considerable number of original ones, of which figs. 85 and 162 are excellent examples, and there are many less frequently copied ones from various standard works. It is not clear to the reviewer, however, that such synthetic diagrams as those of the leaf, stem, and root (figs. 11, 105, 166) are really needed by the average student. They do suggest, it is true, certain more important features of the structure and work of each organ, but because of the omission, for the sake of simplicity, of some other essential structures they are liable to be misleading. Properly selected, accurate drawings would avoid this objection and still be entirely intelligible. The reviewer is inclined to question also whether the substitution of drawings of models showing leaf arrangement, for figures of the stems and leaves themselves, is really necessary as an aid to the imagination of the ordinary college student. The second part, entitled "The kinds and relationships of plants," is expected to be ready during 1917.

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[1] GANONG, WILLIAM F., A textbook of botany for colleges. Part I. The structures and functions of plants. 8vo. pp. xi+401. New York: Macmillan. 1916.

GAGER'S text[2] is evidently intended, like GANONG'S, as a guide for an introductory, cultural course for college students, which shall at the same time serve as a foundational one for students who are to pursue the subject further. The arrangement of topics, however, differs in being professedly physiological, at least in Part II, which corresponds most nearly to the body of GANONG'S book. Part I ("Introduction") deals with the organs of the cormophyte and the structure of the cell. Part II ("The vegetative functions of plants") includes chapters on the loss of water, absorption of water, the path of liquids in the plant, nutrition, fermentation, respiration, growth, and adjustment to surroundings.

Chapter IV, under the title "Loss of water," which does not very adequately forewarn the reader of the nature of its contents, discusses some of the essential facts of the gross morphology, histology, and physiology of the leaf. Chapter V ("Absorption of water") treats of the absorptive function of the soil root, but other functions and the structure of the root, aside from that of the root hairs, are not considered here, nor could anything but the briefest mention of them be found elsewhere in the book. Certain important features of the structure and activity of the stem also are either not referred to at all, or are barely mentioned. Thus, such tissues as bark, phellogen, cork, sieve tubes, etc., are not mentioned in the index, or more than incidentally referred to in the text. Secondary thickening, types of branching, the various habits assumed by the stem, the structure of buds, etc., are not given space for any real discussion or explanation. These omissions are apparently part of the plan of the book and are interesting as showing the author's estimate of the relative importance of these topics among the large number from which selection must be made.

The 26 chapters of Part III ("Structure and life histories") include discussions of the life histories of a considerable number of types, especially of the mosses, ferns, and flowering plants. The fern is made the primary type in these discussions of life cycles, and the whole series is rather copiously illustrated. Important and interestingly written chapters in this part are those dealing with the problem of sex in plants, the economic importance of fungi, evolution, Darwinism, experimental evolution, heredity, and paleobotany. The treatment of these themes, with the series of accompanying portraits of some of the great naturalists, serves to suggest something of the history of certain important botanical theories.

The book is abundantly illustrated with 434 figures, a good share of which are original drawings or halftones. While the appearance, for example, of such illustrations as figs. 127, 198, 263, and 286 is to be welcomed, the same cannot justly be said of some others. The use of such illustrations as figs. 37, 142, 151, 160, 165, or 180 would seem scarcely justified on grounds of mere novelty,

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[2] GAGER, C. STUART, Fundamentals of botany. 8vo. pp. xvi+640. Philadelphia: Blakiston's Sons. 1916.

when much clearer ones of the same objects are already available.—D. S. JOHNSON.

MINOR NOTICES

North American flora.—The third part of Vol. 34 continues the presentation of Carduales by RYDBERG,[3] including the completion of the Tageteae and the Anthemideae. In the Tageteae 22 genera are recognized, the last 5 being presented in this part. Of these the large genera are *Pectis* with 71 species (11 new) and *Porophyllum* with 42 species (10 new). A new genus (*Hydropectis*) is described, based on *Pectis aquatica* Wats.

The recognized genera of Anthemideae number 21, a considerable number of them being segregated from more familiar genera. The largest genus is *Artemisia* with 120 species (29 new), followed by *Achillea* with 24 species (6 new). The other genera are represented by comparatively few species. Three new genera are described as follows: *Vesicarpa*, based on *Artemisia potentilloides* Gray; *Chamartemisia*, based on *Tanacetum compactum* Hall; *Artemisiastrum*, based on *Artemisia Palmeri* Gray.—J. M. C.

The theory of evolution.—SCOTT[4] has made an excellent restatement of the evidences of organic evolution. The somewhat hackneyed subject is enlivened by a forcible and very readable presentation. The book is the result of the organization of 6 lectures (the Westbrook Lectures for 1914). In addition to the evidences from classification, comparative anatomy, embryology, paleontology, and geographical distribution, the author presents evidence derived from domestication, from blood tests, and from experiment.

The opening chapter gives a brief historical review of theories of evolution and a concise statement of the present status of the question. I have seen no better presentation of this body of data for both biologist and general reader than that given in this little book. My only criticism is that it is insufficiently illustrated, although the few illustrations used are well chosen—H. H. NEWMAN.

A moss flora.—GROUT[5] has published a very convenient list of the moss flora of all counties of New York and New Jersey adjacent to New York City. The moss flora of this area has probably been explored more thoroughly than that of any other region of the United States. Numerous keys make the recognition of genera and species relatively easy, and the excellent photographic plates illustrate the genera. Such a publication should stimulate the study of a very interesting flora, for, as the author remarks, "in and around New York

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[3] RYDBERG, PER AXEL, North American Flora **34**:part 3. pp. 181–288. Carduales: Carduaceae (Tageteae, Anthemideae). New York Botanic Garden. 1916.

[4] SCOTT, W. B., The theory of evolution. 8vo. pp. vii+183. New York: Macmillan. 1917.

[5] GROUT, A. J., The moss flora of New York City and vicinity. 8vo. pp. 120. *pls.* 12. New Dorp (N.Y.): published by the author. 1916.

City the moss flora of the north and of the south meet and mingle, and the number of species occurring is large, varied, and interesting."—J. M. C.

NOTES FOR STUDENTS

Transpiration studies.—Among several recent papers dealing with various phases of the study of transpiration, a prominent place should be given to one by LIVINGSTON and SHREVE[6] upon improvements in the use of the cobalt chloride paper method. An improved paper slip has been designed which combines two permanent color standards and an area of carefully prepared cobalt chloride paper. The determinations of the end points are made more definite, therefore, and the probability of error is much reduced. An improved device for furnishing a standard water surface is described also. The temperature relations of the rate of color change in the hygrometric paper and its permanent standardization is discussed also. These improvements will greatly advance the method of study which has already been proved valuable.

Another modification of methods of study is seen in DARWIN'S[7] investigation of the relation of transpiration to relative humidity by the porometer method, using *Prunus Laurocerasus* and eliminating the action of stomata by applying vaseline to the lower surface of the leaves and then placing their intercellular spaces in communication with the external air by means of incisions. Plotting the results, he found that transpiration varies directly as relative humidity when a correction is made for the fact that the transpiration rate is not zero in saturated air. The fact that transpiration does occur in saturated air is due, as pointed out by SACHS, to the production of heat in the leaf by respiration. The experiments showed that for the transpiration to be entirely checked a humidity of 5 per cent above saturation would be necessary, and hence the temperature of the leaf due to respiration is, under the conditions of the experiments, 0°.8C. above that of the atmosphere.

Using similar methods and materials, DARWIN[8] also studied the effect of diffuse light upon transpiration. The results show so remarkable an amount of variation that it seems dangerous to draw any conclusions other than that light tends to increase the water loss for some unknown reason when its influence upon the action of stomata has been eliminated. This increase averages about 33 per cent.

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[6] LIVINGSTON, B. E., and SHREVE, EDITH B., Improvements in the method for determining the transpiring power of plant surfaces by hygrometric paper. Plant World **19**:257–309. 1916.

[7] DARWIN, F., On a method of studying transpiration. Proc. Roy. Soc. London B **87**:269–280. 1914.

[8] ———, The effect of light on the transpiration of leaves. Proc. Roy. Soc. London B **87**:281–299. 1914.

In a more recent report by the same investigator,[9] the corrections previously indicated for humidity and light are used in experiments designed to show the relation between the rate of transpiration and stomatal aperture, the latter condition being determined by the use of the porometer. There was also applied a further correction for cuticular transpiration. The final results show many irregularities, but are regarded by DARWIN as giving substantial support to his thesis that transpiration is regulated by stomatal aperture. He apparently finds nothing corresponding to the incipient drying of LIVINGSTON and BROWN, or the saturation deficit of RENNER, although it seems possible to the reviewer that some of his many irregularities might require some such explanation.

In striking contrast to this theory there comes an account of a study of water relations of cacti. On account of their peculiar behavior, these plants offer special advantages as well as special problems in the general study of transpiration. Their transpiring power differs from ordinary plants in being greater during the night than during the day. This behavior Mrs. SHREVE[10] has investigated, and has found that there is a regular diurnal march of change in the water-holding capacity of the internal tissues that seems both directly and indirectly responsible for the changes in transpiring power; that is, the transpiring power of the cactus is usually greater at night than during the day because the water-holding capacity of the tissues is greater by day than by night. The variations in water-holding capacity act upon transpiring power indirectly by closing the stomata, which in cacti are usually closed during the day and open at night, and they also act directly by resisting the evaporating power of the air. It seems possible, as the author of this paper points out, that a similar change in the tissues of non-succulents may account for the mid-day drop in their transpiring power.

BRIGGS and SHANTZ[11] have made an extensive and detailed study of transpiration as related to growth and to various climatic factors. The measurements were made at Akron, Colorado, during the seasons of 1914 and 1915 and were for 270 pots of 115 kgm. each of soil, including some 25 different crop plants. Continuous automatic records were obtained for air temperature, solar radiation, wet bulb depression, wind velocity, and evaporation from both shallow and deep tanks. Among the comparisons instituted, one of the most instructive is the correlation between transpiration for the small grains and various physical factors. This is expressed by mean correlation coefficients, some of which are those with evaporation from a shallow pan, 0.87; with wet

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[9] DARWIN, F., On the relation between transpiration and stomatal aperture. Phil. Trans. Roy. Soc. London B **207**:413–437. 1916.

[10] SHREVE, EDITH B., An analysis of the causes of variation in the transpiring power of cacti. Physiol. Researches **2**:73–127. 1916.

[11] BRIGGS, L. J., and SHANTZ, H. L., Daily transpiration during the normal growth period and its correlation with the weather. Jour. Agric. Research **7**:155–212. 1916.

bulb depression, 0.88; with temperature, 0.71; with radiation, 0.65; and with wind velocity, 0.22. These serve to emphasize the close relation of transpiration to humidity and evaporation and the comparatively slight influence of wind velocity.

It is also interesting to note that during a 10-day period of maximum transpiration the daily loss of water ranged from 6 to 9 times the dry weight of the crop for millets and corn, from 12 to 16 times for the small grains, and up to 36 to 56 times for alfalfas. During the same 10-day period the annual crop plants lost about one-fourth of the total water transpired during the entire season. The transpiration of the different crop plants per unit area of plant surface shows less variation than the transpiration per unit of dry weight, hence the greater efficiency shown by certain plants in the use of water seems to be due more to a reduction in plant surface than to a reduction of transpiration per unit area of surface. Various other comparisons make this a valuable report for both the botanical and the agricultural investigator.

Another investigation undertaken with a view to economic application of results shows scientific merit of a high order and is comparable in methods and results with that just reviewed. In it KIESSELBACH[12] has limited his research to corn grown under conditions very closely approximating those of crop production. A portion of the experiments was devoted to the development of a satisfactory technique, and errors of former experimenters due to the use of immature plants and small quantities of soil were pointed out. It is impossible to summarize the many data, but it is interesting to note the agreement with the results of BRIGGS and SHANTZ in the very large proportion of total water used by the plant, which is lost during a comparatively short period of maximum transpiration. A rather surprising result is that it was found that corn plants grown for 2 months in a humid greenhouse exhibited no different transpiration rate per unit leaf area when transferred to dry conditions than took place from plants continuously grown under dry conditions. Further it appears that while there were considerable variations in the different varieties in regard to thickness of leaf and epidermis, and also in number of stomata per unit of leaf area, there was no consistent correlation between these structural features and the transpirational rate per unit of dry matter produced. In spite of this it was found that the water requirement of different varieties differed to a marked degree, suggesting that drought-resistant strains may be selected. It also developed as an important result of the investigation that water economy is greatest with neither an excessive nor a deficient soil moisture supply, and further that increasing fertility by the application of fertilizers resulted in still greater water economy.

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[12] KIESSELBACH, T. A., Transpiration as a factor in crop production. Research Bull. no. 7, Neb. Agric. Exp. Sta. pp. 214. *pls.* 4. *figs.* 24. 1916.

The relation of soil moisture to transpiration and to economy in the use of water is also shown by the investigations of YUNCKER,[13] who, using the wax seal method and weighing the sealed pots, has studied the comparative rates of transpiration of young plants of *Zea Mays* growing in soil with 3 different soil moisture contents, all somewhat above the wilting coefficient and showing respectively 25, 45, and 65 per cent of possible saturation. The rate of transpiration and the water requirement for periods up to 1320 hours was least for the driest cultures, which, however, seem to have had no deficiency in water supply, and most for those with the highest soil moisture content. Thus among other things it appears that the experiment demonstrated that the amount of dry matter formed was not at all proportional to transpiration.—GEO. D. FULLER.

Taxonomic notes.—LANGE,[14] in the second part of his studies on the agarics of Denmark, has published his results with *Amanita*, *Lepiota*, and *Coprinus*. The first part, published in 1914, contained a general introduction and an account of *Mycena*. The genus *Amanita* includes 14 Danish species; *Lepiota* 31 species, 1 of which is new; and *Coprinus* 33 species, 3 of which are new. The presentation of each genus is preceded by a full discussion of its characters and an analytical key.

MACBRIDE,[15] in presenting "The true Mertensias of western North America," recognizes 32 species, 4 of which are described as new. GRAY'S *Synoptical Flora* (1886) contains 7 species, 2 of which are restricted to the Atlantic states. Since that time, 74 species have been proposed. In a "Revision of the genus *Oreocarya*," 45 species are recognized, 4 of which are described as new. In "Notes on certain Borraginaceae," *Amblynotopsis* is proposed as a new genus (including 4 species transferred from *Krynitzkia*), and new species are also described in *Lappula* (2), *Cryptantha* (2), *Amsinckia*, *Mertensia*, and *Lithospermum*.

MOORE[16] has described 2 new genera: *Capitanopsis* (Labiatae) from Madagascar, and *Megalostylis* (Euphorbiaceae) from Peru or Brazil (upper Amazon region). He also describes 13 new species from Africa.

OKAMURA,[17] in his second contribution to the bryophytic flora of Japan, describes a new liverwort and 29 new species of mosses well distributed generically.

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[13] YUNCKER, T. G., A study of the relation of soil moisture to transpiration and photosynthesis in the corn plant. Plant World **20**:151–161. 1916.

[14] LANGE, JAKOB E., Studies in the agarics of Denmark. Part II. Dansk. Bot. Arkiv. **2**:no. 3. pp. 53. *pls. 2*. 1915.

[15] MACBRIDE, J. FRANCIS, Contrib. Gray Herb. no. 48, pp. 58. 1916.

[16] MOORE, SPENCER LEM., Alabastra diversa. XXVI. Jour. Botany **54**:249–257. 1916.

[17] OKAMURA, SHUTAI, Contributiones novae ad floram bryophyton Japonicam. Pars secunda. Jour. Coll. Sci. Tokyo **38**:no. 4. pp. 100. *figs. 42*. 1916.

OVERHOLTS[18] has monographed the Polyporaceae of the central states, including the states extending from Ohio to North Dakota and southward to Kentucky and Kansas. He recognizes 132 species in 7 genera, the species of *Poria* and *Merulius* being omitted because "practically nothing is known of them at present." The large genus is *Polyporus*, with 88 species; following it are *Fomes* with 23 species and *Trametes* with 10 species. The keys and contrasted descriptions should make the identification of species comparatively easy. Perhaps the author is to be congratulated that he did not see fit to propose any new species.

SMITH[19] has described 10 new species and 5 new varieties of algae from the lakes of Wisconsin, and also a new genus (*Gloeocystopsis*), which combines the external morphological characters of *Gleocystis* and *Nephrocytium*.

STEVENS,[20] in a synoptical account of the species of *Meliola* occurring in Porto Rico, recognizes 95 species, and describes 62 of them as new.—J. M. C.

Sulphur nutrition.—Although sulphates have little effect on the soil flora, and cannot function therefore as important fertilizers for all crops, the fact that the sulphur content of most soils is rather low, and that certain classes of plants use considerable quantities of sulphur in metabolism, leads to the possibility of sulphur deficiency becoming in certain cases a limiting factor to crop production. HART and TOTTINGHAM[21] have made some greenhouse studies on the relation of elemental sulphur and various sulphates to the nutrition of certain of the Leguminosae, Cruciferae, and Gramineae, groups differing somewhat in their need of sulphur. They find that sulphates may be beneficial to certain crops, even when functioning only as a source of sulphur.

Calcium sulphate in general gave better results than sodium sulphate. It increased the dry weight produced by red clover 23 per cent. With rape the greatest beneficial influence was noted when the calcium sulphate was used in addition to a complete fertilizer. The increase due to the sulphate in this case was 17 per cent. In both plants the roots were much elongated by the sulphate, so that a much larger volume of soil is laid under contribution to the plant, and its ability to withstand drought is much increased. The sulphate therefore not only meets the special needs of these plants for sulphur but improves the general physiological conditions.

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[18] OVERHOLTS, L. O., The Polyporaceae of the middle-western United States. Wash. Univ. Studies **3**:3–98. *pls. 8.* 1915.

[19] SMITH, GILBERT MORGAN, New and interesting algae from the lakes of Wisconsin. Bull. Torr. Bot. Club **43**:471–483. 1916.

[20] STEVENS, FRANK LINCOLN, The genus *Meliola* in Porto Rico. Ill. Biol. Monographs **2**:1–86. *pls. 5.* 1916.

[21] HART, E. B., and TOTTINGHAM, W. E., Relation of sulphur compounds to plant nutrition. Jour. Agric. Research **5**:233–249. 1915.

The grains, barley and oats, showed little effect on the quantity of straw, but a noticeable increase in seed production occurred on plants grown on the soil used (Miami silt loam).

Elemental sulphur, added as flowers, was usually toxic even in the presence of calcium, probably because of its incomplete oxidation to sulphites. Where bases are deficient, the toxicity may be due to accumulation of sulphuric acid from the complete oxidation of the sulphur.—CHARLES A. SHULL.

British Columbia forests.—Mount Robson, British Columbia, situated at practically the present northern known limit of the continental divide, has been visited by COOPER[22] and found to possess 2 climax forest types, one for each of 2 climatic zones. Up to an altitude of 1000 m. the forest is of the Pacific Coast type, with a dominance of *Thuja plicata*. *Picea Engelmanni* is next in abundance, and is followed by *Abies lasiocarpa*, *Tsuga heterophylla*, and *Pseudotsuga mucronata*. The undergrowth shows such truly mesophytic forms as *Acer glabrum*, *Azaleastrum albiflorum*, *Phegopteris Dryopteris*, *Clintonia uniflora*, *Moneses uniflora*, and *Pyrola uliginosa*.

Above this is a subalpine zone extending up to 2000 m., with a climax forest of *Picea Engelmanni*, *Abies lasiocarpa*, and *Pinus albicaulis*. In the undergrowth *Menziesia ferruginea*, *Cornus canadensis*, and several species of *Pyrola* are conspicuous. The successions upon rock surface, talus, moraine, and shingle flat are noted, those of the two last in most detail. Upon the moraine *Dryas octopetala* and *Arctostaphylos rubra* are followed by shrubby species of *Betula* and *Salix*, leading to the third stage, which is the climax forest. A similar set of stages is found upon the shingle flat, although here, probably because of the lack of any fine soil material, the succession advances much less rapidly than upon the moraine.

While COOPER expresses regret at the few data available for this study, it will be welcomed as giving an insight into the vegetation of an almost unknown region.—GEO. D. FULLER.

Large trees.—A recent contest for two prizes of $100 each, offered through the *Journal of Heredity*,[23] for photographs and data regarding the largest trees in the United States, barring conifers, resulted in photographs of 337 trees. The prize for the largest non-nut-bearing tree was won by a *Platanus occidentalis* near Worthington, Indiana, with a circumference, 5 ft. from the ground, of 42.25 ft., and a height of about 150 ft. The largest nut-bearing tree in the competition was a *Quercus lobata* on the foothills of the Sierra Nevada Mountains, in San Benito County, California, with a circumference of 37.5 ft. and a height of 125 ft. The largest specimens of other species were as follows: *Ulmus americana* at Morgantown, West Virginia, with a circumference of 33

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[22] COOPER, W. S., Plant succession in the Mount Robson region, British Columbia. Plant World **19**:211–238. *figs. 8*. 1916.

[23] Photographs of large trees. Jour. Heredity **6**:407–429. 1915.

ft.; *Quercus alba* at Atwood, Indiana, 21 ft.; *Juglans nigra* at Hanover Neck, New Jersey, 24 ft.; and *Liriodendron Tulipifera* at Asheville, North Carolina, 34.5 ft.

The report of the results of the contest also contains other interesting data, while the value of such a competition, as pointed out by LAMB,[24] consists not only in promoting interest in the protection of tree individuals and in the conservation and preservation of forests, but also in affording data for the solution of problems of distribution, of growth, and of duration. In this connection he has prepared maps showing the distribution of 6 of the important species represented and the location of the best specimens reported in the contest. It is hoped that public interest in the subject will not cease with the conclusion of the contest.—GEO. D. FULLER.

American forestry.—Recent changes and improvements have made the magazine known as *American Forestry* valuable not only to the forester but also to the botanical teacher or student interested in trees. An excellent feature is that of devoting special attention to one particular tree species in each issue. Well written articles are given dealing with the identification, characteristics, and habits of the trees, and also with the lumber and its uses. During the first half of the current year the following species have been the subjects of special consideration: *Quercus alba*, *Pseudotsuga Douglasii*, *Thuja plicata*, *Betula papyrifera*, *Ulmus americana*, and *Sequoia sempervireus*. The excellence of the illustrations in these articles is worthy of note.

There are also, in addition to the articles of more particular interest to the professional forester, others upon more general but quite as timely topics. Among these we may note as examples a finely illustrated article upon *Cupressus macrocarpa* under the title of "The tree of legend and romance," in the February issue; and several dealing with forests in time of war, showing some of the devastating effects of the present European conflict. A recognition of various phases of forest and country life is seen in regular departments devoted to children, birds, ornamental and shade trees, and to wood preserving, while quite as important are the very extensive lists of current literature. Finally, as an indication of the international scope of its interests is a page of its notes and news items devoted to Canadian forestry and foresters.—GEO. D. FULLER.

A vegetational map of the United States.—SHREVE[25] has compiled a map of the range of the principal types of vegetation in the United States, basing the boundaries of the various subdivisions upon purely vegetational criteria. The primary classes of vegetation are the well recognized ones of desert, grassland, and forest. Of these the first and last have been subdivided, but the data available for the grassland are not regarded as sufficient to afford a basis

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[24] LAMB, W. H., Value of the contest. Jour. Heredity **6**:424–429. 1915.

[25] SHREVE, F., A map of the vegetation of the United States. Geog. Rev. **3**:119–125. 1917.

for mapping. On the whole, 18 types of plant communities are recognized, briefly characterized, and plotted. An inspection shows, as its author points out, that the areas have in general a north and south rather than an east and west trend, which tends to show that the major differences in vegetation are here determined more largely by conditions of moisture than of temperature.

While the result is decidedly the best map of the sort yet produced, it is also probable that it would be difficult to find an ecologist who would agree with it in every particular. So much of the disagreement would be differences of opinion as to what should be included within a single vegetational type, that diverse criticism would be neither a gracious nor a practical task, and yet the reviewer cannot refrain from expressing a question as to the fitness of including both the *Pinus ponderosa* and *P. Murrayana* forests of the west and the *P. Strobus*, *Tsuga*, and *Abies balsamea* forests of the east in the "northern mesophytic evergreen forest."—GEO. D. FULLER.

History of forest ecology.—A recent study of the historical development of forest ecology is likely to prove of equal interest to foresters and ecologists. In it BOERKER[26] traces the development of plant ecology from its beginnings to the modern phase characterized chiefly by efforts to measure the various habitat factors. The beginnings of forest ecology are seen in the empirical development of silviculture, dating back to the fifteenth century or even earlier; but the founder of the science is considered to be DUHAMEL DU MONCEAU, about the middle of the eighteenth century. About a century later the work of HARTIG and that of a committee of 5 German foresters, appointed in 1868, consisting of WESSELY, HEYER, EBERMAYER, JUDEICH, and BAUR, gave the science further impetus, and resulted in the organization of a series of forest experiment stations throughout Germany. From this beginning the advancement of the science is traced to the present day, as shown in the work of WAGNER, MEYER, and DUESBERG in Germany, and that of FERNOW and ZON in America. It is notable that not until 1909 were forest experiment stations established in the United States.—GEO. D. FULLER.

Permeability.—It is well established that a great variety of stimuli (light, temperature, salts, anesthetics) modify the permeability of protoplasm. In some cases the stimulus decreases the permeability at low intensity and increases it at high intensity. KOKETSU[27] believes he has demonstrated that electrical stimulation increases the permeability of epithelial cells of *Tradescantia discolor*. Cells thus stimulated are less easily plasmolyzed by ordinary plasmolytic agents than are unstimulated cells. After recovery from the stimulus they show a greater degree to plasmolysis by the same concentration of the

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[26] BOERKER, R. H., A historical study of forest ecology; its development in the fields of botany and foresty. Forestry Quarterly **14**:380–432. 1916.

[27] KOKETSU, R., Über den Einfluss der elektrischen Reizung auf die Permeabilität der Pflanzenzellern. Bot. Mag. Tokyo **30**:264–266. 1916.

agent than do the checks. He interprets the first change as due to greater permeability of plasmolytic agents, and the second change as due to loss of solutes during the period of higher permeability. Szücs[28] finds that aluminum salts render cells more difficult to plasmolyze because they harden the protoplasm, although they really decrease its permeability. One must look out for a similar condition with electrical stimuli. The experiments are qualitative but suggest the need of very careful quantitative studies.—Wm. Crocker.

Texas root rot fungus.—Duggar[29] has investigated the causal organism of one of the most destructive of the cotton diseases, an organism which seems to be confined largely to Texas, where the average losses have been variously estimated to be $2,000,000 to $3,000,000. In addition to the attacks on cotton, the fungus damages such crops as alfalfa, beans, sweet potatoes, and certain orchard fruits. As illustrating the omnivorous habit of the fungus, Duggar enumerates a list of nearly 30 host plants (trees, shrubs, and herbs) already noted as used by the fungus. The chief feature of the disease is the sudden wilting and dying of the affected individuals. The fungus was described by Shear as *Ozonium omnivorum*, but Duggar concludes that it should be transferred to *Phymatotrichum*. In the revised description of the species the habitat is stated as follows: "Hyphae on living roots of many plants and in the soil; conidial stage on soil in the vicinity of diseased plants."—J. M. C.

Embryo and seedling of Dioscorea.—Miss Smith[30] has investigated the embryo and seedling of *Dioscorea villosa*, a genus long known through the work of Solms-Laubach as furnishing evidence of a "second cotyledon," or at least a seedling structure quite different from what had come to be regarded as the monocotyl type. Miss Smith traced the development of the embryo to the spherical 4-celled proembryo, and then followed the appearance of the organs. She observed no cotyledonary ring, and claims that the single cotyledon originates as a terminal structure. It may be stated that the course of the vascular strands suggests that the leaf called the "first secondary leaf" occupies the position of a "second cotyledon," which would make the growing point of the stem a terminal structure, and both cotyledons lateral. This, however, is a matter of interpretation in connection with material.—J. M. C.

Vitality of moss protonema.—Miss Bristol[31] has discovered some remarkable cases of the retention of vitality by the protonema of mosses. In samples of soils obtained from various places for the purpose of ascertaining by means

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[28] Rev. in Bot. Gaz. **56**:245. 1913.

[29] Duggar, B. M., The Texas root rot fungus and its conidial stage. Ann. Mo. Bot. Gard. **3**:11–23. *figs. 6.* 1916.

[30] Smith, Pearl M., The development of the embryo and seedling of *Dioscorea villosa*. Bull. Torr. Bot. Club **43**:545–558. *pls. 31–34.* 1916.

[31] Bristol, B. Muriel, On the remarkable retention of vitality of moss protonema. New Phytol. **15**:137–143. *figs. 3.* 1916.

of cultures the algae present in the form of "resting spores," protonema from certain soils began to develop. In these soils the protonema had persisted in the dried condition for 46, 48, and 49 years. A description is given of the appearance of the cells, which seemed to be in vigorous condition. Moss spores contain chlorophyll and are usually short-lived. "Hence the power to produce a resting protonema filament which is able to resume growth, even after half a century, is a great asset to the plant in preventing its extinction through adverse climatic conditions."—J. M. C.

Anatomy of Drimys.—The genus *Drimys* (Magnoliaceae), belonging to the Southern Hemisphere, is very interesting on account of the absence of vessels. JEFFREY and COLE[32] have investigated its wound reactions from material obtained from New Zealand and Java, and also from material at Kew. As a result of injury, the roots develop peculiar tracheary structures, which are regarded as a "reversionary return of vessels" because the markings of the lateral walls resemble those found in the vessels of the Magnoliaceae. They are clearly distinct from ordinary tracheids, but lack the perforations of normal vessels. The authors conclude that these traumatic structures are to be interpreted as a clear indication of the former presence of vessels in *Drimys*.—J. M. C.

A cedar swamp on Long Island.—A swamp on the southern shore of Long Island, New York, about one mile long and half as wide, is, according to TAYLOR,[33] of special interest because (1) it is probably the most northerly grove of *Chamaecyparis thyoides* on the coastal plain of anything like that size; (2) the character of the undergrowth, which includes 77 per cent of species northern in character; and (3) it affords evidence of coastal subsidence in the transition between the swamp and the open salt marsh and in the number of dead and dying trees. This evidence is all the more convincing because of the remoteness of any barrier beach or other possible regulator of exceptional tides, a possible alternative to recent subsidence.—GEO. D. FULLER.

Flora of Isle Royale, Michigan.—COOPER[34] has supplemented his excellent ecological analysis of the vegetation of Isle Royale[35] by a catalogue of its vascular plants. As a list of the mosses of the same island was previously

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[32] JEFFREY, EDWARD C., and COLE, RUTH D., Experimental investigations on the genus *Drimys*. Ann. Botany **30**:359–368. *pl. 7*. 1916.

[33] TAYLOR, NORMAN, A white cedar swamp at Merrick, Long Island, and its significance. Mem. N.Y. Bot. Gard. **6**:79–88. 1916.

[34] COOPER, W. S., A catalogue of the flora of Isle Royale, Lake Superior, Michigan. Acad. Sci. Report **16**:109–131. 1914.

[35] ———, The climax forests of Isle Royale. BOT. GAZ. **55**:1–44, 115–140, 189–235. 1913.

published,[36] this catalogue advances the region to the position of having one of the very few well known floras in the state. The present list includes 40 species of pteridophytes and 479 species of spermatophytes. One happy improvement in the present publication is the ecological definition of the habitat, replacing such time-honored but meaningless phrases as "hillsides," "glades," "woods," and "cool dry woods."—GEO. D. FULLER.

Plants of the Florissant lake beds.—These beds in Colorado have been famous for 50 years for their abundance of finely preserved fossil plants and insects. KNOWLTON[37] has now published a review of the plant material on deposit in the U.S. National Museum. Over 100 plants are presented, and among them 18 new species are described, chiefly woody dicotyledons. Two new genera are proposed; *Palaeopotamogeton* (Potamogetonaceae) and *Florissantia* (Solanaceae). The list of types of fossil plants from Florissant in the U.S. National Museum includes the names of 121 species.—J. M. C.

Mushroom fairy rings.—The occurrence of well developed "fairy rings" formed by a large mushroom known as *Tricholoma praemagnum* in the dry grassland of the open mountain parks of Colorado has been described by RAMALEY.[38] They have been observed in various localities, but all between 6000 and 9000 ft. in altitude. The rings vary much in size, the smallest observed being 3.3 m. across, and seen to increase in diameter at a rate of about 1 dm. per year. One of the interesting characteristics of the fungus is its distinctly xerophytic habit.—GEO. D. FULLER.

Aerating system.—HUNTER[39] has studied the structure of various air chambers in plants of *Vicia Faba* and has found spaces of various sorts in the testa of the seed, the cotyledons, the stem, the leaves, and the root. The study adds to our knowledge of the aerating system as developed in seed plants, even if it rather fails to justify the author's conclusion that the system is "elaborately adjusted in order to insure an efficient gaseous exchange for each living cell no matter where its position may be in the plant tissues."—GEO. D. FULLER.

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[36] COOPER, W. S., A list of mosses collected upon Isle Royale, Lake Superior. Bryologist **16**:3–8. 1913.

[37] KNOWLTON, F. H., A review of the fossil plants in the U.S. National Museum from the Florissant lake beds at Florissant, Colorado, with descriptions of new species and list of type specimens. Proc. U.S. Nat. Mus. **51**:241–297. *pls. 12–27*. 1916.

[38] RAMALEY, FRANCIS, Mushroom fairy rings of *Tricholoma praemagnum*. Torreya **16**:193–199. 1916.

[39] HUNTER, C., The aerating system of *Vicia Faba*. Ann. Botany **29**:627–634. 1915.

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THE
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A STUDY IN PHYSIOGRAPHIC ECOLOGY IN NORTHERN FLORIDA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 229

LAURA GANO

(WITH TEN FIGURES)

Introduction

Ecological investigations of the coastal plain of southeastern United States, with few exceptions, have not been undertaken from the standpoint of the relation of physiography to the successional history of the plant associations, nor has the classification of this region been satisfactorily established in comparison with other forest formations of the United States. SCHIMPER (11) mapped this portion of the coastal plain (with the exception of southern Florida) as temperate rain forest. SARGENT (10) classified it as the southern maritime pine belt. The first classification is obviously inconsistent, and the second is open to question if by pine forest is meant a climax formation.

Except for HARSHBERGER'S detailed treatment of the coastal plain in his *Phytogeographic survey of North America*, studies of this particular region have been scattered, and usually of exceptional localities. On the Gulf Coast, HILGARD in soil surveys in Mississippi and Louisiana paid special attention to plants as soil indicators, outlining associations on this basis. Studies of island plant life in the Mississippi River sound and delta were made by LLOYD and TRACY (8); while MOHR in his *Plant life of Alabama* grouped

various plant associations belonging to the different geological divisions of the coastal region of that state, comparing the flora in its relationships with adjoining regions and with the West Indies and Mexico. More recently, HARPER has contributed numerous publications containing ecological data, as the result of extensive observations and explorations in various parts of the coastal plain, his most complete works being a phytogeographic study of the Altamaha grit formation of Georgia (5), and publications in the reports of the State Geological Survey of Florida (6, 7).

The area described in this study is included mainly in Leon County, Florida. This county is situated half-way between the east and west boundaries of the northern part of the state, bordering the Georgia line and distant about 20 miles from the Gulf of Mexico. The area is approximately 675 square miles, and as a whole is located immediately west of the 84th meridian and between 30 and 31° north latitude.

The topography is diversified, and a soil survey (14) of this county locates and describes 12 soil types (including meadow and swamp), of which the most extensive are those also common to the coastal plain from Virginia south to Florida and west to Texas.

This section of Florida has had a varied history, dating back to early Indian tribes and the first Spanish explorers. According to narratives of DE SOTO's followers, the fame of this country as the "land of plenty" extended to eastern and central Florida, and made it a desirable place to seek for possession and settlement. During the several hundred years of history chronicling invasions and resettlement, therefore, successive clearings of the forest, from the more fertile soils at least, must have been made. About the time of the establishment of the capital at Tallahassee (1823) near the center of the county, WILLIAMS (15) described "abundant groves of oak, hickory, beech, and magnolia crowning the hills and covering their slopes." Such early pictures are of interest now in considering the upland forests.

Physical features

CLIMATE.—Weather records have been kept at Tallahassee since 1885 (3). The mean summer temperature averages 79°.7, autumn

68°, winter 53°, and spring 67°.6 F., indicating a moderate and equable yearly temperature. There are records of severity, however, the lowest temperature for the state being recorded from Leon County, −2° F. on February 13, 1899. Frost may be expected from November 1 until April 1, the frost record for 18 years giving the date of the first killing frost in autumn as November 4, the last killing frost in spring as April 6, the average date of the first killing frost in autumn as December 5, and the average date of the last killing frost in spring as March 3.

The mean annual precipitation for the Tallahassee station (3) is 57.12 inches, and there are 2 marked periods of rainfall; one (the lesser) in winter culminating in March, and the other (more excessive) culminating in July. This summer rainfall averages 26.8 inches, and the winter rainfall 17.9 inches, the summer rains occurring almost daily as afternoon thunderstorms, while the winter rains are more evenly distributed between day and night. The year is divided thus into wet and dry seasons, more or less marked, April and November being the driest months.

There are no reliable records for relative humidity, although for northern Florida the percentage of relative humidity is highest in September and lowest in April.

These climatic effects combined tend to make spring (April period) the hardest season for plants, so far as the moisture relations are concerned.

PHYSIOGRAPHY.—Limestones of the Oligocene period are considered now to be the oldest rocks of Florida and to form the rock basis for most of the state (9). These limestones also are the surface rock over much of northern Florida, and indicate the earliest and most persistent land surface during subsequent geological history. The presence so near the surface of readily soluble and easily disintegrated rock has doubtless had important influence upon the present topography and drainage, as well as upon the character of some of the soil.

Crossing the country from north to south, the most striking topographical feature is the division of the surface into 2 distinct parts, a highland and a lowland. By this division about two-thirds of the surface is included in the highland, which is a portion of the

narrow upland extending along the northern edge of Florida and into Georgia, and which, on its sea-facing side, often drops abruptly to the more recent coastal strip.

The general elevation of the upland is betweeen 100 and 200 ft. above sea level. The surface is gently rolling with series of broadly rounded or flat topped hills, in general extending east and west, and alternating with open, troughlike valleys. Many of the valley streams are mere swampy or boggy tracts, or they may pursue sluggish courses which end blindly, spreading out on the surface of the ground at the lowest part and soaking gradually into the soil. Others end in ponds which occupy basin-like depressions, or may drain into the larger lakes and sinkholes. In this hill region the sinkhole origin of many large lakes as well as small ponds, and the sinkhole formation along the line of some of the valleys at present, suggest that the depressions of this upland division may be due in large part to subterranean erosion. At any rate, no considerable part of the drainage is now carried off on the surface.

Southward from the edge of the highland there is a gradual slope to the Gulf, the surface being varied only by low swells of sandy soil. The St. Mark's River cuts across these sands in the southeastern corner of the county, part of its present course being due to underground solution. The Wakulla River, having its origin in the flatwoods, flows across the southern part of the county as a typical pre-erosion stream, but is soon lost underground, to emerge at length in Wakulla Spring, one of the finest large springs in the state. Some of the small lakes are quite deep and constant, while the majority are mere pine barren ponds, partially or entirely dried out at times. Other depressions are swampy tracts known variously as "bays," "galls," and "sloughs."

SOILS.—As throughout the coastal plain, the soils are chiefly types of sandy series, the Soil Survey (14) stating that only about one-half square mile of soil as heavy as loam or clay would be found in the land surface of the county. The hills are covered by soils described as derived from the Lafayette formation, while the valleys and less elevated portions are covered with Columbia sands deposited during a late period of submergence.

Of the Lafayette derivation, the Orangeburg fine sandy loam and the Norfolk fine sandy loam are the most extensive, and represent 2 types found only within the coastal plain of southeastern United States (1). The former is distributed chiefly in 2 belts across the Gulf Coast states and also west of the Mississippi River, constituting the higher lands. The Norfolk fine sandy loam extends from Virginia to northern Florida and west to Texas. In topography it is similar to the Orangeburg, and together these 2 soils are considered the most important for general agriculture, forming the greater part of the so-called "clay hammock lands" (13), characterized by a telling percentage of clay in their subsoils and esteemed for their fertility. The subsoil of the Orangeburg gives the designation of "red hills" so commonly used in descriptions of southern Georgia and of northern Florida, for the freshly cut or eroded subsoil is a bright red sandy clay. The subsoil of the Norfolk is a yellow sandy clay or clay loam.

Of the sedimentary deposits, the Norfolk sand covers the largest area, being the most widely distributed soil of the coastal plain from New Jersey southward (1). It is characterized by low elevation and generally level surface from the immediate shore line inland.

Corresponding, then, with the 2 general topographic divisions there are 2 general soil divisions, that of the more elevated regions having clayey subsoil, while the rest is nearly pure sand.

Vegetation in relation to physiography

UPLANDS

Clay hammock lands

In the study of the upland on these soils, there are few evidences of primeval forests, but it is easy to follow recent reforestation and thus to gain an idea of the succession under present conditions.

The methods of agriculture as long practiced in these regions soon exhausted the soil and led to clearing of fresh tracts. The use of these for a few years and their abandonment and return to forest afford object lessons of all stages of second growth. Also on

some agriculturally less desirable lands it is possible to see a forest recently undisturbed; likewise on some of the old and extensive estates groves have been preserved and illustrate what the forests may have been before the civil war. Clearing and exhaustive cotton growing soon reduce the humus and bring about soil vitiation, which exposure intensifies, resulting in a more xerophytic state.

The exposed Orangeburg soil readily washes, and plants can get a hold on the steep bare slopes with difficulty. Soil lichens and mosses, however, soon form a gray-green coating, especially if partially shaded. Species of *Cladonia* and *Baeomyces* are among these earth lichens.

Fields relapsing from tillage soon grow a mixture of ruderals and native plants. *Cenchrus carolinianus* Walt., *C. tribuloides* L., *Erianthus divaricatus* Hitch., *E. brevibarbis* Michx., *Andropogon virginicus* L., *A. Elliottii* Chapm., *A. scoparius* Michx., *Gerardia purpurea* L., *G. fasciculata* Ell., *G. tenuifolia* Vahl., *Aplopappus divaricatus* Gray, and *Eupatorium capillifolium* Small, with prostrate species of *Rubus* (*R. trivialis* Michx. and *R. cuneifolius* Pursh) are common plants soon covering the old fields, which in a short time are dotted with the seedlings of pioneer pines. Of these pines, *P. echinata* Mill. (short-leaved yellow pine) is by far the most abundant, although other species occur, as *P. Taeda* L. (loblolly or old-field pine), *P. caribaea* Morelet (Cuban or slash pine), and occasionally *P. clausa* Sarg. (sand or spruce pine), and *P. palustris* Mill. (long-leaved yellow pine). *Quercus virginiana* Mill. (live oak) is a broad-leaved evergreen pioneer, and *Diospyros virginiana* L. (persimmon) and *Liquidambar Styraciflua* L. (sweet gum) are deciduous trees soon growing with the dominant pines. *Sassafras variifolium* Ktze. and *Prunus angustifolia* Marsh. are thicket formers. *Pteris aquilina* L. is an abundant fern characteristic of these xerophytic pioneer stages of the old fields.

The pines which spring up in such numbers, if not disturbed, grow rapidly, and on the whole uniformly, and comparatively soon form a forest of trees of similar height and diameter. Where burning or pasturing does not interefere, a dense shrubbery quickly develops, including a variety of seedling trees and shrubs. Of these

developing trees, oaks are most numerous, *Q. falcata* Michx. (Spanish or red oak) and *Q. stellata* Wang (post oak) being the principal pioneers. *Q. virginiana* Mill. persists, being a tree of almost every habitat, from hydro-mesophytic to xerophytic. When forming groves of large, wide spreading trees, draped with *Tillandsia usneoides* L. (Spanish moss) and supporting on the trunks and

FIG. 1.—*Quercus virginiana* on hammock soil

branches a growth of *Polypodium polypodioides* Hitch., this live oak is the typical tree of the mesophytic "hammock," a term used in these regions of the south to designate lands supporting a forest growth of deciduous and broad-leaved evergreen species, correlated with a rich and fertile soil (fig. 1). The evergreen *Ilex opaca* Ait. grows well in the shade of the pines; and *Cornus florida* L. develops under taller trees, sometimes forming an under forest, with some of the trunks 12–18 inches in diameter at 2 ft. from the ground, the widely spreading tops meeting overhead, while above them rise the pines. *Pyrus angustifolia* Ait. (wild

crab) is a small tree characteristic of the pine wood borders and more open parts.

Mingled with the oaks are hickories, *Carya alba* K. Koch being the prevalent species, and a characteristic member of the developing oak forest. The mixed assemblage of small trees and taller shrubs accompanying the oaks and hickories include *Ilex vomitoria* Ait. (an evergreen), several species of *Crataegus* (especially *C. consanguinea* Beadl., *C. robur* Beadl., *C. panda* Beadl.), *Rhus copallina* L., *Callicarpa americana* L., and *Vaccinium arboreum* Marsh. Low shrubs are *Ceanothus americanus* L., *Gaylussacia dumosa* T. and G., *Rosa humilis* Marsh., and *Yucca filamentosa* L.; while common woody vines with persistent or evergreen leaves are a number of species of *Smilax* (*S. pseudochina* L., *S. bona-nox* L., *S. glauca* Walt.), *Gelsemium sempervirens* Ait., and *Lonicera sempervirens* L.

The herbaceous growth in the pine forest, when undergrowth is not disturbed, is not abundant, but in woodland burnt over or cleared, grasses and sedges spring up and often make pasturage. Blooming early in the spring, *Oxalis stricta* L., *O. corniculata* L., *Phlox pilosa* L., *Scutellaria integrifolia* L., *Salvia lyrata* L., *Houstonia purpurea* L., *Specularia perfoliata* A.DC., *Antennaria plantaginifolia* Rich., *Pyrrhopappus carolinianus* DC., and *Chrysogonum virginianum* L. are herbs which indicate xeromesophytic conditions. In more mesophytic places *Houstonia rotundifolia* Michx. and *Mitchella repens* L. may be found in bloom at almost any date, the latter with flowers and fruits at the same time.

There is no vernal flora, nor can a definite flowering season be set, but there is overlapping and irregularity in the prolongation of the blooming season, conditions related to the spring drought and to the extended growing season due to the climatic causes. The most showy season, so far as the herbs are concerned, is after the summer rains, during the late summer and the fall, when *Agrimonia Eupatoria* L., *Schrankia uncinata* Willd., *Lespedeza hirta* Ell., *L. striata* H. and A., *L. violacea* Pers., *Polygala sanguinea* L., *P. verticillata* L., *Helianthemum carolinianum* Michx., *Oenothera biennis* L., *O. linearis* Michx., *Sanicula canadensis* L., *Gentiana villosa* L., *Asclepias verticillata* L., *A. variegata* L., *Trichostema dichotomum*

L., *Salvia azurea* Lam., *Penstemon laevigatus* Ait., *Gerardia flava* L., *G. purpurea* L., *Galium circaezans* Michx., *Eupatorium coelestinum* L., *E. aromaticum* L., *E. album* L., *Liatris scariosa squarrulosa* Gray, *Chrysopsis mariana* Nutt., *Gnaphalium purpureum* L., and *Solidago petiolaris* Ait., make a representative list for the short-leaved pine wood.

The succeeding stage in upland reforestation is that of the oak-hickory forest, in which the characteristic xeromesophytic oaks are dominant. Of these two oaks, *Q. falcata* Michx. seems the more xerophytic, at least it appears on more exposed and drier situations and soils, and slightly in advance of *Q. stellata* Wang., the other pioneer oak. But together, these with *Carya alba* K. Koch dominate the forest which rapidly follows the short-leaved pines.

With the increasing mesophytic conditions (shade, humus, moisture, bacterial, and fungal development), other oaks (*Q. nigra* L., *Q. laurifolia* Michx., and *Q. alba* L.) appear. Other large trees are *Liquidamber Styraciflua* L. and *Nyssa sylvatica* Marsh. The undergrowth is composed of many seedlings of these species and others, with the small trees and shrubs common to the pine forest, as well as more mesophytic species, such as *Ostrya virginiana* K. Koch, *Cercis canadensis* L., *Aralia spinosa* L., and *Viburnum rufidulum* Raf.

The appearance of young *Magnolia grandiflora* L. and of *Fagus grandifolia caroliniana* Fernald and Rehder indicates the approach of the climax and of the transition to the magnolia-beech forest, in which the broad-leaved evergreens and a variety of deciduous trees assemble.

An undisturbed hammock forest of such mesophytic composition, and apparently representative of the climax capable of development on the uplands, contains abundant magnolias of stately proportions (60–80 ft.), equally large beeches, and Florida sugar maples (*A. floridanum* Pax or *A. saccharum floridanum* Sarg.), with intermixed live oaks, white oaks, red oaks (*Q. texana* Buckley), basket oaks (*Q. Michauxii* Nutt.), sweet gums, big bud hickories, and dogwood, with a few old and large short-leaved and Cuban pines as relics. The abundance is approximately in the order named, and all may be hung with Spanish moss. The shrubbery of

this forest includes *Asimina parviflora* Dunal, *Hamamelis virginiana* L., *Evonymus americanus* L., *Stewartia Malachodendron* L., *Aralia spinosa* L., *Symplocos tinctoria* L'Her., *Osmanthus americanus* Br., *Viburnum rufidulum* Raf., and *V. nudum* L., an assemblage of northern and southern species all about equally indicative of similarly mesophytic habitats; while perhaps the most significant thing is the occurrence of young beeches and magnolias, emphasizing the climax conditions.

The undergrowth and herbage are apparently related to the prevalence of the magnolias and other heavily foliaged trees. If these are dominant, the ground is freer of growth and covered with the heavy and slowly decaying leaves. *Mitchella repens* L. is a common floor covering. Root parasites are *Conopholis americana* Wallr. and *Epifagus virginiana* Bart.; while *Monotropa uniflora* L. and *M. Hypopitys* L. occur in abundance in the damp, shaded soil.

To summarize, the forest succession on clay soil of the upland, as shown in phases of reforestation on limited areas but in all stages, we see (1) pines, (2) oak-hickory forest, (3) deciduous broad-leaved evergreen forest. In the pine forest, *P. echinata* Mill. is the dominant species; in the oak-hickory forest, *Q. falcata* Michx. and *Q. stellata* Wang. with *C. alba* K. Koch; in the climax forest, *Magnolia grandiflora* L., *Fagus grandifolia caroliniana* Fernald and Rehder, and a variety of associates.

Sandy soils

In comparison with the uplands of the northern part of the county, those of the south seem like lowlands. Since their geological history has not been the same and the resultant topography is not so distinct, the vegetational aspect also is different. The two regions seem to exemplify two stages in the coastal plain development, the older and the younger. The southern or younger part typifies the marginal portion of the coast, of comparatively recent emergence, and belonging quite entirely to pre-erosion topography, being level, of low elevation, and covered with loose sandy deposits. Almost the whole surface, therefore, may be considered as upland.

The base leveling of this region, supposing no future oscillatory changes of importance, may require a prolonged period, the ero-

sive forces being capable of slight application, but it will not require extensive work as compared with the more elevated regions to the north. The vegetation seems naturally divided, according to small differences in elevation, into the so-called "scrub," the more or less rolling pinelands, and the flatwoods. In a general way these differences also correspond with the soil types, the scrub being associated with Sandhill soil, the pinelands with Norfolk sands, and the flatwoods with Leon sands. For convenience, these 3 general divisions of the pre-erosion uplands will be discussed separately.

SCRUB OAK FOREST.—The oak association seems to mark the sandhill areas, which, owing to the porous sandy subsoil and the lack of organic matter in the soil, would seem to be a decidedly xerophytic habitat. Three small deciduous oaks and a scattering of pines (*P. palustris* Mill. chiefly) make up the tree growth. Of these oaks, *Q. Catesbaei* Michx. seems to be the most xerophytic, as it is sometimes almost alone on the summits of the knolls or ridges. *Q. margaretta* Ashe (suggested as a possible hybrid between *Q. stellata* Wang. and *Q. alba* L. and sometimes, as noted on the more fertile soils, apparently intergrading into well grown *Q. stellata* Wang.) appears in the intermediate positions; while *Q. cinerea* Michx. grows near the bases of slopes. They intermingle in varying proportions over most of the area, growing to about the same height (15–20 ft.), with many scrubby branches, making when thickly planted a scrubby thicket. *Q. geminata* Small, a scrubby live oak, is another species occurring on sandy soil, usually in situations near water or damp places. *Q. virginiana* Mill. and *Diospyros virginiana* L. also grow on the sandhills.

Shrubs are mostly low and with evergreen or persistent foliage, as *Ceratiola ericoides* Michx., *Leiophyllum buxifolium* Ell., *Vaccinium Myrsinites* Lam., *V. stamineum* L., *V. neglectum* Fernald. *Asimina pygmaea* Dunal (with deciduous though coriaceous leaves), *Ceanothus microphyllus* Michx., and *Vaccinium tenellum* Ait. are other low shrubs of the dry sands.

The herbaceous growth, although sparsely distributed, includes a great variety of coastal plain species. Tufts of scattered wire or poverty grass occur on the spaces of bare sand, the most common

being species of *Andropogon* and of *Aristida*. *Pteris aquilina* L. is abundant also. In the spring, *Cassia Chamaecrista* L., *C. nictitans*, L., *Lupinus perennis* L., *L. villosus* Willd., *Tephrosia virginiana* Pers., *T. spicata* T. and G., *Baptisia simplicifolia* Croom, *B. lanceolata* Ell., *Euphorbia corollata* L., *E. Ipecacuanhae* L., *Croton argyranthemus* Michx., *Jatropha stimulosa* Michx., *Amsonia ciliata* Walt., *Scutellaria integrifolia* L., and *Chrysogonum virginianum* L. are early bloomers, the Leguminosae being most abundantly represented. Through the summer and fall a characteristic and representative list includes *Eriogonum tomentosum* Michx., *Eriogonum longifolium* Nutt., *Polygonella gracilis* Meisn., *Petalostemum corymbosum* Michx., *Desmodium rigidum* DC., *Rhynchosia simplicifolia* Wood, *Hypericum Drummondii* Grev. and Hook., *Angelica dentata* Coult. and Rose, *Asclepias tuberosa* L., *Verbena angustifolia* Michx., *V. caroliniana* Michx., *Gerardia fasciculata* Ell., *Elephantopus tomentosus* L., *Eupatorium aromaticum* L., *Trilisa odoratissima* Cass., *T. paniculata* Cass., *Kuhnia eupatorioides* L., *Liatris tenuifolia* Nutt., *L. elegans* Willd., *Chrysopsis graminifolia* Nutt., *C. gossypina* Nutt., *C. mariana* Nutt., *Berlandiera texana* DC., *Solidago odora* Ait., *Aster lateriflorus* Britt., *A. concolor* L., *Silphium Asteriscus* L., *Helianthus radula* T. and G., *H. mollis* Lam., and *Palafoxia integrifolia* T. and G. Many of these are perennials with prostrate or rosette-forming habit, or with pubescent to flocculent coating on leaves and stems, or, as in the case of the species of *Croton*, a scaly coating or with thick and narrow leaves.

PINELANDS.—Passing to the somewhat lower Norfolk sand, which generally surrounds the islands of Sandhill, the transition is marked by the increase in long-leaved pines. The 3 scrub oaks continue as more or less abundant members of the pine forest (fig. 2). *P. palustris* Mill. and *P. caribaea* Morelet are the pines, both of them valuable species for their turpentine and for their timber. *Quercus virginiana* Mill. and the xerophytic oak *Q. marilandica* Moench. occur occasionally, also *Q. pumila* Walt., a low, shrublike species. *Crataegus panda* Beadl., the common hawthorn of the sands in this vicinity and noticeable for its dark, deeply checked bark and irregular crooked-branched habit, and *Bumelia lanuginosa* Pers. are small trees. *Castanea pumila* Mill. is com-

mon in some places, making groves of trees or a low, shrubby growth, spreading by stolons and rapidly covering a considerable area. *Diospyros virginiana* L. is also a tree of these sands, but more frequent as second growth with the short-leaved pines, live oaks, post oaks, Spanish oaks, and sweet gums, as on cleared land which has been cultivated for a time and allowed to revert to forest.

In this reforesting the early stages thus resemble those on the hills, but to these clearings the long-leaved pines with the scrub

FIG. 2.—Long-leaved pine forest on Norfolk sand

oaks may also return. It is on such more fertile spots or where there has been improvement of the soil that the xerophytic scrub oaks, especially *Q. margaretta* Ashe, appear to grow to better size and may mingle for a time with the xeromesophytic oaks, but cannot long compete with the large trees.

The exact relation of these scrub oaks to this type of pine forest is of interest, as they sometimes appear to replace the pines without apparent difference in topography, soil, or drainage. From the fact that these oaks may appear as xerophytic pioneers, and also that they appear in the more xerophytic situations, as on the summits of the ridges of the sandy soil, it may be that they succeed

better than pines on dry, sterile sand, so that when the pines are removed from such lands, the scrub oaks more quickly take possession, while the pines return more slowly and scatteringly. On the other hand, with improved or more mesophytic conditions, the scrub oaks are soon replaced by pines, xeromesophytic oaks, and the succeeding mixed forest.

There seem, therefore, to be two possible phases of succession on the sandy soils. On the more sterile sands, the scrub oaks may be the pioneers before the long-leaved pines; or, if the pines be removed, these oaks may follow, to give place, with improvement of soil and moisture, to xeromesophytic pines and oaks, and then to the oak-hickory forest, leading toward the climax forest sooner or later. But on soil neither excessively drained nor poorly drained, the scrub oaks will accompany the long-leaved pines, yielding, where more mesophytic growth is favored, to the short-leaved pines and their following as outlined. Groves of short-leaved pines are not uncommon within the long-leaved pine association, especially where there may be some admixture of clay, as when the Norfolk sand is in close association with such types of soils as the Orangeburg and Norfolk fine sandy loams.

The growth of shrubs in these long-leaved pine woods is noticeably scanty and the species relatively few. The frequent burning over of these woods and their utilization for turpentine no doubt prevent a natural growth from starting. However, the contrast with the short-leaved pine forest on the hills is very great in this respect, and the xerophytic conditions are correspondingly greater; hence succession or the renewal of the forest is delayed. The shrubs noted commonly in the pinewoods on sandy soils are *Rhus copallina* L., *Ceanothus americanus* L., *Ilex vomitoria* Ait., *Vaccinium arboreum* Marsh., *V. virgatum* Ait., *V. stamineum* L., *V. Myrsinites* Lam., *V. neglectum* Fernald, *Leiophyllum buxifolium* Ell., *Kalmia hirsuta* Walt., and *Gaylussacia dumosa* T. and G., the Ericaceae being the most numerous. The variety of herbs in these pine forests is striking, many of them being those of the "scrub," the families prominently represented being Compositae, Leguminosae, Euphorbiaceae, Scrophulariaceae, Polygalaceae, and Labiatae, chiefly xerophytic species.

FLATWOODS.—From the dry pinewoods to the flatwoods areas the change is indicated, not by the prevailing tree growth, but by the shrubs and herbs. These mark a most decided difference (fig. 3). The long-leaved pines continue to form the forest, apparently succeeding best on these poorly drained sands. This is

FIG. 3.—In foreground saw palmettos and wire grasses and herbs characteristic of flatwoods, giving way in background to long-leaved pine-scrub oak association.

perhaps the explanation of the specific name of *Pinus palustris* Mill., although this particular pine is by no means a typical swamp tree, as for example is *P. serotina* Michx., nor is it as tolerant of inundation even for a time as is its associate *P. caribaea* Morelet.

The shrubby growth of these flatwoods is made up of dwarf species, seldom rising above 3 ft., and chiefly evergreens. Fires

may be one of the chief causes preventing development of undergrowth, but the presence of these low shrubs adjoining bays and ponds, where fires have been able to do small damage to the natural growth, seems to prove the character of the shrubbery. Dwarf oaks are common (*Q. myrtifolia* Willd., *Q. minima* Small, and *Q. nana* Willd.), with persistent, leathery leaves and mostly bearing abundant fruits. *Myrica cerifera pumila* Michx., *M. carolinensis* Mill., *Ilex glabra* Gray, *Hypericum myrtifolium* Lam., *H. galioides* Lam., *H. aspalathoides* Willd., *H. opacum* T. and G., and *Kalmia hirsuta* Walt. are other shrubs with persistent foliage. *Pyrus arbutifolia* L. f., *Rhododendron nudiflorum* Torr., *R. viscosum* Torr., *Lyonia nitida* Fernald, *Andromeda ferruginea* Walt., *Vaccinium stamineum* L., and *V. Myrsinites* Lam. are also shrubs of the damp to wet sands.

The most conspicuous index, however, of subsoil more or less saturated is *Serenoa serrulata* Hook. f. (saw palmetto). As soon as this palmetto appears with the turpentine pines, poor drainage is to be inferred. The herbs also are strikingly characteristic of undrained soil with its lack of aeration and consequently of assimilable nitrogenous substances. The Leguminosae, so abundantly represented on the Sandhill soil and in the long-leaved pinewoods on the dry sands, do not appear. Besides the grasses and Compositae, the families most in evidence here are Eriocaulaceae, Juncaceae, Liliaceae, Orchidaceae, Sarraceniaceae, Droseraceae, Polygalaceae, Melastomaceae, Onagraceae, Gentianaceae, Scrophulariaceae, and Lentibulariaceae. Representatives of these familes are *Eriocaulon decangulare* L., *E. compressum* Lam., *Juncus Elliottii* Chapm., *J. debilis* Gray, *Xerophyllum asphodeloides* Nutt., *Spiranthes praecox* Wats., *Calopogon pulchellus* R. Br., *Sarracenia flava* L., *S. psittacina* Michx., *S. Drummondii* Croom, *S. minor* Walt., *Drosera brevifolia* Pursh, *Polygala lutea* L., *Rhexia mariana* L., *R. glabella* Michx., *R. virginica* L., *R. ciliosa* Michx., *Ludvigia pilosa* Walt., *L. alternifolia* L., *Viola lanceolata* L., *Eryngium virgatum* Lam., *Sabatia Elliottii* Steud., *S. paniculata* Pursh, *Gentiana Porphyrio* G. Gmel., *Gerardia filifolia* Nutt., *Seymeria tenuifolia* Pursh, *Pinguicula lutea* Walt., *P. pumila* Michx., *Utricularia subulata* L., and *U. cornuta* Michx. The species of *Sarracenia*

are often associated with a luxuriant growth of *Lycopodium alopecuroides* L. or *L. carolinianum* L. and with beds of sphagnum and other mosses. *Osmunda cinnamomea* L., *O. regalis* L., *Onoclea sensibilis* L., *Woodwardia areolata* Moore, and *W. virginica* Sm. are typical bog hydromesophytes and abundant ferns of this habitat. A complete analysis of the flora of these low woods probably would include a longer list than for any other habitat in the county, and would be evidence of the edaphic character of this association.

Summarizing the vegetation as described for the uplands on sandy soils, the long-leaved pines are dominant and constitute the most extensive type of forest. Of these two species, *P. palustris* Mill. and *P. caribaea* Morelet, the latter ranges more widely in habitat, occurring from mesophytic to hydrophytic habitats, even enduring inundation. The former is not a typical swamp tree nor does it succeed well in soil subject to inundation for any length of time. On this account, probably, *P. caribaea* Morelet, a dominant species for the southern Florida pinewoods, is reported to be gradually replacing *P. palustris* farther north. On mesophytic soils these pines are displaced by the more mesophytic species, while on the drier soils or excessively drained sands the scrub oaks succeed better and take possession. *P. palustris* belongs typically, therefore, to sandy soils with subsoil well drained to saturated or forming hardpan, soils in which few other trees would flourish. Since such habitats predominate, owing to the present physiographic conditions on the coastal plain, the present long-leaved pine forest may be looked upon as edaphic, the species of pines, within their respective climatic ranges, being pioneers in these comparatively primitive habitats.

PRE-EROSION DEPRESSIONS

Throughout the coastal plain, depressions not resulting from recent erosive processes present a variety of edaphic studies. Many of these low places are filled for all or part of the time with surface water, or they may be sufficiently depressed below the water table to contain a permanent amount of water. Others may be mere swampy or boggy tracts, or during dry seasons prairie-like. The relation of these surface features to the formation of peat,

especially in Florida, has been investigated and reported by HARPER (**6**), whose descriptive classification of habitats and extensive lists of peat-forming plants present a summary of the plant associations of the various sorts of swamps, marshes, bogs, ponds, lakes, and streams.

The water of these pre-erosion depressions, with their (usually) sandy basins, is characteristically dark-colored, appearing blackish when in quantity, being rich in organic matter, and having a more or less acid reaction.

Lakes, ponds, and streams

The vegetation of the ponds and of the slowly moving waters of the sluggish little streams is not decidedly different, differences depending rather on the depth of water and on the amount of movement. In shallow, permanent water the aquatics are arranged in the usual zonation, from those submerged or floating to those rooted in the muck or sand of the bottoms and to the amphibious plants of the margins.

Lists of aquatics for the ponds and lakes include among the submerged and floating forms *Potamogeton* spp., *Ceratophyllum demersum* L., *Myriophyllum heterophyllum* Michx., *Lemna valdiviana* Philippi, *Castalia odorata* Woodv. and Wood (and the variety *C. odorata gigantea* Fernald), *Nymphaea advena* Ait., *Brasenia Schreiberi* Gmel., *Nelumbo lutea* Pers., *Nymphoides aquaticum* Fernald, *Utricularia inflata* Walt., *U. biflora* Lam., and *U. purpurea* Walt.

In marginal zones, *Panicum hemitomum* Schult., *P. condensum* Nash, *Dulichium arundinaceum* Britt., *Eriocaulon decangulare* L., *E. compressum* Lam., *Mayaca Aubleti* Michx., and *Bacopa caroliniana* Robinson usually grow in shallow water; while the common strand plants are *Fuirena squarrosa* Michx., *Hemicarpha micrantha* Britt., *Rhynchospora corniculata* Gray, *Syngonanthus flavidulus* Ruhland, *Drosera brevifolia* Pursh, *Hypericum virginicum* L., *H. gentianoides* BSP., *Hydrocotyle umbellata* L., *Bartonia* spp., *Diodia virginiana* L., *D. tetragona* Walt., *Spermacoce parviflora* Gray, *Houstonia angustifolia* Michx., *Lobelia glandulosa* Walt., and *Pluchea foetida* DC. The cypresses (*Taxodium distichum* Rich. or

T. distichum imbricarium Sarg.) when present are the chief tree pioneers in the ponds, advancing farthest into the deeper water, reaching from the zone of high water, perhaps, to the extreme limit of the occasional low water, into the zone of water lilies and submerged aquatics (fig. 4). *Cephalanthus occidentalis* L. is a close companion of the cypresses and advances into the standing water

FIG. 4.—Cypresses advancing into deeper water

as a shrub pioneer. The hydrophytic species of *Nyssa* (*N. aquatica* L., *N. sylvatica biflora* Sarg., and the less frequent or local *N. Ogechee* Marsh.), germinating and growing in shallow water, may accompany the cypresses or may spread over the shallow ponds to form the so-called "gum swamps" (fig. 5).

Approaching the shores or in the shallow water of the margins, these trees are joined or surrounded by a zone of marginal shrubs and small trees. Among those which commonly grow in this zone are *Salix longipes* Anders., *Magnolia virginiana* L., *Persea pubescens*

Sarg., *Crataegus viridis* L., *C. aestivalis* T. and G., *Cyrilla racemiflora* L., *Cliftonia monophylla* Britt., *Ilex Cassine myrtifolia* Sarg., *Acer rubrum* L., *A. rubrum tridens* Wood, *Hypericum fasciculatum*

FIG. 5.—Gums (*Nyssa* spp.) forming a gum swamp; trees show swollen bases, and a seedling in center of picture has germinated and is growing in the dark water.

Lam., *H. myrtifolium* Lam., *H. microsepalum* Gray, *Lyonia nitida* Fernald, and *Leucothoe racemosa* Gray.

On the edge of moist but not inundated soil, species of *Myrica* may grow, while *Serenoa serrulata* Hook. and *Ilex glabra* Gray mark

the line of high water. Here live oaks, water oaks, sweet gums, and the swamp and pond pines appear, beginning a meadow or swamp, or quickly giving way to the immediate upland climax (fig. 6). *Smilax Walteri* Pursh is the liana significant of inundated soil, while *S. laurifolia* L. and *S. lanceolata* L. are marginal lianas on moist soil.

FIG. 6.—Lake margin, showing cypresses in water, shrub zone within range of high water, and live oaks on rising ground.

Ponds which dry out during the season are often encircled by hawthorns, *C. viridis* L. being a common marginal species, and *C. aestivalis* T. and G. may fill a shallow or transient pond and convert it into a "mayhaw pond." On areas of clayey soils, willows, maples, sweet gums, and button bushes are the commoner marginal trees and shrubs; while the chief variations in ponds on sandy soils are due to the presence of cypresses or of gums as the tree pioneers,

the composition of the shrubbery about the margin, and in succeeding climax.

Pre-erosion streams, being slow and shallow, do not differ much from the ponds as described. The aquatics in moving water are not so numerous, but the shore growth is more varied, and may grade, with the drainage, into bordering strips of meadow on lowland hammock by which the streamways are conspicuously marked from the adjoining pine forests.

Waters flowing from limestone springs and which are clear and more calcareous have a somewhat different vegetation from that of the acid, brown waters of the other streams. *Liquidambar Styraciflua* L. is a tree of the sometimes inundated margins, and *Ulmus americana* L., *Fraxinus caroliniana* Mill., *F. profunda* Bush, *Quercus nigra* L., *Salix longipes* Britton, *Acer rubrum* L., *Ilex Cassine* L., *Cornus stricta* Lam., and *Cephalanthus occidentalis* L. are common. Canes (*Arundinaria tecta* Muhl.), reeds (*Phragmites communis* Trin.), and saw grass (*Cladium jamaicense* Crantz), with bulrushes (*Scirpus* spp.) are marginal marsh plants.

Swamps and meadows

FLOWING WATERS.—Swampy borders of varying width or overflow strips of meadow are the almost invariable accompaniment of pre-erosion branches, creeks, and rivers, the width of the overflow area depending upon the topography and upon the consequent drainage basin, and upon the volume of the stream. By the accumulation of humus and as improved drainage is secured, these meadow areas in many cases tend to extend outward or upward and often come to occupy wider spaces than would be explained solely by the fluctuations of the stream. From the adjoining vegetation they are marked off by species ranging from hydrophytic to extremely mesophytic. The swampy character extends as far as the soil continues saturated, and in this zone there occur trees of the pond margins, such as cypresses, gums, willows, birches, ashes, water hickory, and water elm (*Planera aquatica* J. F. Gmel.).

On slightly rising ground, but still within range of the high water, there occur pines (*P. caribaea* Morelet, *P. serotina* Michx., *P. palustris* Mill., and *P. glabra* Walt.), with a variety of oaks, such

as *Q. nigra* L., *Q. laurifolia* Michx., *Q. Michauxii* Nutt., also *Carpinus caroliniana* Walt. and *Liquidambar Styraciflua* L. (fig. 7).

Many shrubs and small trees belong to these swampy margins, making a dense growth to the water's edge, with intermingling

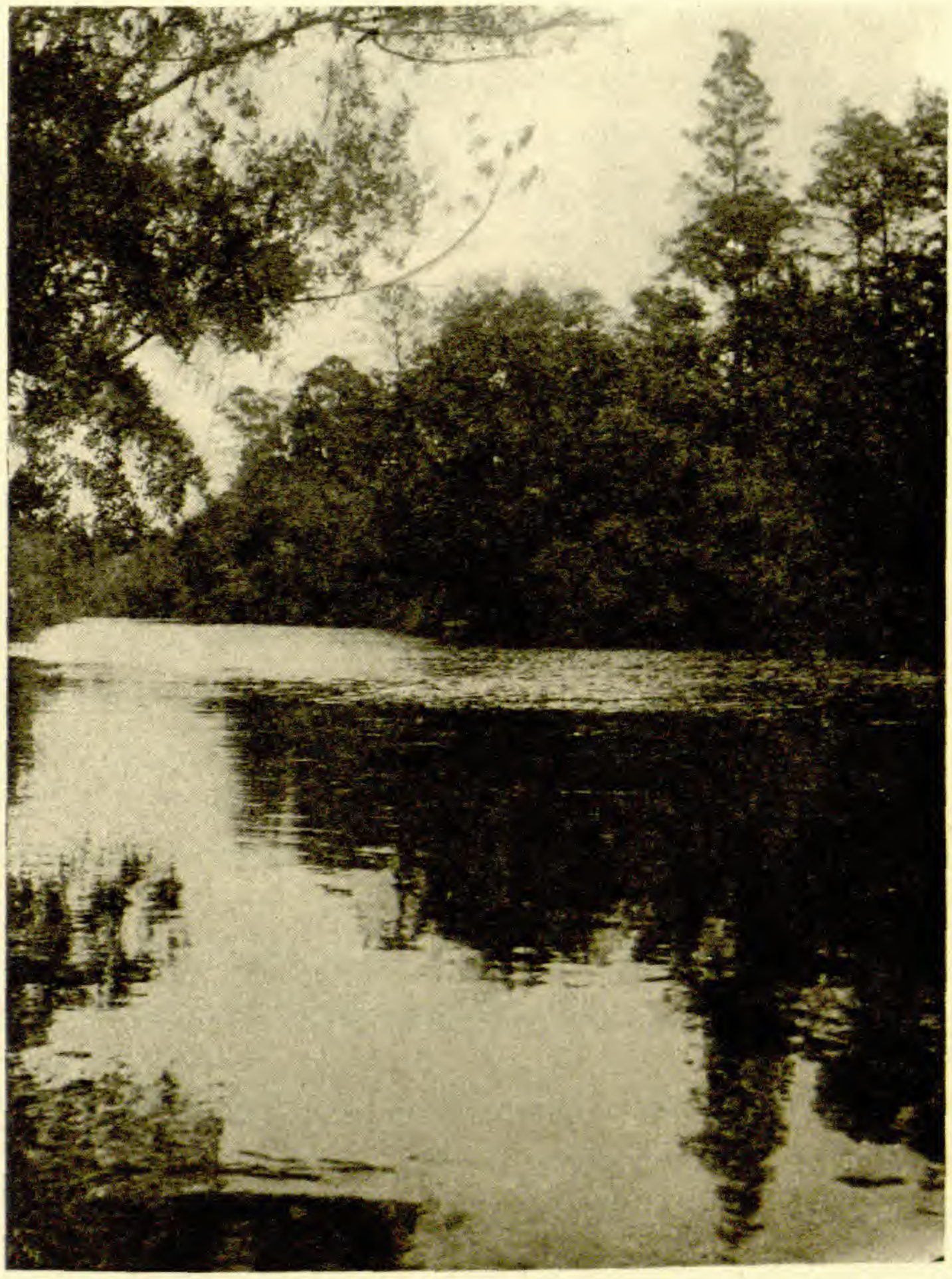

FIG. 7.—Exterior view of Ocklocknee River meadow, showing dense deciduous growth at swampy edges; pines coming in on higher ground.

myricas, bays, and mixed shrubbery, among which are *Itea virginica* L., *Rhus Vernix* L., *Cyrilla racemiflora* L., *C. parvifolia* Raf., *Sebastiana ligustrina* Mill., *Ilex Cassine* L., *I. decidua* Walt., *Cornus stricta* Lam., *Clethra alnifolia* L., *Rhododendron* spp., *Andromeda ferruginea* Walt., *Cephalanthus occidentalis* L., *Pinckneya pubens*

Michx.; with also a variety of lianas such as *Smilax Walteri* Pursh, *S. laurifolia* L., *Berchemia scandens* Trel., *Trachelospermum difforme* Gray, and *Aster carolinianus* Walt.

Beyond reach of frequent inundations, in soil enriched by accumulation of humus, the most mesophytic stage is reached, and the meadow grades into the lowland or river hammock, where a mixed forest of many species develops. *Fagus grandifolia caroliniana* Fernald and Rehder and *Magnolia grandiflora* L. may appear here, with *Celtis mississippiensis* Bosc., *Liriodendron Tulipifera* L., *Halesia carolina* L., *H. diptera* Ell., *Chionanthus virginica* L., and others, forming rich forests of varying composition along the streams. In these forests is an assemblage of mesophytic shrubs, such as *Alnus rugosa* Spreng., *Hamamelis virginiana* L., *Aesculus Pavia* L., *Styrax* spp., and *Viburnum* spp., with lianas and climbing shrubs, such as *Decumaria barbara* L., *Wistaria frutescens* Poir., *Sageretia Michauxii* Brong., *Rhus Toxicodendron* L., *Psedera quinquefolia* Greene, *Cissus Ampelopsis* Pers., *Cissus arborea* Des Moulins, *Vitis rotundifolia* Michx., *V. aestivalis* Michx., *Bignonia capreolata* L., *Tecoma radicans* Juss., and the mesophytic species of *Smilax*. Ferns of the swampy or wetter soils are *Osmunda* spp., *Onoclea sensibilis* L., and *Woodwardia* spp. Of more mesophytic habit are *Aspidium Thelypteris* Sw., *A. patens* Sw., and *Asplenium Filix-femina* Bernh. On the water oaks, black gums, and various other trees *Phoradendron flavescens* Nutt. is abundant, and also *Tillandsia usneoides* L., the ever common epiphyte.

It is to be noted that the character of a well developed stream or river hammock of the region is quite the same wherever occurring and within the boundaries of whatever soils. The telescoping of swamp and hammock complicates the successional phases and is usually extreme, since even slight differences in elevation or drainage are sufficient to modify the vegetation extensively.

QUIET WATERS.—The so-called "bays" are examples of shallow undrained swamps supporting a more or less dense growth of shrubs or small trees. *Magnolia virginiana* L., *Persea pubescens* Sarg., and *P. Borbonia* Spreng. are the real "bays," but the list of plants for these boggy ponds includes a variety of other species, as cyrillas,

grapes, hollies, hypericums, hawthorns, and ericads, and such trees as cypresses, gums, and slash and swamp pines.

There is little suggestion of any definite succession in the composition of the bay or similar swamp. However, *Magnolia virginiana* L., *Persea pubescens* Sarg., and *Ilex Cassine myrtifolia* Sarg. seem to advance into the more hydrophytic portions and appear near the center of the swamp surrounded or followed by the grapes, ericads, and myricas; while *Ilex glabra* Gray, *I. lucida* T. and G., and *Serenoa serrulata* Hook., with *Hypericum* spp. grow beyond the standing water. Frequently *Ilex Cassine myrtifolia* Sarg. is so abundant as to make a fairly impenetrable thicket.

FIG. 8.—Cypresses advancing into waters of an undrained pond, gradually forming a cypress swamp.

An undrained pond may develop into a cypress swamp, the cypresses growing as closely as their swollen bases and groups of projecting knees permit (fig. 8). Around the margin of such a swamp there may be a mingling of oaks, gums, and pines, but more frequently there is a sharp transition to the forest of the upland adjacent, and the edge of the swamp is abruptly marked by the ranks of flat-topped cypresses. When the gums mingle with the cypresses or are the most abundant or only trees, a gum swamp develops, these trees also having swollen or bulging bases. *Tillandsia usneoides* L. gives a characteristic touch to their appearance, especially in winter when the trees are leafless.

The herbs of such swamps are mainly those of pond margins and of the flatwoods, as *Panicum hemitomum* Schutts, *P. condensum* Nash, *Aristida spiciformis* Ell., *Eleocharis* spp., *Fuirena squarrosa* Michx., *Rhynchospora* spp., *Eriocaulon* spp., *Mayaca Aubleti* Michx., *Burmannia biflora* L., *Polygala cymosa* Walt., *P. ramosa* Ell., *Hypericum petiolatum* Walt., *H. virginicum* L., *Ludvigia alternifolia* L., *L. glandulosa* Walt., *Gerardia linifolia* Nutt., *Lobelia amoena* Michx., and *L. glandulosa* Walt.

In certain low places, as at the bases of slopes, water may ooze through the sandy soil to collect on the surface in little pools or pockets, with intervening hammocks of dark muck, or may slowly drain away, sometimes forming the source of a small stream. In this way small branches or considerable tributaries may originate, and by their union form creeks or small rivers. In other cases sloughs and ponds may be formed, such boggy spots often being designated "galls." In vegetation they resemble the bays, often surrounded by or advancing to a hammock stage by the accumulation of humus and the gradual building up of the soil.

Bayheads scarcely differ from these, also being the sources of small branches. In these, typical trees are *Magnolia virginiana* L. and *Persea pubescens* Sarg., with a bordering shrubbery of more or less mesophytic character.

Sloughs are low, flat passageways between swamps or bodies of water. In these passageways the water may be still or but slowly moving, while during the dry season they may be entirely dried out. Cypresses, sour gums, swamp pines, and swamp maples are common slough trees, with live oaks, water oaks, holly, and sweet gums on the edges. Swamp shrubs, including a variety of the ericads, cyrillas, gallberries, hypericums, with the saw palmetto, out of reach of the standing water, are numerous.

Prairies

Prairies are comparable to swamps in being depressions below the general surface and lacking surface drainage. They may be flooded during the rainy seasons and dry at other times, and their vegetation consists typically of herbaceous associations, especially the grasses. No extensive natural prairies exist in connection with the pre-erosion topography under description here, although many of the small ponds and lakes may temporarily become prairie-like, their beds during the dry seasons being overgrown with grasses and other herbage, in which introduced plants, as weeds, make a miscellaneous assemblage.

EROSION TOPOGRAPHY

The northern part of Leon County, having been exposed probably as long as any other section of Florida or of the immediate Gulf

Coast, and being in parts above the general level, should afford illustrations of erosion topography. The conditions are unusual, however, since the presence of limestone so near to the surface has brought about the development of extensive subterranean as well as surface erosion, and the topographic features are thus modified in such ways as to complicate ecological analysis. In considering the region as a whole, it appears that the surface features may be largely due to the underground erosion. The lakes, sinkholes, and enclosed valleys seem evidence of this.

Surface erosion

BRANCHES AND CREEKS.—The trough of almost every valley has a waterway marked by an aggregation of trees and shrubs. The stream is usually an insignificant affair so far as the amount of movement of the water is concerned, and consequently the erosive work accomplished by such a stream is slight. Its course may be found to lead, by a slight rise, to a bayhead where the water is seeping from the base of a slope; or it may issue from a spring whence the water may flow across the ground, spreading out into a miry tract; or, as in the clayey soil of the hills, a definite channel will be cut or gullied down the slope; or the spring will eat back into the hill as a narrow ravine and a small clay canyon thus be cut along the steeper part of the grade. The erosion work lessens as the level is reached, the washing and gullying of the steep banks grade and widen them, and in this way the little streams are gradually bringing the soils of the hills to the valleys.

RAVINES.—In the shady and moist ravines there grow numerous liverworts and mosses, with soil lichens in the upper zone and with ferns along the edges and in the niches. Of the ferns, *Polypodium polypdioides* Hitch. grows on the moist clay banks, also *Asplenium platyneuron* Oakes, *A. resiliens* Kunze., *Polystichum acrostichoides* Schott., *Aspidium Thelypteris* Sw., and *A. patens* Sw. As the stream broadens and shallows and the banks are lowered, reeds, canes, and marsh grasses border the edges, while trees and shrubs develop to form a meadow hammock.

RIVERS.—The Ocklocknee River is an example of an extended stream, rising in Georgia and cutting its way across the latest

deposits of the coast. For most of its course along the western border of Leon County its banks are edged by bluffs of varying elevation (50–100 ft. above sea-level). These are apparently the ancient banks, eroded during a previous period. At places these bluffs approach close to the present low banks, so that the valley varies in width. The erosion work of the river is of small importance, and in its bordering meadow and overflow land it resembles a pre-erosion stream. The low bluffs are generally well wooded and the undergrwoth is often denser and of a more mesophytic type than is that of the upland forest.

Examples of small erosion creeks are to be seen in the southwestern edge of the county, where a series of drainage streams flow from the bays on the Leon sand across the strip of Norfolk sand to empty into the Ocklocknee River, and have cut ravine-like valleys in the sands, in which the most mesophytic trees, including *Magnolia grandiflora* L., *Fagus grandifolia caroliniana* Fernald and Rehder, *Liriodendron Tulipifera* L., *Carya alba* K. Koch, *Acer* spp., *Carpinus caroliniana* Walt., and *Prunus caroliniana* Mill., grow with a rich undergrowth. Entering one of these eroded valleys from the upland of monotonous pine forests, one witnesses the extremes which the region can support.

LAKES.—The surface erosion along the shores of the larger lakes is of small importance, as the shores are usually sloping and the wave action is slight. The trees of the uplands may extend to the water's edge or there may be tracts of fine hammock forest. Other lakes resemble slow fluctuating streams, with cypresses in the shallow water.

Subterranean erosion

The underground solution and the resultant caving in or settling of the ground surface continue to play a part in modifying the topography.

SINKHOLES.—The formation of sinkholes may take place suddenly and expose the limestone, forming depressions, usually circular, varying in size and depth. In case there is no opening through which the water may reach an underground channel, the rainfall and the surface waters may accumulate to form a pond.

In such sinks the water rises and falls with the amount of precipitation and surface drainage. Other sinks are dry, having one or more openings in connection with the underground drainage system.

The cliffs and ledges of limestone, if exposed, soon wear off, soil collects, and gradually the sides become overgrown. The soil collecting in the bottom supports growth of trees and shrubs usually more mesophytic than those of the immediate upland, the shade and the moisture favoring the growth of such seedlings. Liverworts and mosses may be found on the damp soil at the base of a sink and often in the crevices of the sides. *Adiantum capillus-veneris* L. belongs to such situations, as also *Polypodium polypodioides* Hitchc., *Asplenium platyneuron* Oakes, and *Polystichum acrostichoides* Schott. *Panicum dichotomum* L. and *Opelismenus setarius* L. are grasses in the shady ravine-like situations, as pioneers on the sides.

If the sink contains water, pond plants will enter and cypresses or gums may grow. If the base is covered or finally filled with soil, water oaks, live oaks, sweet gums, dogwood, and holly are common sinkhole plants. White oaks, red maples, black gums, and sweet gums are other trees occurring about the sinkhole margins.

SPRINGS.—Many springs are the results of channels in the limestones, occurring where the streams emerge from underground. In the clear, cool, calcareous water of such springs there is not much plant growth, although around the margins are grasses, sedges, sagittarias, and reeds.

LAKES AND PONDS.—The relation of the large lakes to sinkhole formation has been mentioned, sinks or openings occurring in their basins through which the more or less complete drainage of the waters of the lake may take place suddenly or gradually. When these sinks become closed by obstructions or stoppage, the water will again fill the basins (12). By the drainage of such lakes, sometimes large areas of the basins may become prairies, which unless again flooded or otherwise used, may gradually approach the forest stage.

STREAMS.—Underground channels sometimes become surface streams by the caving in of the roof of the cavern. Frequently a section may be left to form a natural bridge. Such is the case

with the St. Mark's River, which emerges from a subterranean course as a series of sinklike ponds, and finally as a surface stream flowing across the sands to the Gulf. At the natural bridge the banks are definite and rise directly from the water level. A rich hammock borders the banks, the trees and shrubs growing close to the water's edge.

Summary

This local study of the Gulf section of the coastal plain may serve to suggest several points in the successional history of the plant associations of the region. Extremes of xerophytic, hydrophytic, and mesophytic societies are to be found. The most xerophytic association is represented by the long-leaved pine-scrub oak forest of sterile, sandy soil. The most mesophytic association is that of the hammocks, occurring on the upland as the climax and also as a temporary climax in the river valleys, being composed of a large variety of species, deciduous and evergreen, of which *Fagus grandifolia caroliniana* Fernald and Rehder is perhaps the most significant deciduous tree, and *Magnolia grandiflora* L. the principal tree among the broad-leaved evergreens. Between these two extremes are the gradations from pioneer pines through the pine-oak and oak-hickory stages. Telescoping and rapid growth in the later stages are characteristic and confusing.

The long-leaved pine-saw palmetto association on the flat, poorly drained sands presents a large edaphic problem. With improvement in drainage, aeration of the soil, and consequent promotion of soil organisms and their work, the change to a mixed forest can take place, as is seen along the streams as well as in local hammocks which have evidently been built up gradually. Drainage of the subsoil brings scrub oaks in place of the saw palmetto into association with the long-leaved pines, and the succession outlined from dry pine woods to the climax forest will naturally follow. With slight depression of the surface a change to a moorlike swamp results.

The various types of swamps, characterized by the prevailing species, as the cypress swamps, gum bogs, pine swamps, and bays, and their transitions to the surrounding forest, furnish opportunities for intensive studies.

Comparative observations

In considering the upland forests in their successional stages, data concerning the evaporation, soil moisture, and certain climatic factors, and their relation to the associations discussed, have been collected. Evaporation records were secured by the use of Livingston atmometers, following the investigations of FULLER (4) and others. Rain-correcting valves were used, the cups were kept standardized to the same unit, and computations made accordingly.

Stations were established in a mesophytic forest of the climax type, in a Spanish oak-post oak-hickory forest, in a short-leaved pine forest, in a beech opening in the short-leaved pine forest, in the dry pine woods (long-leaved pines), in the scrub oaks association, and in the flatwoods. Meadow stations were also placed, but their records are not complete. The stations were located in as nearly typical situations as possible, the atmometers in each case being placed at the surface of the ground. All records demonstrate a constantly high evaporation as one of the climatic results, and all show a general relation between the evaporation and precipitation periods, there being two marked maxima for all the stations, the major one being between the winter and the summer rainfall, corresponding to the April and early May dry season, the other evident in late September and early October, after the summer rains have ceased. All records show a sudden rise in spring from the lowest point in December or January to the April or May maximum (June for the long-leaved pine forest). This corresponds generally to the period of the vernation of the deciduous species and to the renewal of foliage by many of the evergreens. Winter records for the highland stations were uninterrupted by frost through two consecutive winters, but each lowland station suffered once or twice each winter.

Of the upland stations, the average daily evaporation is lowest for the mesophytic climax (magnolia-beech) forest, being 8.5 cc. daily, estimated for a period during which an unbroken record was obtained from December 24 to May 1; this is the most critical period, including from the January minimum to the April maximum. For the same period of time the Spanish oak-post oak forest gave

a record of 9.9 cc.; the beechwood 11.21 cc.; the short-leaved pines 11.67 cc.; the long-leaved pines on Norfolk sand 12.28 cc.; and the scrub oaks 15.3 cc. daily, the reverse of this order being essentially that in which the successional changes as observed occur, from the xerophytic pines and oaks through the xeromesophytic pines and oaks to the climax forest (fig. 9). The beech wood, it must be noted, was subject to pasturing and gave evidence of

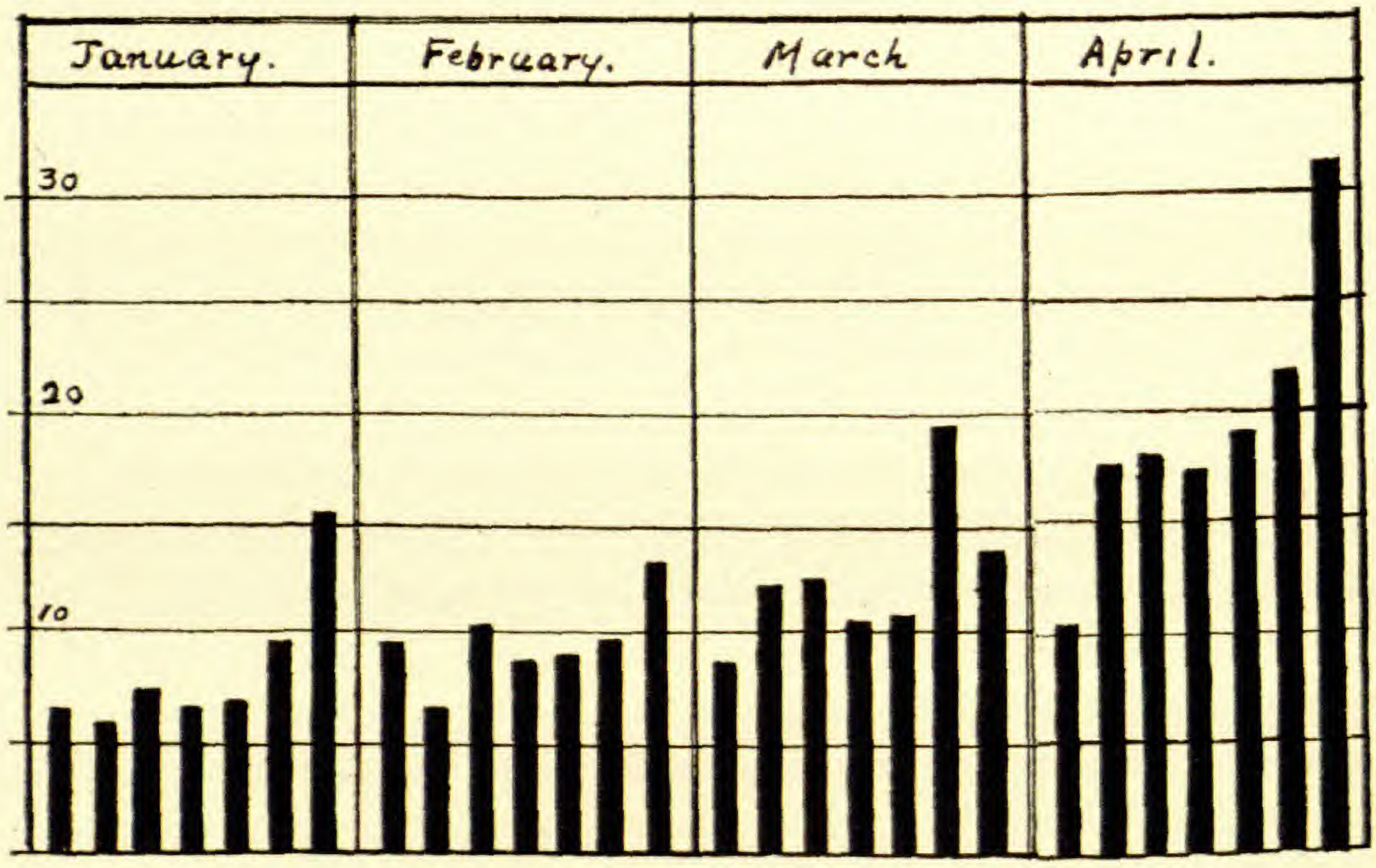

FIG. 9.—Diagram showing comparison in evaporation rate from January to May in (1) mesophytic climax forest; (2) flatwoods; (3) Spanish oak-post oak-hickory forest; (4) short-leaved pine forest; (5) pastured beech forest; (6) scrub oak forest; (7) long-leaved pine forest.

recent burning (probably to promote pasturage), being quite free of undergrowth. Cattle and hogs grazed through this forest and *Erechtites hieracifolia* Raf. appeared among the herbs.

On the basis, however, of the average rate of evaporation estimated for the year, the order is changed. The Spanish oak-post oak association has an evaporation rate very close to that of the short-leaved pines, being 14.00 cc. daily for the former and 14.22 cc. for the latter. Both of these stations were observed without a break from September 1912 to May 17, 1914, and their averages taken accordingly. The two stations are alike in that each has

a dense undergrowth, that of the pine woods being if anything denser than that of the oak forest; indeed, the pine forest is well on its way toward the oak stage. However, there is a difference when the winter and the summer averages are considered. Estimated for the period from June to November, the season during which full foliage of deciduous trees is a large factor, the daily rate for the oaks is 12.49 cc. and for the pines 13.8 cc. In winter (November to June), from the time when the oaks are leafless until they attain full summer foliage, the rates are 15.69 cc. daily for the oaks and 13.70 cc. for the pines. In the beech woods during these seasons, the rates are 13.4 cc. daily for the summer and 17.8 cc. daily for winter, thus showing an approximation to the pines in summer and greater evaporation than either pines or oaks in winter.

The scrub oaks and long-leaved pines behave differently. These oaks average 13.95 cc. for summer (comparable to the short-leaved pines and the open beech woods) and 14.1 cc. for winter; while the long-leaved pines on dry sand show the highest rates, 18.25 cc. for summer and 19.2 cc. daily for winter. The scrub oaks and the long-leaved pines have respectively 15.52 cc. and 17.9 cc. daily average for a period of 18 consecutive months. The scrub oak forest shows less actual variation than any other except the flatwoods, this probably being related to the stunted character of the oaks, their close thicket-like growth, and their habit of retaining the dead leaves most of the winter or until fresh growth starts. In striking contrast, the long-leaved pine forest shows the most extreme variations in range of evaporation of any other station.

The contrast between the two long-leaved pine associations is the greatest of any, as the flatwoods station shows a uniformly lower rate throughout the year than any other and averages 12.99 cc. daily for the 18 months, thus taking the place next in order to the mesophytic climax forest. The summer evaporation for the flatwoods averages 13.24 cc. daily, comparable to that of the scrub oaks and the pastured beech wood. The average winter rate is 11.17 cc. daily, being the lowest, and this is the case although this forest is even more open than any of the others, the shrubs being low and the forest subject to turpentining, burning, and pasturing.

Some experiments to determine the soil moisture relations (by use of the wilting coefficient) were obtained and determined according to the method of BRIGGS and SHANTZ (2). Although incomplete, these tend to confirm the statement that the flatwoods have

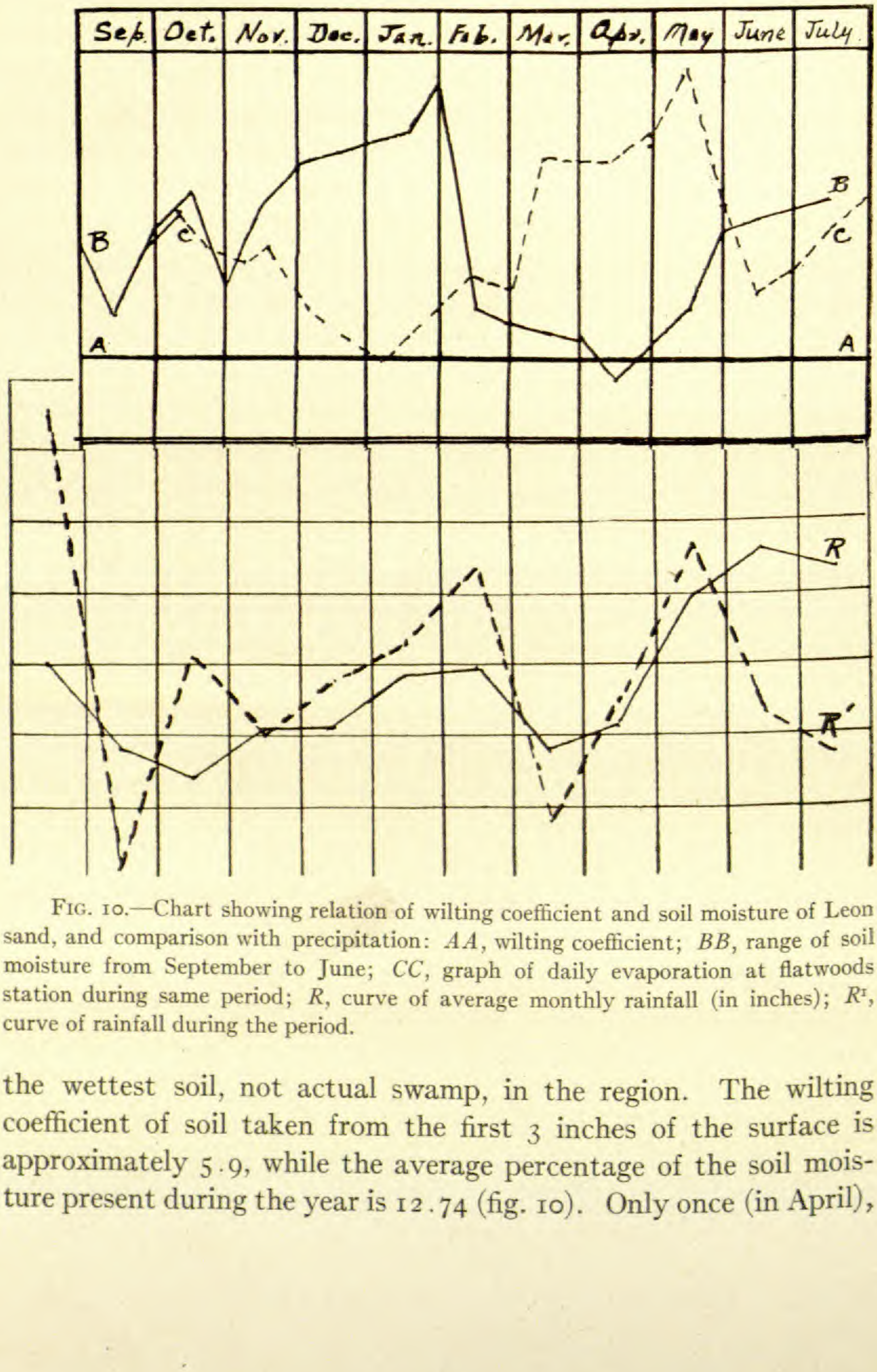

FIG. 10.—Chart showing relation of wilting coefficient and soil moisture of Leon sand, and comparison with precipitation: *AA*, wilting coefficient; *BB*, range of soil moisture from September to June; *CC*, graph of daily evaporation at flatwoods station during same period; *R*, curve of average monthly rainfall (in inches); R^1, curve of rainfall during the period.

the wettest soil, not actual swamp, in the region. The wilting coefficient of soil taken from the first 3 inches of the surface is approximately 5.9, while the average percentage of the soil moisture present during the year is 12.74 (fig. 10). Only once (in April),

at the end of the spring drought, does the percentage of moisture in the first 3 inches of soil fall lower than the wilting coefficient, and then but slightly. The maximum amount is reached in late January and early February, while a summer maximum is reached in July, coinciding with the two periods of rainfall. The curve of the range of the soil moisture agrees quite closely with that of the average daily evaporation, which in this association therefore has a direct relation to the soil moisture and the consequent humidity of the atmosphere at the surface of the soil where evaporation is actively occurring.

Considering the edaphic character of the flatwoods in explanation of its position as determined by the evaporation averages, the order of succession for upland forests as observed seems to have a definite relation to the obtained rate of evaporation. This tends to confirm the observation that in the coastal region studied the present pines are pioneers making a temporary forest, which, owing to present geological, topographical, and soil conditions, may make but slow progress toward the ultimate climax, at least over large areas. When once started, however, the climate favors a rapid mesophytic advance.

To the lectures and teaching of Dr. H. C. COWLES I am indebted for my interest in this subject and for my point of view; to Dr. G. D. FULLER for instructions concerning field work with evaporimeters; and to Dr. J. M. GREENMAN for aid in identification of various plants. Also I acknowledge the assistance of Professor JEROME MCNEILL of Tallahassee, Florida, in the field work and in securing the evaporation and soil moisture readings over an extended time.

RICHMOND, IND.

LITERATURE CITED

1. BONSTEEL, J. A., Soils of eastern United States and their use. Circ. Bur. Soils, U.S. Dept. Agric.
2. BRIGGS, L. J., and SHANTZ, H. L., The wilting coefficient and its indirect determination. U.S. Dept. Agric., Bur. Plant Ind. Bull. no. 230. 1912.
3. Climatological Service Reports, District no. 2, South Atlantic and east Gulf states. U.S. Dept. Weather Bur. Service, 1912–1914.

4. FULLER, G. D., Evaporation and plant succession. BOT. GAZ. **52**:193–208. 1911.
5. HARPER, R. M., A phytogeographical sketch of the Altamaha grit region of the coastal plain of Georgia. Ann. N.Y. Acad. Sci. **17**: 1907.
6. ———, Preliminary report on the peat deposits of Florida. 3d Ann. Report, Fla. State Geol. Surv. 1909–1910.
7. ———, Geography and vegetation of northern Florida. 6th Ann. Report, Fla. State Geol. Surv. pp. 163–431. 1914.
8. LLOYD, TRACY, Insular flora of Mississippi and Louisiana. Bull. Torr. Bot. Club **28**:61–101. *pls. 8–11*. 1901.
9. MATSON, G. G., and CLAPP, F. G., A preliminary report on the geology of Florida. 2d Ann. Report, Fla. State Geol. Sur. 1908–1909.
10. SARGENT, C. S., Forests of the United States. 10th Census. Vol. 9. 1884.
11. SCHIMPER, A. F. W., Plant geography. 1903.
12. SELLARDS, E. H., Some Florida lakes and lake basins. 3d Ann. Report, Fla. State Geol. Surv. 1909–1910.
13. ———, Classification of the soils of Florida. 12th Ann. Report, Comm. Agric. Florida. 1913.
14. WILDER, H. J., DRAKE, J. A., JONES, G. B., and GEIB, W. B., Soil survey of Leon County, Florida. Field Operations, Bur. Soils. 1906.
15. WILLIAMS, J. L., A view of west Florida. 1827.

PERMEABILITY OF CERTAIN PLANT MEMBRANES TO WATER

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 230

F. E. DENNY

(WITH TWO FIGURES)

Introduction

In the exchange of material between the plant and its environment, 3 groups of substances may be considered important, namely, water, gases, and salts. These enter the plant, pass from one portion to another, and some of this material finally passes out into the environment again. In this process a great many membranes must be penetrated. The permeability of these membranes, therefore, is a factor in this material exchange, determining in a measure what substances may enter or leave the plant and at what rate this entrance or exit can take place. For this reason measurements of the permeability of plant membranes become desirable. While much work has been done toward this end from a qualitative standpoint, and while many indirect measurements have been made, direct quantitative measurements in which the results could be referred to known areas of membranes, under a standard set of conditions, have been lacking.

This paper deals with an attempt to get quantitative data on the permeability of certain plant membranes to water; to determine what laws, if any, hold for the rate of penetration of water as related (1) to temperature, (2) to direction of flow through membranes, (3) to concentration of the bathing solutions, and (4) to species of plant under consideration.

Membranes

Non-living membranes such as seed coats and the outer scale of the onion bulb were used, as they were suitable for use with the apparatus employed. The importance of non-living membranes

must not be underestimated. That they perform great physiological functions is coming to be recognized more and more as our knowledge of them increases.

The cell wall may be thought of as a non-living membrane, and its functional importance is emphasized by the work of HANSTEEN-CRANNER (16), in which it is indicated that the antagonism of $Ca^{\cdot\cdot}$ for $Mg^{\cdot\cdot}$ in root toxicity, the action of $Ca^{\cdot\cdot}$ in increasing transpiration and decreasing absorption, and the action of $K^{\cdot}$ in decreasing transpiration and increasing absorption, is due fundamentally to the effect of these ions upon the cell wall. The importance ascribed by WÄCHTER (30) to the cuticle and cork of the outer layers of the beet in preventing the loss of sugar also may be pointed out. Other investigators (4, 10) have shown that the non-living coat plays a dominant rôle in seeds, the coat character being an important factor in determining the respiration, water intake, entrance of toxic materials, delay in germination, longevity, protection from leaching of stored materials, and from mechanical injury, etc.

A study of the permeability of such membranes is desirable in itself, and it was hoped that results so obtained would throw light upon the problem of the permeability of plant membranes in general. In this connection we quote PFEFFER (24): "the physiological process itself will first have to deal with the experimental study of lifeless material, studies which may perhaps in their turn make clear processes taking place in the organism."

A non-living semipermeable plant membrane was discovered in 1907 by BROWN (5) in the barley grain, and later BROWN and WORLEY (6) measured its permeability to water. SCHROEDER (27) reported a similar membrane in the wheat grain. GOLA (13) found such membranes in the seeds of a great many different species. SCHROEDER and GOLA did not measure the permeability of the membranes to water. SHULL (28) found that the seed coat of *Xanthium* is semipermeable to certain substances, and pointed out the distinct advantage this membrane had for experimental purposes, in that it could be removed from the seed, and its permeability characters studied directly, without other structures becoming factors in the experiment. He constructed an osmometer

in which a portion of the seed coat was used as the membrane, and made preliminary measurements on the rate of penetration of water. The problem of getting quantitative measurements of the permeability of various plant membranes was then undertaken by the writer with the results here reported.

The rate of penetration of water through membranes was measured with an osmometer of the design shown in figs. 1 and 2. *A*, *B*, and *C* are hard rubber discs, 3.5 cm. in diameter and 2 mm. in thickness. Brass discs also were used, but are not suitable for

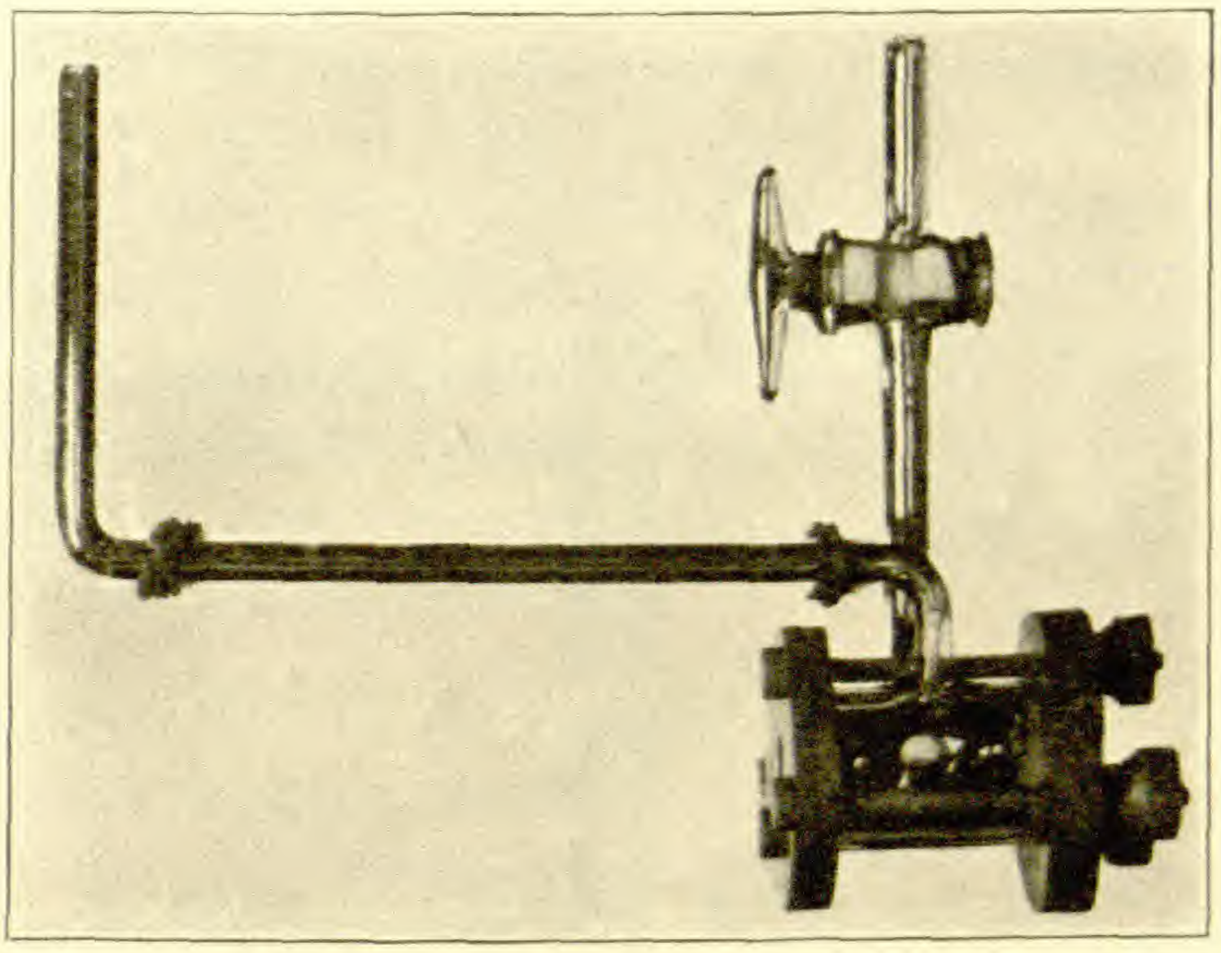

FIG. 1.—Photograph of osmometer: explanation in text

use with salt solutions. In the centers of *A* and *B* at *K* is a hole of known diameter. Between *A* and *B* and over this hole the membrane to be studied is placed; thus the area of the membrane used can be calculated. *D* is a hard glass cylinder with ground edges fitting snugly against the hard rubber discs. Soft rubber gaskets are interposed between the glass cylinder and the discs at *E*, and the apparatus made tight by screwing up the bolts at *H*. F is the tube for admitting water into the internal chamber. The latter is filled with distilled water until water appears in the horizontal capillary tube. The position of the meniscus in *G* may be set at any desired spot by means of the stopcock in *F*. *G* is a capillary tube with about 10 cm. horizontal length and with a capillary bore of approximately 1 mm. Scale divisions on *G* were

calibrated by weighing with mercury. One scale division on G = 0.000337 gm. of water at 25° C. The whole apparatus is then immersed in a vessel containing a solution of cane sugar or sodium chloride and the vessel placed in a water bath regulated to constant temperature. The osmotic force of the bathing solution pulls water through the membrane from the internal chamber, and this causes the meniscus in the capillary tube to recede. By successive

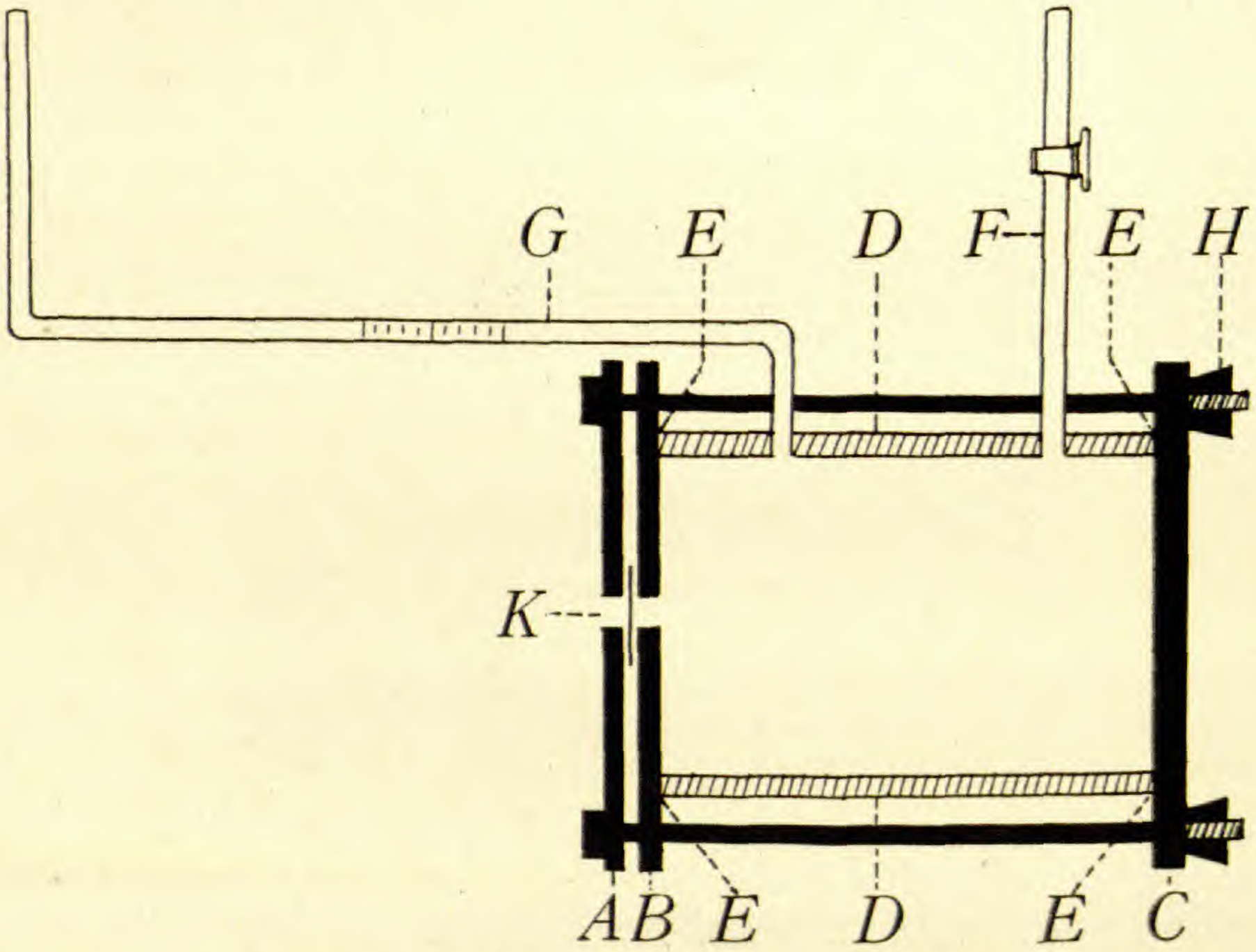

FIG. 2.—Drawing of osmometer: explanation in text

readings of the position of the meniscus in G at various intervals of time the rate of water movement through the membrane can be determined.

As water passes through the membrane, it has a tendency to dilute the bathing solution at K. This tendency is overcome by a stirrer whirling in front of the membrane at K which keeps the concentration constant there. The amount of water passing into the bathing solution is so small as compared with the large volume of the latter that the concentration of the solution exerting the osmotic pull is maintained constant.

At the end of an experiment it is possible to record the quantity of water passing through a known area of membrane, in a known interval of time, at a constant known temperature, and under the constant osmotic pull of a solution whose osmotic pressure in atmospheres is known. This gives a measure of the permeability of the membrane under the conditions of the experiment.

The sources of error and the precautions taken were as follows:

Temperature errors.—It was found that when the apparatus was immersed in the bathing solution it reached the temperature of the latter in 6–8 minutes. An interval of at least 10 minutes was allowed before readings were begun. The temperature of the bathing solution was constant to ±0°.1 C. The effect of the possible deviation of the temperature of the internal liquid upon the readings of the meniscus in the side arm was calculated.[1] The volume of the osmometer is 4421.13 cu. mm. Assuming it filled with water at 4° C., then changes in temperature of 0°.1 show the relation between temperature errors and scale division of the osmometer as indicated in table I.

TABLE I

Temperature	Volume error (in mg.) of water	Scale division value (in mg.) of water	Error of readings in scale divisions
5° ±0°.1.....	0.01326	0.338	<1
15° ±0°.1.....	0.17665	0.3378	<1
25° ±0°.1.....	0.18525	0.337	<1
35° ±0°.1.....	0.30764	0.336	<1
45° ±0°.1.....	0.37212	0.3347	1.11

Accurate readings of the side arm could be made only to one scale division. Temperature deviation therefore introduced no error except at temperature as high as 45°, and then only a slight error resulted.

Variation in membranes.—It was early found that there was a large variation in the permeability of different membranes of the same species. One membrane could not be compared with another, but only with itself under the various conditions of the experiment.

[1] Calculated from data taken from Smithsonian tables, Smithsonian Misc. Coll. **63**: no. 6.

The permeability of the same individual membrane was measured under the different conditions studied, therefore, and the same set of observations made with a number of other membranes of the same species. In some cases it was possible to allow the same membrane to remain in the osmometer during a whole series of readings. When it was necessary to remove the membrane from the osmometer, care was taken in replacing it that the same portion of the membrane was used in the next reading.

Constancy of semipermeability.—When a membrane gave constant rates for 1 hour, readings being taken at intervals of 10 minutes, it was assumed that its permeability to salt or sugar had not changed during the experiment, or at any rate that any change in permeability that had occurred did not affect the readings taken. No serious attempt was made to determine the completeness of semipermeability. Preliminary experiments indicated that the membranes were slightly permeable to sodium chloride, but a passage of cane sugar through the membrane was not detected. Conductivity measurements are to be made to determine the permeability of these membranes to salts, and these results will be reported in a later paper.

Preparation of solutions.—Cane sugar solutions were prepared in accordance with tables given by FINDLAY (11), rock candy being used. Sodium chloride was made up on the volume molecular basis and its osmotic pressure figured from the data given by RENNER (25).

Capillary tube errors.—Although according to the law of POISEUILLE the flow of water through capillary tubes is affected by temperature, it is not believed that this was a factor in these experiments. In POISEUILLE's experiments the liquid was subjected to a head of pressure and flowed through the capillary tube with rapidity, whereas in these experiments there was no hydrostatic pressure applied and the rate of movement through the tube was very slow. According to BARKER (3), when water is the liquid in question this law applies only to tubes with less than 0.5 mm. diameter of capillary; while the capillary used in these experiments was 1 mm. It is not believed that the results obtained are affected by the influence of temperature on the flow of water through the capillary tube of the apparatus.

Other precautions taken.—All membranes were heated in boiling water before being used for experimental purposes. The distilled water used inside the osmometer was previously boiled to drive off dissolved gases. Tests were made to determine that no leakage was occurring in the apparatus. In filling the osmometer care was taken to drive out all air bubbles from the internal portion of the apparatus. Special pains were taken to see that no air bubbles were lodged on the membrane.

Effect of temperature

Membranes of the seed of *Arachis hypogaea* were placed in the osmometer and measurements were made of the rate of penetration of water at the temperatures indicated. After being measured at the various temperatures, each membrane was checked back

TABLE II

EFFECT OF TEMPERATURE UPON PERMEABILITY OF SEED COAT (MEMBRANES OF *Arachis hypogaea*)

Number	Osmotic pressure at 25°C.	Water (in mg.) passing through 19.635 sq. mm. of membrane per hour				
		5°.2 C	15°.2 C	25°.2 C	35°.0 C	45°.0 C
1	27.65	11.01	18.59	29.09	.....	.....
2	"	22.93	38.08	58.19	.....	.....
3	"	.....	.....	56.48	78.71	104.85
4	"	.....	.....	68.46	91.18	126.78
5	41.48	16.51	28.32	42.79	.....	.....
6	"	33.02	53.12	82.15	.....	.....
7	"	42.19	69.07	102.69	128.69	161.83
8	"	37.91	59.03	88.99	106.87	142.83
9	13.82	.....	.....	28.52	40.59	52.96
10	"	.....	.....	35.37	50.82	71.68
11	"	13.45	21.25	34.23	47.22	61.09
12	"	15.41	24.79	35.94	46.40	64.20

From left to right, the figures are readings obtained from the same individual membrane at different temperatures; each line represents a different membrane.

against a previous temperature to be certain of constant behavior. In transferring from one temperature to another the membrane was not removed from the osmometer, so that the results indicated in table II show a comparison of the rates indicated by the same membrane at different temperatures. When the bathing solution was changed from one temperature to another its osmotic pressure also changed, and a correction was made for this change in osmotic

pressure of the bathing solution due to changes in temperature. The actual osmotic pressure of the solution at the temperature used was calculated on the basis of proportionality between osmotic pressure and absolute temperature. The observed rate was corrected on the basis that the rate was proportional to the pull applied, which will be shown later to be the case for solutions of sodium chloride. When cane sugar was used as the bathing solution the observed rate was not corrected, because proportionality between rate and pull applied does not exist with such solutions.

TABLE III

VALUE OF Q_{10}

5.2–15.2 C.	15.2–25.2 C.	25.2–35.0 C.	35.0–45.0 C.
1.688	1.564	1.374	1.332
1.661	1.528	1.339	1.390
1.716	1.512	1.258	1.259
1.609	1.546	1.206	1.364
1.637	1.487	1.433	1.305
1.530	1.507	1.448	1.411
1.579	1.610	1.388	1.294
1.608	1.449	1.298	1.384
Average, 1.628	1.525	1.343	1.344

TABLE IV

EFFECT OF TEMPERATURE UPON PERMEABILITY OF SEED COAT (MEMBRANES OF *Arachis hypogaea*)

Number	Osmotic pressure of cane sugar solution at 25° C.	Water (in mg.) passing through 19.635 sq. mm. of membrane per hour			
		3.6 C	13.6 C	23.6 C	33.6 C
1	21.25	7.98	13.69	20.54	26.81
2	"	13.12	21.11	30.07	42.21
3	"	11.64	19.40	28.92	38.79
4	"	6.39	10.27	14.38	19.51
5	"	14.55	23.39	35.09	46.89

From table II we may estimate the coefficients for 10° rise in temperature (see table III), hereafter referred to as Q_{10}. Experiments were carried out also in which cane sugar was used as the bathing medium instead of sodium chloride. The results with cane sugar are given in table IV. The figures in table IV give

coefficients for 10° C. as recorded in table V. These data were not corrected for change in osmotic pressure due to change in temperature, because, as will be shown later, the rate is not exactly proportional to the pull applied. But that this correction would

TABLE V

VALUE OF Q_{10}

3.6–13°.6 C.	13.6–23°.6 C.	23.6–33°.6 C.
1.716	1.541	1.305
1.610	1.424	1.403
1.667	1.492	1.342
1.607	1.400	1.357
1.607	1.500	1.336
Average, 1.641	1.463	1.348

be a small one and that it would not affect the general results, is indicated by the following results obtained when a correction is figured on the assumption that the rate is proportional to the pull. Making this correction, the first column of table IV, showing values of Q_{10}, becomes:

1.649
1.547
1.604
1.545
1.546

Average, 1.578

In addition to this, preliminary results were obtained from measurements of 6 other membranes. While the conditions of the experiment were not so accurately controlled, the average coefficients obtained were as follows:

Approximately 5 to 15° Q_{10}=1.617
" 15 to 25° Q_{10}=1.470
" 25 to 35° Q_{10}=1.422

Temperature coefficients.—The effect of temperature upon a process has been much used to obtain information as to its nature, that is, whether chemical or physical. Generally speaking, chemical processes follow the van't Hoff law (Q_{10}=2 to 3), but the effect of

temperature upon the process of diffusion is such that Q_{10} is approximately 1.3. Applying the results of these experiments to this case it is found that the coefficient obtained does not correspond with either the van't Hoff coefficient or the diffusion coefficient. Measurements of the permeability of membranes made heretofore have shown in general a temperature coefficient approximating that of the van't Hoff law, but there is no evidence in these experiments that in the passage of water through the seed coat of the peanut chemical processes are exclusively involved. Apparently also the effect of temperature is not merely upon the rate of diffusion of water. Probably we are not justified in using the numerical coefficients obtained to form any conclusion as to the nature of the process by which water passes through the peanut membrane.

Comparison with temperature coefficients obtained by others.— KRABBE (**19**) measured the effect of temperature upon the permeability of the living cells of cylinders of pith of *Helianthus annuus* and pieces of roots of *Vicia Faba*, etc. As criteria he took the rate of increase in length of pieces of plasmolysed tissue, allowed to absorb water at temperatures in vicinity of 0° and 20° C, and the length of time for plasmolysis to occur at these temperatures. He found that the velocity of water movement increased 3–5 times when the temperature was increased 20° C. (Q_{10} approximately 2.0–2.5). He believed that this high coefficient indicated that purely physical forces were not operative, but that it was due to a specific property of living protoplasm.

RYSSELBERGHE (**26**) investigated the effect of temperature upon the permeability of the living protoplasm, using pith cells of *Sambucus nigra*, lower epidermal cells of *Tradescantia*, and filaments of *Spirogyra*. He made use of 3 methods: the rate of shortening of a tissue in a plasmolysing solution at different temperatures, the rate of elongation of plasmolysed tissue in water at different temperatures, and the rate of plasmolysis of a tissue under microscopic observation. His general results are as follows:

Temperature	0	6	12	16	20	25	30
Comparative rate	1	2	4.5	6	7	7.5	8

This gives an average value for Q_{10} from 0°–30° of 2.0. RYSSELBERGHE does not agree with KRABBE that this high coefficient necessarily indicated the special activity of vital matter.

BROWN and WORLEY (**6**) determined the speed of intake of water by barley grains immersed in water at different temperatures. Their results gave a temperature coefficient of 1.8–1.9. Since this approached closely the van't Hoff coefficient (2–3) for the effect of temperature on the rate of chemical reaction, they considered that chemical processes were involved in the penetration of water through the semipermeable membrane of the barley grain. This chemical reaction, according to their view, took place in the water itself, that the effect of temperature was to split the larger aggregates of water into simpler ones, and that only these simpler molecules were transmitted by the differential septum. This was offered as evidence in favor of the hydrone conception of ARMSTRONG (**1**) as to the composition of water.

PFEFFER (**24**) measured the rate of water movement across the copper ferrocyanide membrane at different temperatures with the following results:

Temperature	In stream per hour
7°.1	5.9 mm.
17°.6	9.4 "
32°.5	13.3 "

The above figures give values of Q_{10} as follows:

$$7.1\text{–}17°.6,\ Q_{10}=1.558$$
$$17.6\text{–}32°.5,\ Q_{10}=1.266$$

The writer's observed values are in fair agreement with these figures. For purposes of comparison, a summary is given in table VI. It will be noted from this table that no parallel exists between

TABLE VI

Observer	Nature of membrane studied	Temperature range	Q_{10}
Krabbe	Living pith cells of *Helianthus*	0–4 to 20–26°	2.0 to 2.5
Rysselberghe	Living cells	0 to 30°	2.0
Brown and Worley	Semipermeable membrane of barley seed	3.8 to 34°.6	1.9 to 1.8
Pfeffer	Copper ferrocyanide	7.1 to 32°.5	1.558 to 1.266
The writer	Seed coat of *Arachis hypogaea*	3.6 to 45°	1.641 to 1.343

the nature of the membrane studied and the value of the coefficient observed. If this were the case, the barley and peanut membranes

should be expected to give similar results, while the similarity of results given by the copper ferrocyanide membrane and peanut should not be expected. We may note, however, a parallel between the method of observation employed and the coefficient obtained. The first 3 observers studied the permeability of the membrane indirectly, other structures such as cell contents and seed contents being present. In the last two cases the membrane was measured directly, without other structures being factors in the rates observed.

It is questionable to what extent results obtained by the indirect method may be referred to the membrane alone. There is the possibility that the temperature effect may have been, not upon the membrane merely, or upon the water exclusively, but also upon the cell contents or seed contents. The latter effect may have contributed to the total results from which the coefficients were calculated. The chemical reaction indicated by the coefficient 2–3 may have taken place in that phase of the system that was internal to the membrane studied.

In these experiments the temperature may have exerted an effect on the water, but if so the temperature coefficient does not indicate that this was related to a chemical reaction. There is no evidence of a temperature action in splitting the larger water aggregates into simpler hydrone molecules as found by BROWN and WORLEY with the semipermeable membrane of the barley grain.

Tendency of temperature coefficients to fall in value with increased temperatures.—An inspection of the temperature coefficients obtained in these experiments shows that the coefficients are higher at the lower temperatures and lower at the higher temperatures. This has been found to hold for a great many different processes. KANITZ (18) noted a number of physiological processes that show this tendency. SNYDER (29) has pointed out that some purely chemical reactions also exhibit a falling value of Q_{10}, and COHEN-STUART (8) has shown that according to the van't Hoff law itself values of Q_{10} are not constants and that the velocity is not an exponential function of the temperature. Table VII indicates the general tendency of Q_{10} for different processes. Falling values of Q_{10} are thus shown to occur in measurements made (*a*) with living matter, (*b*) with non-living matter, (*c*) with a physical

process, and (*d*) with a chemical process. These figures also emphasize the fact that temperature coefficients should not be averaged for a large interval of temperature, but that the range of the values of Q_{10} for each temperature interval should be shown for which experimental data are available.

TABLE VII

RYSSELBERGHE'S RESULTS WITH LIVING PROTOPLASM		RESULTS OBTAINED WITH NON-LIVING PLANT MEMBRANES		VAPOR PRESSURE OF WATER AT VARIOUS TEMPERATURES		REMSEN AND REID'S RESULTS WITH HYDROLYSIS OF NITRO-BENZAMIDE*	
Temperature	Q_{10}	Temperature	Q_{10}	Temperature	Q_{10}	Temperature	Q_{10}
0–6°	3.2	5.2–15.2°	1.628	5–15°	1.943	60–70°	1.84
6–12	3.8	15.2–25.2	1.525	15–25	1.854	70–80	1.72
12–16	2.0	25.2–35.0	1.343	25–35	1.776	80–90	1.65
16–20	1.5	35.0–45.0	1.344	40–50	1.675	90–100	1.59
20–25	1.1	.....	.....	.....	.....	.....	.....
25–30	1.1	.....	.....	.....	.....	.....	.....

* From data given by SNYDER (*29*, p. *169*).

Relations of permeability of membranes to vapor pressure of water.—The experiments of BROWN and WORLEY (**6**) showed that Q_{10} approximated in numerical value the vapor pressure coefficients of water at those temperatures. From table VII it will be noted that similar results were not obtained with the peanut membrane; that while the coefficient of permeability rates and vapor pressure are not equal, they both show the same tendency to fall in value at higher temperatures. It may be noted in this connection that the coefficient obtained lies between the diffusion coefficient and the vapor pressure coefficient.

Rate as related to flow through capillary tubes.—According to the law of POISEUILLE, as reported by KRABBE (**19**), the quantity of water flowing through a glass tube increases from 1 to $1+0.0336793\,t+0.0002209936\,t^2$, where t is the temperature in degrees Centigrade (KRABBE **19**, p. 477). This would make the coefficient for 10 rise in temperature about 1.358. Since this law applies only to capillary tubes with a length above a certain minimum amount, and since the temperature coefficients obtained in these experiments are not constants but vary with the temperature, it is not believed

that the results obtained indicate that the passage of water through the membrane is analogous to the passage of water through capillary tubes.

Rate as related to previous heating or cooling.—When the permeability of a membrane is measured at one temperature and the membrane then transferred to another temperature, the question is raised as to whether or not there is any "after effect" of the previous temperature. To determine this point membranes were fitted into 2 osmometers and a measurement was made of the permeability of each membrane. One osmometer was then placed in a beaker of water in an ice chest at 2.5 C., and the other in an oven at 46° C. The next day the two were again placed in the original osmotic solution at the original temperature and readings again taken. The results obtained are given in table VIII. No after effect of a previous temperature, or hysteresis, was observed at the temperatures used in these experiments.

TABLE VIII

Intervals of 10 minutes	25° C.	25° C.
First membrane		
First	27 spaces	29 spaces*
Second	29 "	29 "
Third	28 "	29 "
Fourth	29 "	29 "
Fifth		29 "
Second membrane		
First	23 spaces	23 spaces†
Second	23 "	24 "
Third	23 "	24 "
Fourth	24 "	22 "
Fifth		23 "

* After 14 hours at 2°.5 C.

† After 15 hours at 45° C.

Rate as affected by direction of flow of water through membranes.—A peanut seed coat membrane was placed in an osmometer and a measurement made of the rate at which water passed through it. The membrane was then removed from the osmometer and its position reversed, the opposite surface being turned toward the

inside of the osmometer. The latter was then placed again in the original solution and a reading made of its rate of water passage. Table IX indicates the results obtained. The peanut membrane therefore is more permeable for water in one direction than in the other, and the favorable direction is from the outside toward the inside.

TABLE IX

RATE AS AFFECTED BY DIRECTION OF FLOW OF WATER THROUGH MEMBRANE OF *Arachis hypogaea*

NUMBER	DIAMETER OF HOLE	OSMOTIC PRESSURE	WATER (IN MG.) PASSING THROUGH MEMBRANE PER HOUR				
			In	Out	In	Out	Percentage decrease from in to out
1.....	8 mm.	67	139.52	95.92	135.16	90.91	32
2.....	"	"	137.34	87.20	111.18	84.58	31
3.....	"	"	124.26	93.09	.....	.....	25
4.....	"	"	99.25	43.21	98.86	.....	56
5.....	"	100	396.21	207.94	402.67	205.97	49
6.....	"	"	236.88	158.20	224.80	146.12	34
7.....	"	"	164.38	121.27	202.32	127.59	32
8.....	5 "	25	36.89	20.82	30.04	24.70	32
9.....	"	"	30.55	27.70	38.02	26.98	20
10.....	"	48	.....	70.17	106.11	.....	33
11.....	"	"	.....	79.87	111.82	.....	38
12.....	"	"	.....	35.65	53.77	.....	33
13.....	"	"	.....	44.75	63.69	44.50	29
14.....	"	"	.....	49.06	71.88	.....	31

"In" means direction outside of seed toward inside; "out" means direction inside of seed toward outside.

Measurements with the seed coats of *Prunus Amygdalus dulcis* gave the following results:

Rate in =48.5 mg. per hour
Rate out=42.6 " " "
Rate in =48.5 " " "
Rate out=40.4 " " "

Measurements made with the onion scale and with the seed coat of *Dioon edule* did not indicate any observable difference in the rate of penetration in opposite directions. This difference in the behavior of the two types of membranes may be correlated with their differences in structure. The peanut and almond seed coats are composed of two or more distinct layers, and have surfaces of different physical and chemical nature on opposite sides; such is

not the case with onion and cycad membranes. The differences in rate in opposite directions through a membrane have long been known to workers with animal membranes. MATTEUCCI and CIMA (**21**) in 1845 observed it with the skin of the frog and eel. COHNHEIM (**9**), according to HAMBURGER, ascribed the same phenomenon to the living action of the intestinal membrane. HAMBURGER (**14**) showed that this behavior was not restricted to living membranes, but that non-living animal membranes gave similar results; in fact, he prepared artificial membranes from parchment with layers of collodion, chromgelatin, and chromalbumen that were more permeable in one direction than in the other. He ascribes this to the "double" nature of the membrane, and the writer's results offer evidence in favor of HAMBURGER'S interpretation. If this difference in rate is due to the presence of double membranes of different nature, or to differences in surface on opposite sides, may not the plant cell itself show a difference in permeability in opposite directions, since such a system of double membranes is represented by the cell wall and ectoplast?

Rate as related to the concentration of the external solution.— Solutions of different osmotic pressures were used as the external solution in order to determine whether or not the rate of water

TABLE X

RELATION BETWEEN OSMOTIC PRESSURE APPLIED AND RATE (SODIUM CHLORIDE SOLUTION)

Number	Water (in mg.) passing through membrane per hour in atmospheres of osmotic pressure		
	13.82	27.65	41.48
1	22.53	44.37	67.89
2	21.96	44.37	66.75
3	.....	28.09	42.79
4	.....	58.18	82.15
5	28.53	56.48	.....
6	35.37	68.93	.....
7	34.23	.....	102.69
8	35.94	88.0	88.99

movement was proportional to the pull applied. Two solutions were used, sodium chloride and cane sugar. The results with sodium chloride are shown in table X. A comparison of the

osmotic pressure and rates from the data in table X is given in table XI.

TABLE XI

Ratio of pressures 27.65:13.82	Ratio of rates	Ratio of pressures 41.48:27.45	Ratio of rates	Ratio of pressures 41.48:13.84	Ratio of rates
2.000.....	1.969	1.500.....	1.530	3.000.....	3.013
2.000.....	2.020	1.500.....	1.504	3.000.....	3.040
2.000.....	1.980	1.500.....	1.471	3.000.....	3.029
2.000.....	1.949	1.500.....	1.412	3.000.....	2.977

TABLE XII

RELATION BETWEEN OSMOTIC PRESSURE APPLIED AND RATE (CANE SUGAR SOLUTION)

Number	Water (in mg.) passing through membrane in atmospheres of osmotic pressure					
	5.15	10.30	15.62	21.25	29.22	48.00
1.....	.....	23.39	29.44	33.66	.....	.....
2.....	.....	29.44	37.65	.....	.....	.....
3.....	9.70	21.11	27.09	35.37	.....	.....
4.....	8.55	18.25	24.53	31.95	.....	.....
5.....	8.16	16.83	24.25	28.43	.....	.....
6.....	7.70	13.35	18.17	21.96	.....	.....
7.....	14.09	27.04	36.62	45.18	.....	.....
8.....	14.73	25.84	35.26	42.79	.....	.....
9.....	11.81	19.40	23.96	28.24	.....	.....
10.....	12.83	23.45	31.66	35.37	.....	.....
11.....	10.84	21.11	27.95	33.66	.....	.....
12.....	13.69	24.53	31.95	39.36	.....	.....
13.....	5.53	9.69	11.98	13.69	.....	.....
14.....	6.84	11.72	13.69	16.54	.....	.....
15.....	11.98	23.96	29.66	36.97	.....	.....
16.....	.....	23.10	28.07	34.80	.....	.....
17.....	.....	.....	.....	.....	41.07	53.05
18.....	.....	.....	.....	.....	50.49	63.32
19.....	.....	.....	.....	.....	64.75	84.72

A comparison of the ratios of pressure and the ratios of the rates indicated in table XII is given in table XIII.

When the ratios of pressure applied were 2.000, 1.515, 1.360, and 1.642, the average ratio of rates observed was 1.888, 1.297, 1.211, and 1.285 respectively. It will be seen that the rate of water penetration is nearly proportional to the pull applied when sodium chloride is used, but that when cane sugar is used as the external solution, the rate is not proportional to the pull, but the

coefficient falls off with the higher concentrations. It is believed that this lowering of the rate is due to the increasing viscosity of more concentrated sugar solutions.

TABLE XIII

RATIOS OF RATES

5.15:10.30	10.30:15.62	15.62:21.25	29.22:48.0
2.176	1.259	1.144	1.292
2.133	1.279	1.305	1.254
2.063	1.284	1.302	1.308
1.734	1.344	1.172	.....
1.919	1.441	1.209	.....
1.756	1.361	1.234	.....
1.634	1.354	1.214	.....
1.825	1.364	1.179	.....
1.947	1.235	1.117	.....
1.792	1.351	1.204	.....
1.753	1.325	1.233	.....
1.712	1.302	1.143	.....
2.000	1.235	1.208	.....
"	1.168	1.246	.....
"	1.238	1.249	.....
"	1.215	1	.....
Average, 1.888	1.297	1.211	1.285

It was found that it was not possible to increase the concentration of the solutions on opposite sides of a membrane by an equal amount on each side without changing the rate at which water passed through the membrane. This was done in the following way: distilled water was placed inside the osmometer, the apparatus surrounded by a solution of sodium chloride having a known osmotic pressure, and the rate of water movement measured. Then the osmotic pressure was increased on each side of the membrane by an equal amount. The results are given in table XIII. Thus, although the effective osmotic pressure exerting an influence upon water movement was practically the same, the rate of water movement was not the same, but much less. With the same membrane the same osmotic pull does not give the same rate, but the rate depends upon the distribution of the concentration on opposite sides of the membrane. When the concentration of the external solution was kept constant and the concentration of the internal solution was varied, the results given in table XIV

were obtained with sodium chloride solutions. From these data the writer has not been able to formulate any mathematical relation between differences in concentration on opposite sides of the membrane and the rate of water movement through it. Another

TABLE XIII

RATE OF WATER MOVEMENT AS RELATED TO DIFFERENCES IN CONCENTRATION OF SOLUTIONS ON OPPOSITE SIDES OF MEMBRANE

Membrane	Solution	Osmotic pressure of external solution	Osmotic pressure of internal solution	Effective osmotic pressure	Rate per hour
First	Na Cl	18.43	0	18.43	48.29
"	"	36.86	18.43	18.43	35.94
Second	"	13.82	0	13.82	41.07
"	"	27.65	13.82	13.83	23.96
Third	Sugar	10.30	0	10.30	20.54
"	"	21.25	10.30	10.95	10.84
Fourth	"	10.30	0	10.30	29.09
"	"	21.25	10.30	10.95	15.63

TABLE XIV

FALL IN RATE OF WATER MOVEMENT WHEN CONCENTRATION OF INTERNAL SOLUTION WAS INCREASED

OSMOTIC PRESSURE OF EXTERNAL SOLUTION	OSMOTIC PRESSURE OF INTERNAL SOLUTION	WATER (IN MG.) PASSING THROUGH MEMBRANE PER HOUR	
		First	Second
46.10	0	67.74	61.67
"	4.61	49.54	45.21
"	9.22	45.49	39.43
"	13.82	39.39	32.99
"	18.43	26.96	24.27
"	23.05	22.92	18.86
"	27.65	12.13	10.11

set of readings was taken in which cane sugar solutions were used. The results are given in table XV, and show that the relation between concentration and rate is complex.

From the data given in tables XIII–XV we may conclude: (1) that the rate is greatly affected by changes in the concentration of the internal solution; (2) that equal osmotic differences do not necessarily produce equal rates; and (3) that no mathematical relation has been noted between the concentration on opposite

sides and the rate of water movement through the membrane. This emphasizes the caution that must be used in plasmolytic experiments on the rate of water movement through a membrane. Plasmolysis deals with solutions of different concentrations on opposite sides of a membrane. The concentration of only one of the solutions, the plasmolysing solution, is known. In such experiments the internal concentration of the cells of plant or seed is not known and is subject to change, that is, to variations in pulling power. Results should not be referred to changes in the permeability of the membrane alone until it has been found that the internal concentration has remained constant during the experiment (it is to be understood that this statement is intended to apply to *rate of water movement* and not to the final equilibrium attained by the two solutions).

TABLE XV

RATE OF WATER MOVEMENT AS RELATED TO DIFFERENCES IN CONCENTRATION OF SOLUTIONS ON OPPOSITE SIDES OF MEMBRANE

OSMOTIC PRESSURE OF EXTERNAL SOLUTION	OSMOTIC PRESSURE OF INTERNAL SOLUTION	EFFECTIVE OSMOTIC PRESSURE	WATER (IN MG.) PASSING THROUGH MEMBRANE PER HOUR	
			First	Second
73.69.....	0	73.69	41.07	37.65
48.0.....	0	48.0	37.65	34.23
21.25.....	0	21.25	27.38	21.39
10.30.....	0	10.30	13.59	17.11
73.69.....	10.30	63.39	21.39	20.54
73.69.....	21.25	52.42	9.58	11.13
48.0.....	10.30	37.70	17.11	16.26
21.25.....	10.30	10.95	6.84	8.56

Comparison of permeability of membranes of different species

Membranes from different species showed large differences in permeability, as indicated by table XVI. Equal areas (19.635 sq. mm.) of membranes were measured; saturated sodium chloride of approximately 375 atmospheres osmotic pressure was used as the external solution. It will be seen that membranes of different species and different membranes of the same species show large differences in permeability. The causes of these differences in the

rate of penetration will be dealt with in a later paper. It may be stated here, however, that thickness of membrane is not the limiting factor. The thinnest membrane is that of *Cucurbita*, and the thickest is that of *Prunus Amygdalus*.

TABLE XVI

RELATIVE PERMEABILITY OF VARIOUS MEMBRANES

Membrane	Water (in mg.) passing through per hour	Membrane	Water (in mg.) passing through per hour
Citrus grandis	0	Allium Cepa	39.2
" "	0	" "	12.9
" "	0	" "	12.3
" "	0	" "	31.2
Cucurbita Pepo	7.3	" "	22.4
" "	4.8	Prunus Amygdalus dulcis	120.0
" "	0	" " "	144.0
" "	4.0	" " "	72.0
" maxima	11.0	" " "	86.0
" "	0	" " "	60.0
" "	9.0	" " "	72.0
" "	11.3	" " "	72.0
" "	15.3	Arachis hypogaea	328.0
Xanthium pennsylvanicum	20.0	" "	530.0
" "	16.0	" "	564.0
" "	14.7	" "	710.0
" "	22.0	" "	528.0
Juglans regia	32.0	" "	672.0
" "	22.7	" "	584.0
Allium Cepa	25.7	Dioon edule	777.5

Structures of membrane used

The layers of tissue represented in the ripened seed coat of the various species and their origin have not been accurately determined by an examination of successive stages of the development of the seed. A study of the histology of the seeds of *Cucurbita* has been made by BARBER (2), of *Prunus Amygdalus* by PÉCHOUTRE (23), of *Xanthium* by HANAUSEK (15), of the Leguminosae by PAMMEL (22), and of the seed coats of various species in many families by LONAY (20), BRANDZA (7), GUIGNARD (12), and HARZ (17). From an examination of sections of the membranes used, and from a comparison made with the reports of these investigators, it is believed that the following structures are involved in these membranes: (1) an outer integument, a much compressed and hardly

distinguishable inner integument and nucellus, and a single layer of endosperm in *Arachis hypogaea* and *Prunus Amygdalus dulcis;* (2) a single integument and a layer of endosperm in *Xanthium pennsylvanicum* and *Juglans regia;* and (3) a portion of the integument, a layer of perisperm, and a layer of endosperm in *Cucurbita Pepo* and *C. maxima.* Details of the structure of these membranes, and a microchemical and chemical study of their composition will be given in a later paper.

Summary

1. Quantitative measurements were made of the permeability to water of certain non-living semipermeable plant membranes under experimentally controlled conditions.

2. The apparatus and method employed had the following advantages over osmometers ordinarily employed: (1) the passage of as small a quantity of water as 0.000337 gm. could be detected; (2) the exact area of the membrane used could be calculated; (3) the concentration of the solution exerting the osmotic pressure could be kept constant.

3. The effect of temperature upon the permeability to water of the seed coat of *Arachis hypogaea* was measured and the temperature coefficients for 10° rise in temperature were obtained. An average coefficient was not calculated. Since the temperature coefficients are not constant, but vary with the temperature, an average coefficient is without significance.

4. The temperature coefficient is lower than that according to the van't Hoff law, and is higher than the diffusion coefficient. There is no evidence that either chemical or physical processes are exclusively involved in the passage of water through the membrane.

5. The temperature coefficients showed higher values at lower temperatures and lower values at higher temperatures, and this is in agreement with the behavior of temperature coefficients in other processes.

6. A comparison is made with the temperature coefficients obtained in the permeability experiments of (1) KRABBE with living membranes, (2) RYSSELBERGHE with living membranes, (3)

BROWN and WORLEY with non-living seed coat membranes, (4) PFEFFER with copper ferrocyanide membrane.

7. No hysteresis or after effect of a previous temperature was observed.

8. It was found that the seed coats of peanut and almond showed a difference in permeability to water in opposite directions through the membrane, the faster rate being from the external toward the internal portion of the seed.

9. When distilled water was placed on one side of the membrane, the rate of water movement was proportional to the osmotic pressure applied upon the other side, when sodium chloride solutions were used; but this proportionality did not exist when cane sugar solutions were used.

10. When solutions of varying concentrations were placed on opposite sides of the membrane, it was found that the relation between rate and concentration difference was complex, and that in general equal osmotic differences do not necessarily produce equal rates; the rate is greatly affected by changes in the concentration of the internal solution; no mathematical relation was noted between the concentration on opposite sides and the rate of water movement through the membrane. The bearing of these facts upon plasmolytic experiments based on rate of water movement through membranes was pointed out.

11. A comparison of the permeability of several plant membranes under similar conditions was made, large differences appearing.

In conclusion, I wish to express my appreciation to Dr. WILLIAM CROCKER for suggesting the problem and rendering valuable assistance during the course of the experiments.

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LITERATURE CITED

1. ARMSTRONG, HENRY E., Hydrolysis, hydrolation, and hydronation as the determinants of the properties of aqueous solutions. Proc. Roy. Soc. London A. **81**:80–95. 1908.
2. BARBER, KATE G., Comparative histology of fruit and seeds of certain species of Cucurbitaceae. BOT. GAZ. **47**:263–310. 1909.

3. BARKER, GEO. F., Textbook of physics.
4. BECQUEREL, PAUL, Recherche sur la vie latent de la graine. Ann. Sci. Nat. Bot. IX. **5**:193–320. 1907.
5. BROWN, A. J., On the existence of a semipermeable membrane inclosing the seeds of some of the Gramineae. Ann. Botany **21**:79–87. 1907.
6. BROWN, A. J., and WORLEY, F. P., The influence of temperature on the absorption of water by seeds of *Hordeum vulgare* in relation to the temperature coefficient of chemical change. Proc. Roy. Soc. London B. **85**:546–553. 1912.
7. BRANDZA, M., Développement des téguments de la graine. Rev. Gén. Bot. **3**:1–32, 71–84, 105–126, 150–163, 229–240. 1891.
8. COHEN-STUART, C. P., A study of temperature coefficients and van't Hoff's rule. Konn. Akad. Wetens. Amsterdam. Proc. Sec. Sci. **14**:1159-1172. 1912.
9. COHNHEIM, O., Über die Resorption im Dünndarm und der Bauchhöhle. Zeitschs. Biol. **37**:443–480. 1908.
10. CROCKER, WM., Rôle of seed coats in delayed germination. BOT. GAZ. **42**:265–291. 1906.
11. FINDLAY, ALEX, Osmotic pressure. Longmans, Green, & Co. 1913.
12. GUIGNARD, L., Recherches sur le développement de la graine. Rev. Gén. Bot. **7**:1–14, 21–34, 57–66, 97–106, 141–153, 205–214, 241–250, 282–296, 303–311. 1893.
13. GOLA, G., Richerche sulla biologia e sulla fisiologia dei semi a tegumento impermeabiles. Mem. Acc. Reale Sci. Torino II. **55**:237–270. 1905.
14. HAMBURGER, H. J., Permeabilität von Membranen in zwei entgegengesetzten Richtungen. Biochem. Zeitschr. **11**:443–480. 1908.
15. HANAUSEK, T. F., Die "Kohleschicht" im Perikarp der Kompositen. Sitzber. Kais. Akad. Wien I. **116**:3–32. 1907.
16. HANSTEEN-CRANNER, B., Über das Verhalten des Kulturpflanzen zu der Boden Salzen. Jahrb. Wiss. Bot. **53**:553–599. 1913–1914.
17. HARZ, C. O., Landwirthschaftliche Samenkunde. Berlin. 1885.
18. KANITZ, ARISTIDES, Temperatur und Lebensvorgange. 1915.
19. KRABBE, G., Über den Einfluss der Temperatur auf die osmotischen Processe lebender Zellen. Jahrb. Wiss. Bot. **29**:441–498. 1896.
20. LONAY, H., L'Anatomie des téguments seminaux. Arch. Inst. Bot. Univ. Liege 3 and **4**:1–142. 1904.
21. MATTEUCCI, CH., and CIMA, A., Mémoire sur l'endosmose. Ann. Chim. et Phys. **13**:63–86. 1845.
22. PAMMEL, L. H., Anatomical characters of Leguminosae, chiefly genera of Gray's Manual. Trans. Acad. Sci. St. Louis **9**:91–274. 1899.
23. PÉCHOUTRE, F., Contribution à l'étude du développement de l'ovule et de la graine des Rosacées. Ann. Sci. Nat. Bot. **16**:1–158. 1902.
24. PFEFFER, W., Osmotische Untersuchungen. 1877.

25. RENNER, O., Über die Berechnung des osmotischen Druckes. Biol. Centralbl. **32**:486–504. 1912.
26. RYSSELBERGHE, FR. VAN, Influence de la température sur la perméabilité du protoplasme vivant pour l'eau et des substances dissoutes. Bull. Acad. Roy. Belg. Cl. Sci. 173–221. 1901.
27. SCHROEDER, C., Über die selection permeable Hülle des Weizenkernes. Flora **102**:186–208. 1911.
28. SHULL, CHAS. A., Semipermeability of seed coats. BOT. GAZ. **56**:169–199. 1913.
29. SNYDER, CHAS. D., On the meaning of variation in the magnitude of temperature coefficients of physiological processes. Amer. Jour. Physiol. **28**:167–175. 1911.
30. WÄCHTER, W., Untersuchungen über den Austritt von Zucker aus den Zellen der Speicherorgane von *Allium Cepa* und *Beta vulgaris*. Jahrb. Wiss. Bot. **41**:165–220. 1905.

ARBORES FRUTICESQUE CHINENSES NOVI. I

CAMILLO SCHNEIDER

5-5620 **Deutzia** (Sect. EUDEUTZIA, subsect. STENOSEPALAE Schn.) **Rehderiana,** sp.n.—Frutex ut videtur mediocris, dense breviter ramosus; ramuli annotini biennesque dense scabriter stellato-pilosi, rubro-fusci, vetustiores glabrescentes, cortice detersili; gemmae parvae, perulis pluribus lanceolatis acuminatis fusco-rubris pilosis cinctae. Folia matura papyracea, ovato-oblonga vel pleraque satis late ovata, apice satis sensim acuta, basi rotundata, margine breviter et subdistanter argute serrata, 1.5–2.5 cm. longa, 0.8–2 cm. lata vel surculorum ad 3.8:2.5 cm. magna, superne obscure viridia, scabra, pilis stellatis (4–)5–7 (–8)-radiatis subdense vel sparsius conspersa, subtus pallidiora, subcinerascentia vel in sicco interdum quasi caesio-viridia, in facie pleraque densius in nervis laxius pilosa pilis 5–7–9 radiatis, nervis lateralibus utrinsecus 4–5; petioli 1–3 mm. longi, superne sulcati, undique dense stellato-pilosi. Cyma 3–8-flora, pleraque subsessilis, ramulos laterales breves 1–2 rarius ad 4 cm. longos terminans, plus minusve dense stellatopilosa; pedicelli graciles, fructiferi ad 5–6 mm. longi; calyx stellatotomentosus pilis homomorphis 9–12-radiatis, dentibus triangulari-lanceolatis tubum aequantibus vel subaequantibus rarius subsuperantibus acuminatis intus glabris; petala alba (vel lactea?), extus sparse stellato-pilosa, ovato-vel elliptico-oblonga, apice satis acuta, 7–9 mm. longa, 3–4 mm. lata; stamina exteriora longiora petalis circ. duplo breviora, interiora exterioribus paullo breviora; filamenta exteriorum apice manifeste bidentata dentibus latis rectangularibus vel subtriangularibus antheram breviter stipitatam subaequantibus vel parum superantibus interdum anthera breviori-bus, interiorum lata, apice truncata et irregulariter denticulata vel versus apicem sensim acuta, antheram faciei interiori circa medium affixam gerentia; styli 3 laciniis calycis vix longiores. Capsula hemisphaerica, circ. 3 mm. crassa, lobis erectis vel incurvis plus minusve deciduis.

Yunnan occidentalis: inter Talifu et Tengyüeh, probabiliter in regione inter flumina Mekong et Salween, Octobri 1914, *C. Schneider* (no. 2613; typus in Herb. Arb. Arn. et in Hb. Schneider).

This is a very distinct looking plant, with its small, broadly ovate, almost subsessile leaves, and its few-flowered inflorescences which are borne on short lateral branchlets. It seems to be most closely related to *D. subsessilis* Rehd., which may easily be distinguished by its much longer (up to 6 cm.), more oblong leaves whose stellate hairs of the under surface have only 4–6 rays, by its larger and richer inflorescences, and bv its larger flowers.

It is with great pleasure that I connect with this distinct species the name of Mr. ALFRED REHDER, the well known dendrologist of the Arnold Arboretum, who (SARGENT, Pl. Wils. 1:1913) has given an excellent contribution to the knowledge of the genus *Deutzia*.

Spiraea (Sect. CHAMAEDRYON Ser.) **teretiuscula,** sp.n.—Frutex latus, ad 2 m. altus, laxe ramosus, ramis subnutantibus; ramuli hornotini flavescentes vel violascentes, vix sulcato-striati, puberuli, annotini rubro-brunnei vel fusci, teretiusculi, vetustiores cinerascentes; gemmae ut videtur parvae, ovatae, perulis imbricatis pilosis obtectae. Folia decidua, ovalia, obovalia vel ovato-elliptica, apice rotundata, interdum fere subemarginata, minutissime apiculata, basi plus minusve late cuneata, 6–11 mm. longa, 3–7 mm. lata, margine integerrima, superne laete viridia, tantum novella minutissime puberula, cito glaberrima, subtus discoloria, cinerascentia, initio paullo distinctius pilosa, dein etiam glabra, sub microscopio ut in *S. canescenti* papillosa, nervis utrinsecus 3–4 vix visibilibus; petioli flavescentes, vix 1.5 mm. longi, puberuli. Corymbus circ. 25-florus, convexus, tomentosulus, ramulos normaliter plurifoliatos 1–3 cm. longos terminans, 1.5–2.5 cm. diametiens; flores albi, circ. 5 mm. diametientes; pedicelli graciles, floribus breviores, tomentosuli; receptacula late turbinata, extus tomentosula, intus pilosula; sepala late triangularia, receptaculis aequilonga, extus glabra, intus ad apicem et ad marginem fulvo-villosula; petala suborbicularia, circ. 1.5 mm. lata, sepala duplo superantia; stamina 20, petalis subaequilonga; discus distinctus 10-lobatus, lobis apice dorsoque leviter sulcatis; carpidia extus versus basim intus ad ventrem sparse villosa, stylis apicalibus quam stamina subduplo brevioribus. Fructus maturi ignoti.

Szechuan australis: in regione Yen-yüan Hsien, inter viculos Ka-la-pa et Liu-ku, in dumetis montanis, alt. circ. 3000 m., 17 Maji 1914, *C. Schneider*

(no. 1256); eadem regione, prope Kua-pie, in declivibus calcareis montium, alt. circ. 3000 m., 23 Maji 1914, *C. Schneider* (no. 3546; typus in Herb. Arb. Arn. et Hb. Schneider).

At first sight, this species seems to be much like *S. ovalis* Rehd., which also has terete branchlets and similar leaves and flowers, but which is easily distinguished by the glabrous branchlets, leaves, and inflorescence, as well as by the leaves being not papillose beneath. According to the papillose leaves, *S. teretiuscula* is more closely related to *S. canescens* Don, but all the forms of this variable species have the branchlets distinctly angular.

Here may be mentioned another interesting form I collected in southern Szechuan "in dumetis montium inter viculos Hun-ka et Wo-lo-ho, alt. circ. 3300 m., 13 Junii 1914 (no. 3525; frutex circ. 2 m. altus, alabastra rosea)," the flower buds of which are pink. In its angular branchlets it resembles *S. canescens*, but the young ovate or ovate-elliptic leaves are not distinctly papillose beneath. Judging by its pinkish flowers it seems to represent a new species, but, unfortunately, the flowers are too young to furnish sufficient characters for a description. The young branchlets, leaves, and inflorescences are not quite so distinctly puberulous as in *S. teretiuscula*, and they seem to become very soon almost glabrous. The leaves are entire, and measure up to 15 mm. in length and 7 mm. in width.

5-5622 MALUS PUMILA Mill., var. **subsessilis,** n.var.—A typo praecipue recedit fructibus immaturis subsessilibus iis *Docyniae Delavayi* similibus ovato-ellipticis circ. 2.5 cm. longis et 2 cm. crassis sparse villosis apice concavis sepalis persistentibus conniventibus.

Szechuan australis: inter pagos Hoh-si et Te-li-pu, alt. circ. 2300 m., 7 Maji 1914, *C. Schneider* (no. 1132; typus in Herb. Arb. Arn. et Hb. Schneider; tantum arborem unicam mutilatam probabiliter cultam ad 5-metralem vidi).

The subsessile fruit of this apple suggests a *Docynia*, but the leaves and flowers are that of a true *Malus*. So far as I can judge by the material before me, it represents only a form of *M. pumila*, the variability of which needs a careful study. To *M. pumila* sensu meo (Ill. Handb. Laubh. 1:715. 1905) certainly belongs *M. asiatica* Nakai in Matsumura, Icon. Pl. Koisik. 3:19. *pl. 155*. 1915.

6808 **Malus** (Sect. DOCYNIOPSIS Schn.) **docynioides,** sp.n.—Arbuscula squarrosa, ad 6 m. alta; ramuli novelli griseo-villosi, floriferi laxius villosuli ut vetustiores glabrescentes fuscescentes; gemmae satis evolutae ignotae. Folia partim sempervirentia, tenuiter coriacea, biennia elliptico-oblonga vel obovato-elliptica, apice plus minusve rotundata sed apiculata, basim versus sensim attenuata, cuneata, margine subintegerrima vel a medio ad apicem indistincte

glanduloso-crenulata, 2–5.5 cm. longa, 0.7–2 cm. lata vel latiora ad 4.5:2.3 cm. magna, superne intense viridia, nitidula, glabra, subtus pallidiora, laxe villosula, costa nervisque lateralibus utrinsecus plerisque 5 prominulis flavescentibus glabrioribus, petiolis superne sulcatis saepissime laxe villosulis ad 1 cm. longis; folia novella versus apicem pleraque distinctius crenato-dentata vel irregulariter sublobulato-dentata, ad 3:1.7 cm. magna, superne in costa sparse lanuginosa, subtus satis dense griseo-villosula, in costa nervisque tomentella, petiolis ad 8 mm. longis tomentellis. Flores ad 1–3 fasciculati, fere sessiles, albi, circ. 2.5 mm. diametientes; sepala 4–5 mm. longa, late triangularia, subito breviter acuminata, utrinque satis dense lanuginosa, receptaculo dense griseo-villoso-tomentello subaequilonga; petala ovalia, apice rotundata, basi breviter unguiculata, circ. 13 mm. longa et 7 mm. lata; stamina circ. 30, longiora petalis triente breviora, antheris flavis; styli 5, parte inferiore connati, paullo supra basim villosuli, staminibus longioribus breviores; ovarium 5-loculare, loculis in stylorum basi distincte productis 2-ovulatis ovulis plus minusve superimpositis vel appositis. Fructus ignoti.

Szechuan australis: inter Kua-pie et Ta-tiao-ko, alt. circ. 2700 m., 23 Maji 1914, *Schneider* (no. 1349; typus in Herb. Arb. Arn. et Hb. Schneider).

The old leaves of this strange *Malus* are very much like those of *Docynia Delavayi* (Fr.) Schn., which are almost entire but sometimes show a similar dentation. The flowers, however, of *M. docynioides* are different from those of a true *Docynia* in having only 2 ovules in each carpel, while in *D. Delavayi* as well in *D. indica* Decne. I have always found 4–6 ovules. Otherwise the structure of the ovary of our new species agrees with that of the ovary of *M. Tschonoskii* (Max.) Schn. which, as I have pointed out (FEDDE, Rep. spec. nov. 3:179. 1906), may represent the type of a new section for which I proposed the name *Docyniopsis*. The figure given in SARGENT'S *Trees and Shrubs* 1: *pl. 37, fig. 2.* 1903 is incorrect, and has been copied by myself in my *Ill. Handb. Laubh.* 1:*fig. 403h;* the cells of the ovary are distinctly protruding into the base of the styles. As REHDER has stated (SARGENT, l.c. 74), the separation of the genus *Docynia* from *Malus*, especially from the group formerly regarded as genus *Eriolobus*, is rather an artificial one. But, after all, I hesitate to unite the true species of *Docynia* with *Malus*, and I refer to this genus all the species which possess only 2 (very rarely 3) ovules in each cell of the ovary, while *Docynia* may be distinguished by its 4–6-ovulate carpels.

Sorbus (Sect. ARIA) **Ambrozyana,** sp.n.—Frutex elatus vel arbor parva, habitu *S. Ariae;* ramuli annotini glabri, fusco-purpurei,

lenticellis flavis sparse obtecti, vetustiores fusco-nigrescentes; gemmae ovato-oblongae, acuminatae, perulis paucis fusco-purpureis margine dense longiciliatis cinctae, divaricatae, laterales 7–8 mm., terminales circ. 10 mm. (vel ultra?) longae. Folia decidua, subchartacea, pleraque elliptico-oblonga, minora interdum ovato-elliptica et maxima obovato-oblonga, apice acuta vel plus minusve rarius distincte acuminata, basi satis acute vel late cuneata, rarius subrotundata, minora latiora 6–7 cm. longa et 2.5–3.5 cm. lata, oblonga majora 7:2.5 ad 15:4 cm. vel latiora ad 14:6 cm. magna, margine irregulariter vel dupliciter subglanduloso-denticulata vel sublobata, superne saturate viridia, paullo nitidula, glabra (vel juniora ut videtur in costa nervisque subimpressis sparse pilosa), subtus valde discoloria, pulchre albescentia vel leviter flavescentia, facie tomento lanuginoso adpresso obtecta, costa nervisque lateralibus utrinsecus 9–10 subrectis in dentes exeuntibus angulo circ. 45° a costa divergentibus prominentibus sparsius lanuginosis vel fere glabrescentibus colore flavescente conspicuis; petioli 1–2 cm. longi, flavescentes, superne canaliculati, sparse lanuginosi vel fere glabri. Inflorescentia valde deflorata vel fructifera ramos laterales normaliter 2–3-foliatos ad 3 cm. longos terminans, corymbosa, circ. 5 cm. longa et lata, sparse pilosa vel glabra, fructibus 3–6; pedicelli circ. 5 mm. longi; sepala florum valde defloratorum late vel satis anguste triangularia, partem liberam receptaculi aequantia, initio ut receptaculum lanuginosa, deinde ambo glabra; petala ignota; stamina ut videtur circ. 25; discus cupularis, glaber; styli 2, 2/3 connati, basi parce lanuginosi; ovarium totum inferum, carpellis ventre ut videtur tantum basi connatis in parte libera parce lanuginosis; fructus rubri, obovato-globosi, ad 12:12 vel 15:13 mm. magni, apice parte libera receptaculi et parte inferiore persistente stylorum coronati sepalis plus minusve deciduis, sparse punctati; semina obovalia, valde compressa, apice rotundata, basi sub hilo apiculata, 5–6 mm. longa, 3–3.5 mm. lata, flavo-brunnea.

Yunnan boreali-occidentalis: ad latera orientalia montium niveorum prope Lichiang-fu, alt. circ. 3200 m., Octobri 1914, *C. Schneider* (no. 3913, typus in Herb. Arb. Arn. et Hb. Schneider).

The nearest relatives of this species seem to be *S. Aria* Crtz. and *S. lanata* Koch, from both of which it may at once be distinguished by its much shorter sepals and the different serration and lobation of the leaves. The shape of

its rather narrow and long leaves is different from that of all the other Asiatic species of this group, and I cannot identify it with any species mentioned by REHDER in his *Conspectus specierum Asiae orientalis* (SARGENT, Pl. Wils. 2:272. 1915), nor with any other form known to me.

The name is given in honor of Count ISTVAN AMBROZY, a very successful garden maker on his famous estate at Malonya, Hungary, as a slight return for all his help in my dendrological studies.

8246 SORBUS HUPEHENSIS Schn., var. **aperta,** n.var.—*S. aperta* Koehne in SARGENT, Pl. Wils. 1:465. 1913.—A typo praecipue recedit foliis (4–)5, non 6–8-jugis, foliolorum paribus in rhachide interstitiis plerisque 1.8–2.3 cm. longis separatis.

See my remarks under the following variety.

8250 SORBUS HUPEHENSIS, var. **obtusa,** n. var.—A typo praecipue recedit foliis 4–5-jugis, foliolorum paribus in rhachide interstitiis 1.5–2.5 cm. longis separatis, foliolis apice distincte obtusis margine tantum triente superiori dentibus utrinsecus 3–9 serratis maximis lateralium ad 5.5:2.2 cm. magnis subtus sub microscopio undique satis dense papillosis.

Yunnan boreali-occidentalis: prope Yung-ning, 19 Junii 1914, *C. Schneider* (no. 1166; typus in Herb. Arb. Arn. et Hb. Schneider; arbor circ. 8 m. alta).

In determining the *Sorbus* of the *Aucuparia*-group collected by myself in southern Szechuan and northwestern Yunnan, I cannot refer the above form to any species or variety enumerated by KOEHNE in his *Sorborum chinensium conspectus analyticus* (SARGENT, Pl. Wils. 1:475. 1913). It seems to me most nearly related to *S. aperta* Koeh., from the type of which it differs by its 5–6 (instead of 4–5) pairs of leaflets which are distinctly obtuse at their apex and also distinctly papillose beneath. As in *S. aperta*, the pairs of leaflets are more distant on the rhachis, and the leaflets are somewhat larger than in typical *S. hupehensis*. Otherwise, var. *obtusa* seems to connect the latter with *S. aperta*, and I am unable to detect sufficient differences to keep *S. aperta* a distinct species. I make it, therefore, a variety of *S. hupehensis*, of which it represents the most northern form, chiefly distinguished by its fewer pairs of leaflets.

To the typical *S. hupehensis* Schn. (in Bull. Herb. Boiss. II. 6:316. 1906; and Ill. Handb. Laubh. 1:680, *fig. 374r, 775n.* 1906), I refer the following specimens of my own collections which, partly, may represent var. *laxiflora* (see later) if it is possible to keep this form even as a variety.

Szechuan australis: inter pagos Wo-lo-ho et Hun-ka, in silvis apertis montium, alt. circ. 3000–3400 m., 13 Junii 1914, *C. Schneider* (no. 3532; arbor circ. 10 m. alta, trunco circ. 0.6 m. crasso; flores odore valde ingrato).

Yunnan boreali-occidentalis: ad latera orientalia montium niveorum prope Lichiang-fu, in dumetis apertis, alt. circ. 3500 m., Octobri 1914, *C. Schneider* (no. 2829 et 3912; fructus maturi carnei); eodem loco et tempore (no. 2811; fructus carnei; gemmae apice distinctius rufo-lanatae); in angustiis montium inter Sung-queh et Teng-chuan, 29 Septembris 1914, *C. Schneider* (no. 2905; arbor ad 8 m. alta; fructus carnei; gemmae ut in no. 2811 rufo-lanatae; rhachis foliorum ad 9-jugorum apicem versus distinctius alata; folia surculorum a me in eadem arbore abscissorum minora ad 12-juga foliolis tantum ad 2:0. 7 cm. magnis iis *S. Prattii* non absimilibus).

The fruiting branch of no. 2905 agrees well with that of no. 2811, both showing the buds distinctly fulvous at the apex, and the narrow wings of the rhachis. I do not know whether these two numbers represent another form because I have not yet seen fully developed buds of typical *S. hupehensis*.

In nos. 2829 and 3912 the buds are much more glabrous, and the rhachis is almost wingless. I am at a loss how to distinguish these specimens from the type of *S. laxiflora* Koehne collected by E. H. WILSON in western Szechuan, northeast of Tachien-lu, on the Ta-p'ao-shan, July 4, 1908 (no. 3008), and therefore I propose the following variety:

5-5624 SORBUS HUPEHENSIS, var. **laxiflora,** n. var.—*S. laxiflora* Koeh. in SARGENT, Pl. Wils. 1:466. 1913.

It needs further investigation to determine how this variety may really be distinguished from typical *S. hupehensis*. KOEHNE himself says that *S. laxiflora* forms with *S. hupehensis* and *S. aperta* "a special group distinguished by its small stipules, medium-sized leaves with 4–7 pairs of medium-sized leaflets, and by a remarkably loose inflorescence."

There is another group of species described by KOEHNE which I cannot separate because the characters on which they are founded by the author are too variable according to my own observations. I therefore propose to unite them in the following manner:

8311 SORBUS PRATTII Koeh., var. **tatsienensis,** n. var.—*S. munda* Koeh. in SARGENT, Pl. Wils. 1:469. 1913, includ. f.a. *tatsienensis* et f.b. *subarachnoidea*.—*S. pogonopetala* Koeh., l.c. 473.—A typo nonnisi foliolis paullo majoribus saepissime basi tantum integerrimis argutius et paullo profundius serratis differre videtur.

After a careful comparison of all the type numbers before me, I do not even know how to distinguish *S. munda* as a variety from *S. Prattii*. In describing *S. pogonopetala* the author apparently overlooked the fact that the type of this species (*E. H. Wilson's* no. 3003), a flowering specimen, and the only one the author has seen, came from the same locality (Pan-lan-shan, west of Kuan Hsien) as *Wilson's* no. 4323 which KOEHNE makes the type of his *S. munda* f. *subarachnoidea*. But this fruiting specimen agrees in every respect with the

flowering one, the only difference I can detect being that the pubescence is somewhat fulvous, while it is greyish in no. 3003. KOEHNE says: "*Sorbus pogonopetala* differs from all the other Chinese species with numerous small leaflets in its strongly bearded petals; it is also remarkable in the purplish black color of its petioles and rhachis." The last mentioned character is, in my opinion, judging by the co-type before me, of no value, and apparently only due to an effect of drying. The hairy petals are also present in *S. Prattii*, in the description of which the author himself says "petala . . . medio supra parce tenere lanato-barbata." I fail to see any difference between the "beards" of *S. Prattii* and of *S. pogonopetala*.

Of both *S. Prattii* and *S. munda*, KOEHNE has described two forms, the first one, of course, representing nothing else than the type. In reducing *S. munda* to a variety of *S. Prattii*, the name of KOEHNE's f.a. *tatsienensis* has to be used, according to international rules, as the new varietal name. It may also be mentioned that the presence or absence of papillae on the under surface of the leaves is a rather doubtful character to base any varieties or even species upon. In our case, the younger leaves of *S. pogonostyla* are "subtus epapillosa," while of *S. munda* they are described as "subtus sat valide papillosa, inter papillas parce v. haud reticulato-striata." In the specimen of *S. munda* (no. 4323) before me the leaves may better be described as "subtus satis indistincte papillosa," and the kind of papillae observed on the leaves of these species of *Sorbus* seems to be always much more indistinctly developed on the younger leaves than on the mature ones, and they are quite often entirely absent "non nisi circa stomata." After all, I believe we ought not to lay too much stress upon the development or absence of these papillae.

ARNOLD ARBORETUM
JAMAICA PLAIN, MASS.

PECULIAR EFFECTS OF BARIUM, STRONTIUM, AND CERIUM ON SPIROGYRA

S. S. CHIEN

(WITH TWO FIGURES)

It has been pointed out by OSTERHOUT[1] that dilute solutions of $BaCl_2$ (0.001–0.0001 M) have a specific effect on certain species of *Spirogyra*. They produce a peculiar contraction of the chloroplasts in the middle of the cell which is very characteristic. This effect was not produced at this dilution by any of the other salts examined. As specific effects of this kind are uncommon, it seemed desirable to investigate the matter further.

Two species of *Spirogyra* were investigated, a large form of the *S. crassa* type, which was used by OSTERHOUT, and a smaller species. The method of contraction differs somewhat in the two species. In both kinds contraction usually begins in the region near the ends of the cell, but in the larger kind the chloroplasts sometimes begin to contract in the central region. In a large percentage of the cells of the larger kind the central region shows the greatest contraction. The chloroplasts in this region may either shrink away from one side of the cell wall more than from the other, or equally from all sides. In the latter case the final shape (fig. 1, *B*) assumed by these bodies is quite typical for this kind of *Spirogyra*. In the smaller kind the greatest shrinking occurs in regions between the center and the ends of the cell (fig. 2, *B*). These are cells of the larger kind, however, whose chloroplasts contract like those of the smaller kind, and vice versa. In general the longer cells of each species are apt to contract most toward the ends, while the shorter cells are apt to have the greatest contraction in the middle. In either case the contractions of the chloroplasts are very characteristic.

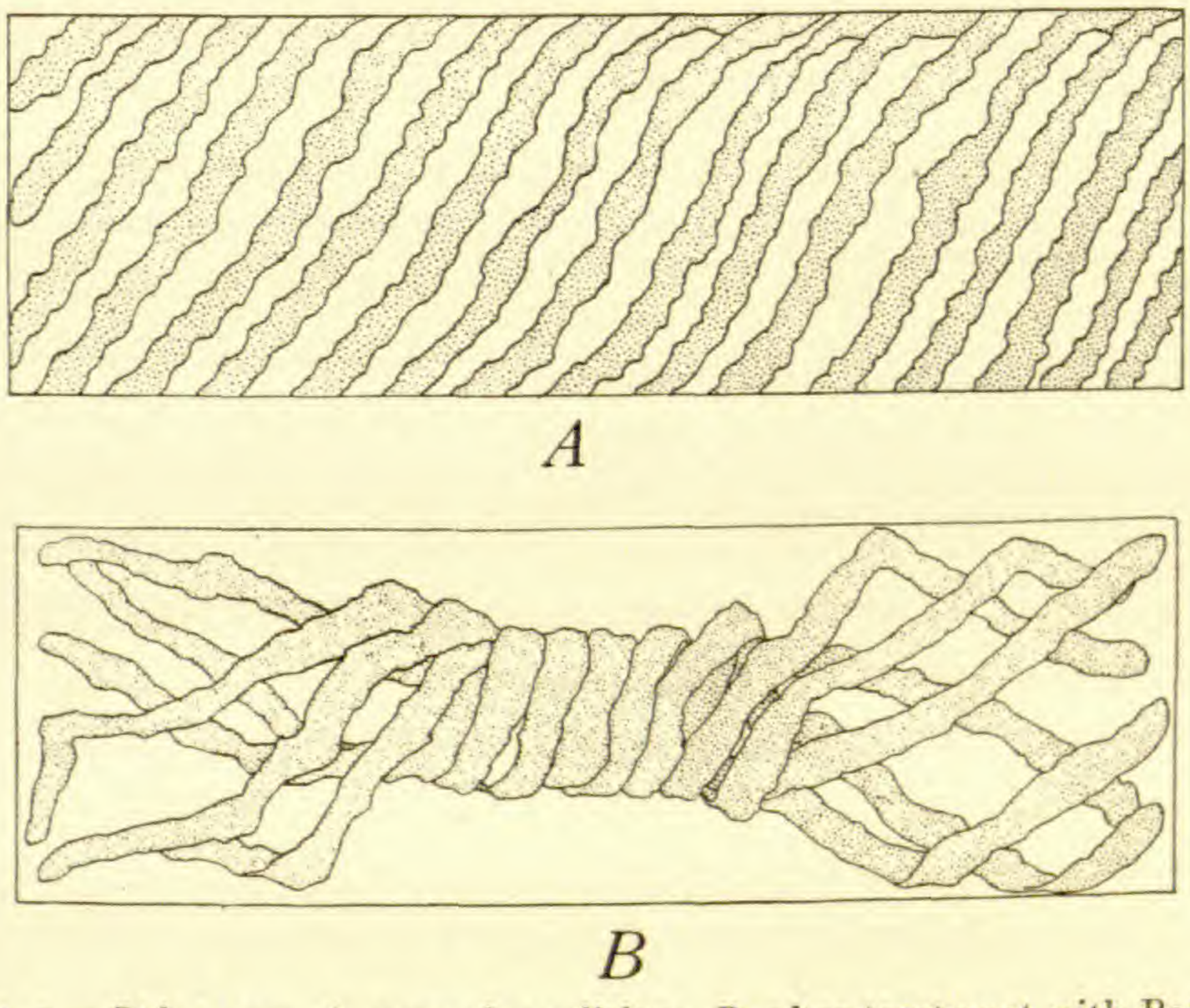
Contraction usually takes place a few minutes after the application of the solutions. Table I shows the time necessary for producing the effect at different solutions.

[1] OSTERHOUT, W. J. V., Specific action of barium. Amer. Jour. Botany 3:481–482. 1916.

The last named dilution of each salt is the lowest dilution at which contraction appears. $CeCl_3$ produces contraction of the larger kind only. It is also seen from the table that the lower

TABLE I

For the larger kind of *Spirogyra*		For the smaller kind of *Spirogyra*	
Solution	Time in minutes	Solution	Time in minutes
$CeCl_3$ 0.005 M	8	$BaCl_2$ 0.05 M	1–2
0.001 M	9	0.01 M	2–5
0.0005 M	9	0.001 M	15
0.0001 M	9		
0.00005 M	10	$SrCl_2$ 0.05 M	2–3
$BaCl_2$ 0.005 M	4	0.01 M	3–4
0.001 M	5		
0.0005 M	7		
$SrCl_2$ 0.01 M	2.5		
0.005 M	12		



A

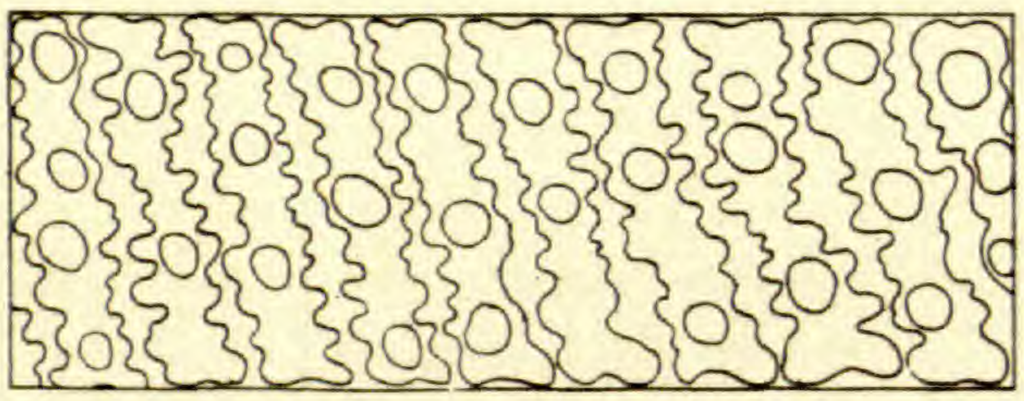
B

FIG. 1.—*Spirogyra: A*, normal condition; *B*, after treatment with $BaCl_2$

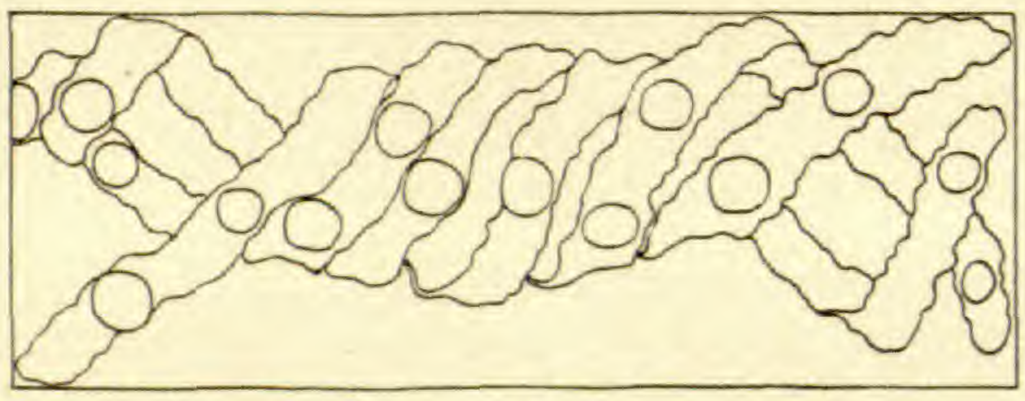
limit of $CeCl_3$ solution which produces the effect is less than that of the $BaCl_2$ solution, but the table shows that the effect is produced more rapidly in $BaCl_2$ and in $SrCl_2$ than in $CeCl_3$. These

two kinds of *Spirogyra* are different also in respect to the phenomena of antagonism, which will be discussed later.

If a contraction appears only after some hours or days, it is disregarded, as in such long experiments complicating factors are present. Solutions of barium and strontium salts in lower concentrations than those indicated will not produce the effect in 24 hours. With other salts which usually do not produce the effect contractions sometimes appear after 3 or 4 hours (or after several days), but this may be due to other agencies than the salts, because in material dying from natural causes cells with contracted chromatophores are sometimes found.



A



B

FIG. 2.—*Spirogyra:* large form, *crassa* type, pyrenoids omitted; *A*, normal condition; *B*, after treatment with $BaCl_2$.

The material used for this purpose must be fresh and in good condition, or it will lose its sensitiveness and fail to contract when the salts are applied. For example, material was taken at first from a glass jar kept in the greenhouse and was found to be sensitive to both barium and strontium. Later this material deteriorated because of the increased sunlight and heat of the greenhouse, and did not respond to $SrCl_2$, although still responding to $BaCl_2$. Still later it became insensitive to $BaCl_2$ solution, even at the concentration of 0.1 M. The same thing occurred with the small form which grew in a pond. This decrease of sensitiveness seems to be due to some chemical or physical change in the cell when its vitality is injured, which prevents the chromatophores from contracting.

As this visible effect of barium, strontium, and cerium is well suited to the study of antagonism, attempts were made to see what salts hinder or prevent these effects.

Some experiments were made with the smaller kind of *Spirogyra* in order to see whether antagonism occurs. $BaCl_2$ and $SrCl_2$ have no antagonistic action on each other. $CaCl_2$ and $CeCl_3$ (neither of which causes contraction of the chromatophores by itself) are able to antagonize $BaCl_2$, and $CeCl_3$ is more effective than $BaCl_2$. The chromatophores do not contract when placed in a mixture made by adding 10 cc. of 0.04 M $CeCl_3$ to 90 cc. of 0.08 M $BaCl_2$ in an hour and a half (after which the experiment was discontinued); while if 10 cc. distilled water is added to 90 cc. of the same $BaCl_2$, contraction begins after 1.5 minutes. Antagonism is obtained also by mixing 60 cc. of 0.1 M $Cacl_2$ with 40 cc. of 0.1 M $BaCl_2$. In this mixture no contraction occurs in 1 hour, while if 60 cc. of distilled water is used in the mixture in place of $CaCl_2$, the contraction occurs in 2 minutes. The proportions here mentioned are approximately the optimum ones for each mixture.

Summary

1. The chloroplasts of certain species of *Spirogyra* contract away from the cell wall in a peculiar and characteristic fashion in solutions $CeCl_3$, $BaCl_2$, and $SrCl_2$ (in the case of the smaller kind in the last 2 only). The effect is observed in dilutions as great as 0.00005 M $CeCl_3$ (in the case of the larger species), and in 0.0001 M $BaCl_2$. $SrCl_2$ also produces this effect, but not at such great dilutions as $CeCl_3$ and $BaCl_2$.

2. In the smaller species of *Spirogyra* the effect of $BaCl_2$ is inhibited when $BaCl_2$ is mixed with $CeCl_3$ or $CeCl_2$ in proper proportions.

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BRIEFER ARTICLES

MANIPULATING MICROSCOPIC ORGANISMS IN STAINING[1]

Perhaps the chief limiting factor in the successful preparation of permanent microscopic mounts of unicellular and colonial organisms is the difficulty of manipulation during the staining, with the incidental loss of the material in the necessary changing of the staining and washing solutions. The small size of such forms as *Sphaerella*, *Pandorina*, *Volvox*, *Pediastrum*, and the desmids renders these organisms especially liable to loss in these parts of the process. When handling the very small forms, the usual precautions taken in staining filamentous algae are generally wholly inadequate to prevent the loss of the material.

The difficulties in the way of success, however, may be overcome by using a funnel, filter paper, and a wash bottle, and combining the careful manipulations of quantitative chemistry, to prevent loss of valuable material, with the dehydration and staining methods of the Venetian turpentine method of mounting algae.[2] After the organisms have been killed in the usual 1:1:400 chromacetic acid solution, the entire contents of the vessel can be filtered, leaving the material to be stained on the filter paper in the funnel. Complete washing to remove the killing fluid can be secured by the use of the wash bottle. Should it be desirable to let the material stand in water for a few hours, the filter paper may be punctured and the material washed out into a beaker (this will save the work of washing). Refiltering later will leave the material on the filter paper and, with a little washing with water from the wash bottle, ready for the staining.

This manipulation is especially adapted to use with the iron-alum haematoxylin stain. The staining method itself may be greatly shortened, and in the modified form gives splendid results with these lower forms. The iron-alum should be dissolved in distilled water and all water treatments of material should be made with distilled water to avoid precipitates. Weak solutions of iron-alum should be used, 0.1 per cent solution at most. Frequently a few drops of a 1 per cent

[1] Contribution from the Department of Botany, Pennsylvania State College, no. 8.

[2] CHAMBERLAIN, CHAS. J., Methods in plant histology, 3d ed. Chicago, 1915.

solution in 100 cc. of water are sufficient. The iron-alum solution is applied slowly to the material on the filter paper in the funnel. After a short time, 15–30 minutes or even less being sufficient, the material is washed thoroughly with distilled water from the wash bottle. A weak haematoxylin stain is then applied slowly to the material, and repeated observations of specimen mounts under the microscope will determine when the staining is complete. A 0.1 per cent haematoxylin stain is strong enough, and 30 minutes or less time is long enough for it to act.

When the staining is complete, the differentiation of the stain is accomplished by a thorough washing in distilled water and the customary destaining with the 0.1 per cent iron-alum solutions. This latter step is the one requiring the greatest care, since it can be overdone most easily; the light haematoxylin stain used in the method is easily lost by prolonged treatment with even a very weak iron-alum solution. One application of the destaining solution is generally sufficient if care be taken that the material is thoroughly saturated with the solution. As usual when using the iron-alum solution for differentiating a stain, the process should be closely observed under the microscope. It is to be understood that no directions concerning the time limits can be given for the use of either the staining or differentiating solutions, since success depends upon the proper balancing of these two processes. When the stain has been properly differentiated, the material should be thoroughly washed with distilled water from the wash bottle. At any stage of the process, should it be desired to allow the material to remain covered by the solution being used, this can be accomplished by fitting a short piece of rubber tubing over the stem of the funnel and using a clamp to stop the flow of the solution.

Dehydration is accomplished by the glycerine dehydration method in general use. The transfer to the 5 per cent glycerine solution is made by puncturing the filter paper and washing with the glycerine solution into an open vessel, such as a Petri dish. The minimum amount of glycerine should be used, since it is difficult to remove. After a few days' exposure to evaporation, the glycerine solution is concentrated. The material is again poured into the filter paper in the funnel and complete dehydration accomplished by washing all the glycerine out with 95 per cent alcohol, followed by absolute alcohol to complete dehydration. There should be no doubt about the thoroughness of this part of the process, since complete dehydration is essential to success. When completely dehydrated the material is transferred quickly to a 10 per cent Venetian turpentine solution and placed in a

desiccator and allowed to concentrate according to the directions given for the Venetian turpentine method.

Should it be desired to use some of the other stains, such as the Magdala red-anilin blue combination recommended for algae, it will be necessary to modify this manipulation to suit the method. Since these stains are used in strong alcoholic solutions, the material to be stained is washed after killing by the method already described, and then dehydrated by the glycerine method before staining. The glycerine is washed out with 95 per cent alcohol and the stains applied.

Summary

1. Treat the material the proper length of time in a suitable killing solution.

2. Filter the material to remove killing solution, leaving the material on the filter paper in the funnel.

3. Wash with distilled water from a wash bottle.

4. Treat with a 0.1 per cent (or less) iron-alum solution.

5. Wash with distilled water, using wash bottle.

6. Stain by application of 0.1 per cent (or less) aqueous haematoxylin stain.

7. Wash with distilled water.

8. Differentiate the stain with 0.1 per cent iron-alum solution, washing with distilled water very thoroughly after the treatment.

9. Dehydrate with glycerine and mount by Venetian turpentine method.

10. Vary the treatment, when alcoholic stains are to be used, by dehydrating before staining.—J. BEN HILL, *Pennsylvania State College, State College, Pa.*

THE BOTANICAL STATION AT CINCHONA

The Botanical Station at Cinchona, in the Blue Mountains of Jamaica, which from 1903 to 1913 was leased by the New York Botanical Garden, has now been leased by the Smithsonian Institution, on behalf of 14 American botanists and botanical institutions that have contributed the rental. These botanists and institutions believe that there is need in the American tropics for a counterpart of the famous Buitenzorg Garden of Java. They hope that the opening of this laboratory at Cinchona may prove as stimulating to the development of botany in

this country as the opportunities afforded at Buitenzorg have been to the advance of this science in Europe.

The equipment available at the Station consists of the residence with its furnishings, 3 laboratory buildings, 2 glass propagating houses, and a garden of 10 acres containing many species of exotic shrubs and trees, besides many native plants from the highlands of Jamaica. The occupant of Cinchona is also free, within reasonable bounds, to study and collect plants over the many thousand acres of the whole Cinchona reservation, as well as in the neighboring valleys belonging to private owners. He will likewise be given every available facility for study at Hope Gardens, where he will find an herbarium, a library, and an extensive collection of tropical plants. The same privilege will be his at Castleton Garden, which contains fine collections of cycads and palms, and of *Ficus* and other dicotyledonous trees.

The many different types of native vegetation accessible from Cinchona and from Hope include a number of great ecological interest and numerous species of importance for the morphologist, cytologist, and physiologist. The ecological types range from the cool mountain forest with its tree ferns, epiphytes, and water soaked filmy ferns, to the hot, steaming woods of the lowlands of the north side at one extreme, and to the dry savannas and cactus deserts near Kingston at the other. Fuller statements of the opportunities for research in various lines, written by men who have worked there, may be found in *Science* **43**:917. 1916 (see also *Popular Science Monthly*, January 1915).

Any American investigator may be granted the use of the Cinchona Station by the Cinchona Committee, which consists of N. L. BRITTON, JOHN M. COULTER, and DUNCAN S. JOHNSON. Applications for this privilege and for information regarding the conditions under which it is granted should be sent to the writer.—DUNCAN S. JOHNSON, *Johns Hopkins University, Baltimore, Md.*

CURRENT LITERATURE

BOOK REVIEWS

Vegetation of Paraguay

CHODAT[1] has issued the first of a series of bulletins upon the plants of Paraguay. The work on which the series is based includes investigation continued at intervals since 1889 and culminating in an expedition made in 1914 by CHODAT and his former pupil VISCHER, and authorized by the Federal Department of the Interior of Switzerland. Sketches, water colors, and photographs were made in the field, as were also some chemical tests. A large quantity of material was brought home for later study.

The first chapter treats of the climatology and physiography of the country. The discussion of climate is based upon records covering 30 years, made by BERTONI at Asuncion on the Paraguay River and at Puerto-Bertoni on the Alto-Parana. The eastern part of Paraguay has a subtropical climate of the Chinese type; the western part is more like the Mediterranean region. Topographically, the state may be divided into the depressions along the Paraguay River and the mountains of the east. A lower highland of about 300 m. elevation separates the 2 main depressions, that about Lake Ypacarai and the lowland around the Ypoa lagoon. This cordillera extends nearly east and west between the Rio Salado and Rio Manduvira, and these 2 depressions are the regions under discussion in this paper.

CHODAT then takes up the Solanaceae, a group of intermediate importance, which compose several distinct formations, and gives somewhat in detail the variations in adaptation for climbing found in the liana forms, and the anatomical changes which occur during curvature. Only a few species are insect pollinated, those having large tubular flowers being visited by lepidopterous insects and humming birds. The genera *Sessea* and *Grabowskia* are here reported for the first time for the Paraguay flora. Indigo was found present in 2 species not previously known to contain the pigment. A few species of the family find in Paraguay their southern limit, while a somewhat larger number reach here their most northern extension. Several are mentioned as endemic and some of these are extremely local. In the third chapter the author discusses the Hydnoraceae, largely from a morphological standpoint. The one genus given (*Prosopanche*) is reported as parasitic on the roots of *Prosopis* and some of the Solanaceae.

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[1] CHODAT, R., and VISCHER, W., La vegetation du Paraguay. 1st fascicle. 8vo. pp. 157. *pls. 3. figs. 123.* Genève. 1916.

The last chapter deals with the dominant group of the region, the Bromeliaceae. The 2 main divisions considered are the cistern plants and the epiphytic *Tillandsias*. The latter are divided into those which lean against the support and those having some means of attachment to it. The different adaptations for climbing are illustrated. The structure and function of the hairs of *Tillandsia* and of the hairs on the submerged leaf bases of the cistern species are given particular attention. The presence of cortical roots in the attached lianas is also noted and their value to the plant discussed. Here, as in the Solanaceae, insect pollination is not very common, but the humming bird is a regular visitor to some large-flowered species. A few of the Bromeliaceae, as *Tillandsia usneoides* and *T. recurvata*, have a range from southern United States or Mexico to the southern part of South America. Most of the species mentioned, however, are limited to South America, 9 being given as endemic. The author also includes in this chapter a very interesting description of the xerophytic rupicole species belonging to various families which are found on the rocks of Cerro San Tomas and Sierra d'Acahay.—ARAVILLA TAYLOR.

NOTES FOR STUDENTS

Puget Sound algae.—A fascicle of papers[2] from the Puget Sound Marine Station at Friday Harbor, Washington, gives the results of work done on algae at the station, largely during the summer of 1916.

Miss HURD finds that young bladder kelps (*Nereocystis*) can adapt themselves to 55 per cent of fresh water in their environment if the change is made gradually. She concludes that rapid elongation of this plant is due to low light intensity in the water, and that growth of the stipe is greatly retarded by strong light when the bulb approaches the surface of the water. The fact that this does not act as a very exact determiner of length is readily understood, when we remember that the variation from extreme high tide to extreme low tide during the growing season in this region is more than 12 ft. She reaches the conclusion that there is no relation between rate of growth and mechanical stretching in the stipe of the plant. The experimental evidence given seems to justify this conclusion, providing that nothing else (for example, light) was a limiting factor in both experiment and control.

In another paper Miss HURD decides that the *Codium adhaerens* (Cabr.) Agardh reported from San Juan Islands and probably that from all of Puget Sound is *C. dimorphum* Sved., since it has no utricle hairs and has two types of utricles, the one with unmodified end wall and the other with thickened, striated end wall. She believes that the variation in the predominance of thick or of thin end walls in the utricles is probably due to differences in environment. The thick-walled type sometimes predominates over the whole

[2] Puget Sound Marine Station Publications 1:nos. 17–24. 185–248. *pls. 33–466.* 1916.

thallus, sometimes is found only around the margin and on the under side of the lobes, and sometimes is wanting entirely.

MUENSCHER reports a list of marine algae found on Shaw Island (one of the San Juan group), with notes as to zonal distribution and relative abundance, and a discussion of the ecological factors involved. He finds 54 Rhodophyceae, 31 Phaeophyceae, 15 Chlorophyceae, and 3 Myxophyceae. The plates give the distribution at various points on the island and will be very useful to collectors of algae in the region.

Miss KIBBE reports the presence of a parasitic fungus (*Chytridium alarium*, sp. nov.) on *Alaria fistulosa* collected in Alaska. She examined all of the species of brown algae that were readily available at the Puget Sound Marine Station, and also specimens of *Alaria valida* from Alaska, and did not find any trace of this fungus in any of them. In *A. fistulosa* she found the fungus in various forms in all parts of the plant except the heavy older portions of the stipe.

Miss KARRER finds that some light is thrown on the metabolism of *Nereocystis* by chemical reactions whose results are seen under the microscope. She finds that the cell walls are made up of cellulose and algin, the latter being probably the substance that holds the cells together. She finds that the presence of the inorganic substances (calcium, magnesium, sodium, potassium, chlorine, sulphates, carbonates, phosphates, and iodine) whose presence in the plant have often been shown by analytical chemists can be demonstrated in the cell by using the methods suggested by TUNMANN[3] and MOLISCH[4] with slight modifications.

Miss CLARK reports the acidity of marine algae as determined by titration. She reports that all of the 31 species tested were acid.

LANGDON[5] finds that carbon monoxide is present in the float of the bladder kelp (*Nereocystis*), the quantity varying considerably in different individuals. He finds the presence of carbon dioxide to be only occasional and the quantity minute. He does not find confirmation of previous work tending to show that the quantity of carbon dioxide and of oxygen vary with the time of day. He suggests that since theories of photosynthesis have largely been concerned with carbon monoxide and its reduction product formaldehyde, and with formic acid, of which carbon monoxide may be considered the anhydride, it is possible that the occurrence of carbon monoxide in plant tissues may be more general than has been supposed. Apparently LANGDON'S work is the first demonstration of free carbon monoxide in a living plant. A large plant cavity surrounded by rapidly growing tissue furnishes an unusually favorable opportunity for the investigation of gases taking part in metabolism. The sieve tubes in this plant are in the mycelium-like pith web on the interior surface of

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[3] TUNMANN, O., Pflanzenmicrochemie. Berlin. 1913.

[4] MOLISCH, H., Microchemie der Pflanzen. Jena. 1913.

[5] The substance of this paper has also been published in Jour. Amer. Chem. Soc. **39**:149–156. 1917.

the float. Since the whole surface of the sieve tubes in this portion of the plant is thus exposed to the gas contained in this float, it would seem possible that considerable oxidation of foods is carried on in this internal atmosphere. The gas in this float is shown to contain a little larger percentage of oxygen than air. It may possibly be worth while to consider the presence of carbon monoxide in plants in connection with the wide distribution of oxidases in plant tissue and the possible mechanism of their reaction.[6] LANGDON's thorough demonstration of the presence of carbon monoxide in this cavity is a very important piece of work, and great interest attaches to the possible relation of this gas to the metabolism of the plant.—G. B. RIGG.

Quantitative characters in beans.—By means of a statistical study of pole and bush beans, EMERSON[7] has analyzed the characters causing height variation in *Phaseolus vulgaris*. They are 3 in number and apparently segregate independently after crossing. First is the manner of growth, which is either "determinate" (bush type) or "indeterminate" (pole type), with the indeterminate habit completely dominant in the F_1 generation, and showing the typical 3:1 splitting in the F_2 generation. Such behavior he interprets as the result of a single pair of freely segregating factors behaving in a Mendelian fashion.

TSCHERMAK, using the hybrids *Phaseolus vulgaris*×*P. multiflorus* and the reciprocal, found anomalous splitting in the F_2, since some of the "short" segregates produced "talls" in succeeding generations. He makes no mention of habit of growth, and merely classifies the progenies as "talls" and "shorts." The results of TSCHERMAK need not be compared with EMERSON'S, however, because in the former case the hybrids are interspecific, and in the latter intervarietal (intraspecific).

The second character operative in determining height is number of internodes. The presence of this character was deduced from the fact that different varieties of both pole and bush beans differed in the number of internodes produced when grown under the same conditions. The question then arose as to whether this tendency to produce few or many internodes could be inherited independently of habit of growth. Suitable crosses were made and the results seemed to answer the question in the affirmative, although the evidence is admittedly incomplete. The factors determining this difference could not be shown to be perfectly dominant, but apparent segregation followed hybridization. This segregation was attended in the F_2 generation by a range of variability exceeding that of the 2 parents. EMERSON interprets this result as due to the action of multiple segregating factors.

The third character involved in height is length of internode. The modification of this character by habit of growth made its behavior difficult to study.

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[6] REED, G. B., BOT. GAZ. **62**:53–64. 1916.

[7] EMERSON, R. A., A genetic study of plant height in *Phaseolus vulgaris*. Research Bull. no. 7. Nebr. Agric. Exp. Sta. pp. 73. 1916.

In order to have a standard of comparison between pole and bush bean types, the first 5 internodes were measured and the means computed. For comparison between different varieties of pole beans the mean of the first 5 internodes was used. It was thought that the actual internode length found for some of the bush varieties might not be representative of the potential length which would have been attained by the upper internodes had not the production of a terminal inflorescence hindered further growth. To test this supposition crosses were made between a bush bean with long internodes and a pole bean with short internodes. The resulting hybrid showed an intermediate development in the F_1 and a wide range of variation in the F_2 generation. Bush beans with shorter internodes and pole beans with longer internodes than the parent types exhibited were obtained. Here again the variations were attributed to the action of multiple, non-dominant, independently segregating factors.

In conclusion, the author points out that the results of other investigators tend to show that quantitative characters in plants are inherited in two ways: (*a*) they are due to the action of a single Mendelian pair of factors showing complete dominance in the F_1 and a 3:1 ratio in the F_2 generation; (*b*) they exhibit an intermediate development in the F_1 and a wide range of variation in the F_2 generation. In class (*a*) belongs the determinate as opposed to the indeterminate habit of growth. Characters such as length and number of internodes fall into class (*b*). Such characters as those of class (*b*) have been interpreted in 2 ways. EMERSON, TSCHERMAK, EAST, and others attribute them to the interaction of many independently segregating factors, a theory in accord with the multiple factor hypothesis of NILSSON-EHLE. CASTLE, however, has interpreted such behavior as due, in some cases, to the modification of a unit factor through hybridization. In the case of the bean crosses, EMERSON implies that the factor involved would be that which determines habit of growth. After discussing this latter hypothesis and the assumptions its adoption would necessitate, he rejects it in favor of the multiple factor hypothesis.

Since, therefore, the characters involved in producing an effect seem to behave in different manners in inheritance, the author explains the variation in height following hybridization between pole and bush beans as due to the modification of the expression of a unit factor by the presence or absence of a number of factors producing other effects (as, for example, the effect of the determinate habit of growth on the potential length of internodes and on the number of internodes, etc.). However, the author disavows any intention of maintaining that this is the only possible explanation, and suggests that it may have to be modified to suit the results of further selection and hybridization experiments.—WILBUR BROTHERTON.

Philippine forests.—Our knowledge of the economic importance and the environmental conditions of some tropical forests has been advanced by a

recent publication,[8] the joint product of a botanist and a forester. The former seems to have contributed many details concerning the floristic and ecological composition of the many variations in the dipterocarp forest. The quantitative data regarding the physical climatic factors are among the first to be collected in tropical forests according to modern methods. Soil moisture determinations for every month in the year, although unfortunately not accompanied by the wilting coefficient of the soil, show that the soil is quite uniformly moist throughout the year. Atmometer records throughout the year give for the first time the data for an adequate comparison with the evaporating power of the air in mesophytic forests elsewhere. In the dipterocarp forests of Mount Maquiling the maximum, minimum, and average daily rates of evaporation upon the floor of the forest are respectively 5.3, 0.7, and 2.5 cc., as compared with 10.6, 3.3, and 7.1 cc. obtained by the reviewer[9] in the mesophytic beech-maple forest of northern Indiana. The evaporation data are especially good because they are given not only for the floor of the forest but also for the second story trees, where there is protection by the general canopy of foliage, and give a maximum, minimum, and average daily rate of 7.5, 1.8, and 5.3 cc. respectively, and for the atmosphere above the tree tops, where the maximum, minimum, and average daily rates are 22.1, 8.4, and 15.7 cc. The leaves of the tree tops are thus exposed to an evaporating power of the air 6 times as great as that obtaining for the ground vegetation.

The forester's part of the report contains many data of the distribution, composition, volume, and rate of increment of these forests. The results show that they may, when cut and logged by modern methods, make a very important contribution to the lumber supply of the world. In this connection it is interesting to note that the average rates of growth of the dipterocarps are about the same as those of the hardwoods in the central deciduous forest region of the United States; while one of the most rapid growers, *Parashorea plicata*, appears to grow about twice as fast as *Liriodendron*. The relative advantages of various cutting systems are discussed, and the opinion expressed that planting of dipterocarps is not likely to be successful.

This article, together with the earlier reports of WHITFORD, gives a good general ecological knowledge of these interesting forests, and should furnish a good basis for the rapid evolution of methods of forestry which will render these natural resources a permanent source of wealth for these islands.—GEO. D. FULLER.

Distribution of species.—WILLIS in two recent papers[10] attempted to show that the geographical distribution of species within Ceylon is to be explained, not by natural selection, but by the relative local age of each species, basing his

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[8] BROWN, W. H., and MATHEWS, D. M., Philippine dipterocarp forests. Phil. Jour. Sci. Sect. A. **9**:413–561. *figs. 16*. 1914.

[9] BOT. GAZ. **58**:193–234. 1914.

[10] Rev. in BOT. GAZ. **61**:82. 1916; **62**:160. 1916.

argument upon statistics. In accordance with his theory he made a number of predictions as to the distribution of species in New Zealand. These predictions have been verified by statistics which he has collected there, and which he presents in his latest paper, furnishing a very striking verification of his theory.[11]

Supposing a given species to have entered the islands at a certain point, and spread at an even rate, the area of its distribution at any time would be a measure of its local age. It is reasonable to suppose that this species would give rise to endemics, in increasing number as time went on, and as the area occupied became greater. At the limits of the islands farthest from the point where the species entered, the local age of the species, and consequently the number of endemics to which it had given rise, would be least. Following out such a conception, WILLIS predicted that the middle zones of the islands should show a greater number of endemics than the outer zones, and this proved to be the case.

Following the same line of thought, those endemics which were produced early would have most nearly reached the limits of the islands in their distribution, while those produced later in the local history of the parent species would be more limited in their distribution to the middle zones of the islands. Consequently, the author predicted that "the range of an endemic species would on the average be greater the nearer that one of its limits was to either end of the islands." This also was verified.

Another prediction made and verified was that widely distributed species would be more widespread within the islands than endemics, as in the case of Ceylon. On the basis that the land connection with New Zealand both ended earlier and began earlier than that with Ceylon, the author predicted that the average area occupied by a species in New Zealand would be greater than in Ceylon, that is, that both "wides" and endemics would be comparatively fewer in the lower or earlier stages in the scale. These predictions also were verified.

Those who wish to examine the exact mathematical statement of the author's method and conclusions are referred to the paper.—MERLE C. COULTER.

A floating reed swamp.—Occurring in the delta of the Danube River is a remarkable form of floating swamp formed by the reed *Phragmites communis* var. *flavescens* Gren. and Godr. It has been described by Miss PALLIS,[12] who visited and studied it in 1912 and again in 1913. She found that this swamp, known as Plav, differs from a closed reed swamp chiefly in the fact that it floats, the surface of the mat of soil and vegetation remaining constantly about

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[11] WILLIS, J. C., The distribution of species in New Zealand. Ann. Botany **30**: 4–457. *fig. 1.* 1916.

[12] PALLIS, MARIETTA, The structure and history of Plav, the floating fen of the delta of the Danube. Jour. Linn. Soc. **43**:233–290. *pls. 11–25.* 1916.

4 cm. above the fluctuating surface of the water. These fluctuations of the water level are great, as there are usually 3 floods each year, 2 in spring and 1 in autumn, the water at such times rising 1–6 m. The floating mat is made up almost entirely of vertical rhizomes of the reed, which, with the aid of their roots, retain much soil, the whole attaining a thickness of 0.8–2 m. The aerial shoots vary in height from 1.2 m. to 5.15 m. This mat originates attached to the soil, but becomes floating with the death of the basal rhizomes and the action of such floods as are accompanied by only small depositions of silt. The maximum size of units becoming detached is given as 2500 sq. m. In the shallower water much of the reed mat remains permanently attached. Little other vegetation is mingled with the *Phragmites*, its only competitor being *Typha angustifolia*, which is apparently only able to inhibit its growth for a short time. The reed seems to be succeeded by *Cladium mariscus* or by an aggregation of species of *Carex*.

The most remarkable part of this paper is the hypothesis offered to explain the difference in size of the reed, varying as it does from 1.2 m. to 5.15 m. This Miss PALLIS ascribes, not to any difference of variety, but to a difference in age. She believes that the giant shoots, 5 m. in height, have arisen earliest and at the base of the branch system of the rhizoids, and that with progressive advancement toward the higher parts of the branch system the aerial shoots have become gradually smaller and shorter. The change in size is thus a senile degeneration which ultimately results in the death of the individual. Unfortunately, the necessary experimentation to prove this theory would extend over many years and hence could not be undertaken. Many of the facts appear to support Miss PALLIS' hypothesis, and most of her argument seems sound, but some of the evidence seems to point to overcrowding being at least one factor in the reduction in size of shoots. More data regarding the germination and early growth of the reed should shed light upon the question of the duration of life of the *Phragmites* and its final senescence and death.—GEO. D. FULLER.

Taxonomic notes.—DUCKE,[13] in a presentation of new and little known plants of the Amazon region, discusses 146 species, 102 of which are Leguminosae. There are 34 new species described, 21 of which are Leguminosae. Among the new species there is a *Zamia* (*Z. Lecointei*). The paper appears in the initial number of a journal issued by the Botanical Garden of Rio de Janeiro.

GREENMAN[14] has published the second part of his monograph of *Senecio*, including the AUREI (§ 6). He recognizes 48 species and describes 5 of them as new. Of the new species, 2 are from the region of Newfoundland and

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[13] DUCKE, A., Plantes nouvelles on peu connues de la région amazonienne. Archiv. Jard. Bot. Rio de Janeiro 1:1–159. *pls. 19.* 1915.

[14] GREENMAN, J. M., Monograph of the North and Central American species of the genus *Senecio*. Part II. Ann. Mo. Bot. Gard. 3:85–194. 1916.

northern Maine, 2 from Utah and Nevada, and 1 from Mexico. In addition to the full descriptions and synonymy, the citations of stations and exsiccatae are very complete.

GRIFFITHS[15] has described 9 new species of *Opuntia*, which have been growing under his observation for 5–8 years.

PITTIER[16] has published a revision of *Inga*, a large American genus of leguminous trees, which has not been revised since 1875. He recognizes 212 species, 40 of which are new, representing 5 sections, which are further subdivided into series.

RENDLE[17] has published *Maidenia* as a new genus of Hydrocharidaceae from West Australia. It is a delicate water plant 5–6 cm. high, covered with numerous threadlike leaves, and belongs to the Vallisnerieae.

WERNHAM,[18] in a seventh paper on the Rubiaceae of the American tropics, has published an analytical key to the genera. The extensive display of Rubiaceae in this region is indicated by the fact that 182 genera are recognized, distributed among 21 tribes.

WRIGHT[19] has published a new genus (*Thuranthos*) of Liliaceae from South Africa, related to *Drimia* Jacq.—J. M. C.

Excretion of acids by roots.—HAAS[20] has taken up the much controverted question, do roots give off acids other than carbonic? He grew roots of early sweet corn in distilled water for 5 and for 19 days and tested the H+ concentration of the water against standard buffer solutions of phosphates with phenolphthalein as the indicator. He concludes that no acid other than carbonic is excreted by roots, but that decay of the roots does give a slight increase in the alkalinity of the water. The author says "The problem is important not only because acids dissolve plant food from the soil, but also because it involves the fundamental questions of the reaction of protoplasm and of the mechanism of excretion." This is true, but to answer the question in a way applicable to natural conditions one should not put them in the abnormal conditions offered by distilled water.[21] One might also expect the

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[15] GRIFFITHS, DAVID, Additional species of *Opuntia*. Bull. Torr. Bot. Club **43**: 523–531. *pl. 30*. 1916.

[16] PITTIER, HENRY, Preliminary revision of the genus *Inga*. Contrib. U.S. Nat. Herb. **18**:173–223. *pls. 81–105*. 1916.

[17] RENDLE, A.B., A new genus of Hydrocharidaceae. Jour. Botany **54**:313–316. *pl. 545*. 1916.

[18] WERNHAM, H. F., Tropical American Rubiaceae. VII. Jour. Botany **54**: 322–334. 1916.

[19] WRIGHT, C. H., Diagnoses Africanae. LXIX. Kew Bull. 1916:no. 9. p. 233.

[20] HAAS, A. R., The excretion of acids by roots. Proc. Nat. Acad. Sci. **2**:561–566. 1916.

[21] TRUE, R. H., The harmful action of distilled water. Amer. Jour. Bot. **1**:255–273. *fig. 1*. 1914.

author to relate his work to the rather extensive work done on the differential absorption of ions by plant structures and the resulting changes in the reaction of the substratum.[22] This promises explanation of the corrosive action of roots, their great power to absorb salts from soils, as well as their ability to redden neutral litmus. On account of this process some method other than that used by the author will probably need to be employed for investigating acid secretion in natural growth conditions, in the presence of nutrient solutions or soil. The value of this work as a basis for a general conclusion is doubtful, considering that only two experiments were performed on a single species, and these in an abnormal condition.—Wm. Crocker.

Subantarctic and New Zealand floras.—Skottsberg[23] has continued the series of comparisons made between the floras of portions of the southern hemisphere characterizing the previous work of Hooker, Diels, Schimper, Werth, Cheeseman, and Chilton, and revising the list of bicentric types by taking recent additions to the flora of Subantarctic America and New Zealand into consideration. The list includes 49 orders. These may be referred to groups comprising (1) an Australian and New Zealand element in America, (2) an Andine element in New Zealand and Australia, and (3) an old Antarctic element which is more strictly bicentric. Of the last group *Nothofagus* is a striking example, with 6 species in New Zealand, 1 in Tasmania, 1 in Tasmania and New South Wales, 1 in New South Wales, and 8 in Chili with 3 extending to Fuegia.

He includes some recent evidence from fossil plants found in Graham Land, and concludes that there existed an Antarctic Tertiary flora resembling the present floras of Subantarctic America, New Zealand, and Australia, and that the Antarctic continent may have been a center of evolution from which plants and animals wandered north. The present flora is due therefore to a combination of old wanderings, the extinction of certain species during the Ice Age, the survival of others, and finally transoceanic migrations, which, if they ever took place, are still going on.—Geo. D. Fuller.

Subalpine plants of the Rocky Mountains.—Adding to a series of phytogeographical papers upon the Rocky Mountain region already noted,[24] Rydberg[25] has analyzed the subalpine flora of the region. It consists of about 800 species, of which only 10 per cent are entirely restricted to the subalpine zone. About 20 per cent of the whole number are transcontinental plants,

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[22] Skene, M., The acidity of *Sphagnum* and its relation to chalk and mineral salts. Ann. Botany **29**:65–87. 1915.

[23] Skottsberg, Carl, Notes on the relations between the floras of Subantarctic America and New Zealand. Plant World **18**:129–142. 1915.

[24] Bot. Gaz. **62**:83–84. 1916.

[25] Rydberg, P. A., Phytogeographical notes on the Rocky Mountain region. VI. Distribution of the subalpine plants. Bull. Torr. Bot. Club **43**:343–364. 1916

while another 20 per cent are found also in the Pacific mountains, leaving 60 per cent peculiar to the Rockies. Of these, fully one-half are restricted to the southern Rockies, and less than one-fourth to the northern Rockies. Of the locally endemic species, which are all herbaceous, 6 are confined to the Canadian Rockies, 3 to Montana, 3 to Idaho, 14 to Wyoming, 13 to Utah, and 16 to Colorado. *Viola biflora* is noted as having the most remarkable distribution, having been found only in a few places in Colorado, in Alaska, and in Europe.—GEO. D. FULLER.

A polycotyledonous bean.—HARRIS[26] has secured a race of the common garden bean which shows steadily more than 2 cotyledons as tested by 3 offspring generations, comprising thousands of individuals. Since the race appears in a "pure line" and has remained constant in several differential features, he concludes that its origin and behavior are characteristic of mutation as defined by DEVRIES. The cotyledons are highly variable in number, ranging from 2 to 7, but have a modal frequency of 4. For this reason the embryo is described as tetracotyledonous. This persistent tendency of a dicotyledonous type to develop polycotyledony is an interesting confirmation of the claim that the number of cotyledons developed depends upon conditions rather than upon inevitable inheritance.—J. M. C.

Illinois Academy.—The volume of *Transactions* of the Illinois Academy of Science for 1915 has just appeared. It contains the following botanical papers: Comparison of a Rocky Mountain grassland with the prairie of Illinois, by GEORGE D. FULLER; Studies in *Phyllosticta* and *Cercospera*, by ESTHER YOUNG; Method of prophesying the life duration of seed, by JAMES E. GROVES; Peculiar examples of plant distribution, by H. S. PEPOON; The grass flora of Illinois, by EDNA MOSHER; A Florida smut, *Ustilago sieglingiae*, in Illinois, by MARGARET MEHLHOP. A symposium on colloids includes the following papers: Outline of the chemistry of colloids, by D. A. MACINNES; Significance of colloidal chemistry in physiology, by WILLIAM CROCKER.—J. M. C.

Bog theories.—The vegetation of peat bogs exhibits such remarkable peculiarities of habit and structure that it has called forth a number of varied and somewhat conflicting explanatory theories. These theories have been summarized carefully by RIGG,[27] especially in so far as the xerophily of the plants is concerned, in a manner that is likely to prove very useful. A good bibliography adds to the value of the paper.—GEO. D. FULLER.

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[26] HARRIS, J. ARTHUR, A tetracotyledonous race of *Phaseolus vulgaris*. Mem. N.Y. Bot. Gard. **6**:229–244. 1916.

[27] RIGG, G. B., A summary of bog theories. Plant World **19**:310–325. 1916.

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THE
BOTANICAL GAZETTE

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DEVELOPMENT OF DUMONTIA FILIFORMIS[1]

II. DEVELOPMENT OF SEXUAL PLANTS AND GENERAL DISCUSSION OF RESULTS[2]

GRACE A. DUNN

(WITH PLATES XIX–XXII AND SEVEN FIGURES)

Introduction

Dumontia filiformis (Huds.) Grev. is a red seaweed which is widely scattered in the temperate zones. It has been reported as occurring on the Auckland and Falkland Islands (**2**), on the shores of Alaska, and is very common in northern Europe. This species was first found on the Atlantic coast of North America, by the writer, at South Harpswell, Maine, in June 1913. Tetrasporic and cystocarpic plants were collected at that time. Sterile plants were collected by THAXTER at Kittery Point, Maine, in April 1914.[3] These are the only two points on this coast where plants of *Dumontia* have been reported to occur.

In all probability *Dumontia* has become established on the coast at South Harpswell some time between 1909–1913. F. S. COLLINS collected at South Harpswell in the early part of July for 6 years (1902–1905 and 1908–1909) in the same pools in which *Dumontia* was abundant in July 1913 and 1914. He states that he has never found a single specimen of *Dumontia* in any of these pools, and if

[1] Botanical contribution from the Johns Hopkins University, no. 55.

[2] First paper entitled "The development of the tetraspores." Plant World, **19**: 271–281. *figs. 2.* 1916.

[3] Personal letter from F. S. COLLINS.

the plants were then present they must have been extremely scarce. The plants were very abundant in the early part of July 1913. If a few solitary plants were present in 1909, it is apparent that they must have multiplied rapidly in the following 4 years. It is highly improbable, therefore, that any plants of *Dumontia* were present at South Harpswell as early as 1905.

GREVILLE (1) in 1830 described fructifications which he had observed in *Dumontia filiformis*. These fructifications were attached to the inner surface of the wall of the thallus and consisted of "clusters of large ovate seeds." It is evident from GREVILLE's description and figures that these "seeds" were carpospores. KÜTZING (5) published illustrations and a very brief description of the tetraspores. HARVEY (2) pictures a group of carpospores and states that "clustered spores are common." THURET (17) refers to the antheridia of *Dumontia*, so at that time these bodies were known to exist. The writer has not been able to find any description of the antheridia. All the papers published on the red algae previous to 1883 dealt chiefly with the distribution and seasonal occurrence of the various genera and the gross morphology of the individuals. SCHMITZ's (13) paper in 1883 marks a greater step in advance in the study of the red algae than has since been made by any one investigator. Although his descriptions are not complete, his general conception of the structure of the female reproductive organs of *Dudresnaya*, *Gloeosiphonia*, and other members of the Cryptonemiales is essentially correct in regard to the cell history. His observations on *Dudresnaya*, *Polyides*, and *Petrocelis* concerning the behavior of the nuclei in the ooblastema filaments and auxiliary cells are correct. In *Gloeosiphonia* and some other genera SCHMITZ reports that the nucleus in the cell which forms the carpospores is the product of two fusions.

The structure of the female reproductive organs of the red algae is quite complicated. The auxiliary cell, the cell which produces the carpospores, in nearly all the genera is formed by the fusion of the cytoplasm of two or more cells. The behavior of the nuclei in these cells fusing to form the auxiliary cell proved to be a stumbling block to SCHMITZ and many other workers, some of whom regarded the nucleus in this cell as the product of as many as 6 fusions (HAUPT-

FLEISCH 4). The next epoch making paper in the study of the red algae was that by OLTMANNS (9). OLTMANNS worked out very carefully and in much detail the nuclear and cell history during fertilization and carpospore formation in *Dudresnaya*, *Gloeosiphonia*, and *Dasya*. OLTMANNS' chief contribution was the convincing evidence that the nucleus functioning in the auxiliary cell at the time of the formation of the carpospores is a descendant of the fusion nucleus in the carpogonium, and that no other nuclear fusion has occurred. OLTMANNS' descriptions are detailed and his illustrations are remarkably clear, but nevertheless some present day botanists question his observations concerning the absence of a fusion between the nucleus in the auxiliary cell and that nucleus which enters it from the sporogenous filament. These botanists are inclined to believe that in the members of the Crytonemiales, as in certain of the Ascomycetes, there are two nuclear fusions at the time of fertilization. *Dumontia* and *Dudresnaya* belong to the same family, Dumontiaceae, and it is to be expected therefore that the two genera will have similar reproductive organs. In view of the fact that OLTMANNS' results have been questioned by some workers, the present investigation of *Dumontia filiformis* was undertaken for the purpose of gaining all possible information concerning the behavior of the nuclei during fertilization and the formation of the carpospores. It was also desired to gain information concerning the general structure of this alga, the cytology of its tetraspores, and the structure of its male reproductive organs.

This study was begun in June 1913, at the Harpswell Laboratory, South Harpswell, Maine, where the plants were abundant. It was continued during 1913, 1914, and 1915 at South Harpswell and at Johns Hopkins University.

The writer wishes to thank Professor J. S. KINGSLEY for the privileges of the Harpswell Laboratory, and also Dr. M. A. HOWE and Mr. F. S. COLLINS for identifying this alga. This investigation was undertaken at the suggestion of Professor D. S. JOHNSON, under whose directions it has been carried out, and whose criticisms have been a constant source of aid. Dr. W. D. HOYT also has kindly examined many of the preparations.

Methods

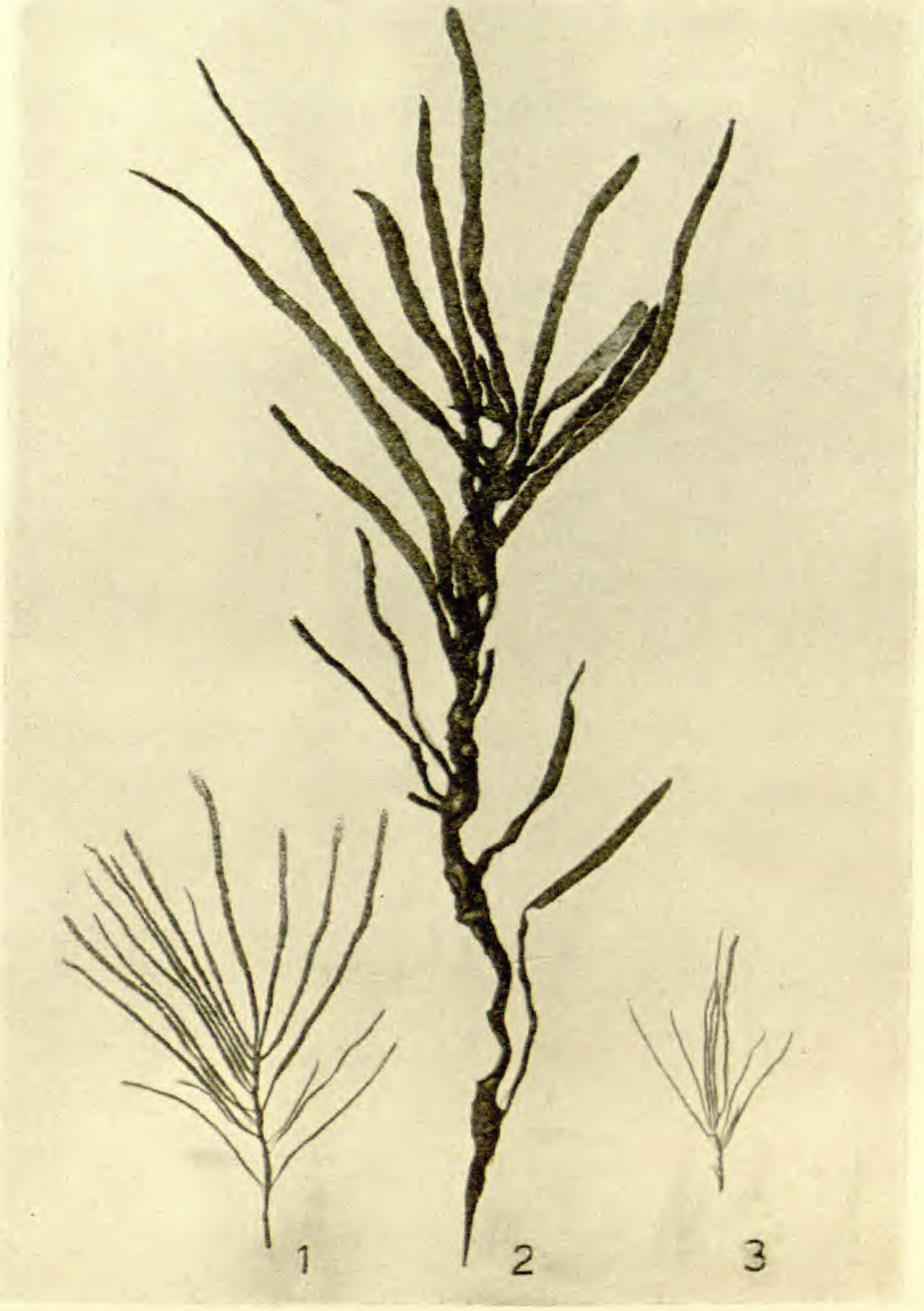
Plants of this alga, either whole or cut into lengths of 5–10 mm. each, were fixed in medium chromo-acetic solution, or in Flemming's fluid, within a few minutes after being collected. As the alga is very gelatinous, great care was taken that all changes in the alcohols should be made very gradually. The material on which the alcohol was changed in 5 per cent grades showed considerably less shrinkage than that on which the changes were made in 10 per cent grades. Most of the paraffin sections used were 10 or 12 μ thick. Sections 2 μ thick were also used for cytological details. For staining, Heidenhain's iron alum hematoxylin (1 hour in alum solution, 2 hours in hematoxylin) gave the best results. Acid fuchsin and methyl green stained the spores very well, but were not satisfactory for the vegetative structure. The triple stain, safranin, gentian-violet, and orange G, was also used. The slipping from the slide of sections of material fixed in Flemming's fluid occurred somewhat frequently in consequence of bleaching the sections in hydrogen peroxide. This difficulty was finally largely overcome by dipping the slides into 0.5 per cent solution of celloidin in a mixture of equal parts of alcohol and ether.

Description

HABITAT AND APPEARANCE

Dumontia, at South Harpswell, grows in abundance in tufts in the small tide pools and also on the rocks that are exposed to the air at low water. On large round rocks which were much exposed to the surf, female and tetrasporic plants of *Dumontia* were found growing down almost to the lower limit reached by *Chondrus crispus*, that is, just below the mean low water level. There is considerable variation in the size of the plants. The larger plants were found in the more exposed places. The plants in the tide pools near the low water mark were larger than the plants in the higher pools, and the largest plants of all were those growing at low levels on the round rocks. The color of the plants varies from a rich dark red to a pale reddish yellow. Mature tetrasporic and female plants ranged in height from 4 cm. to 23 cm. There is

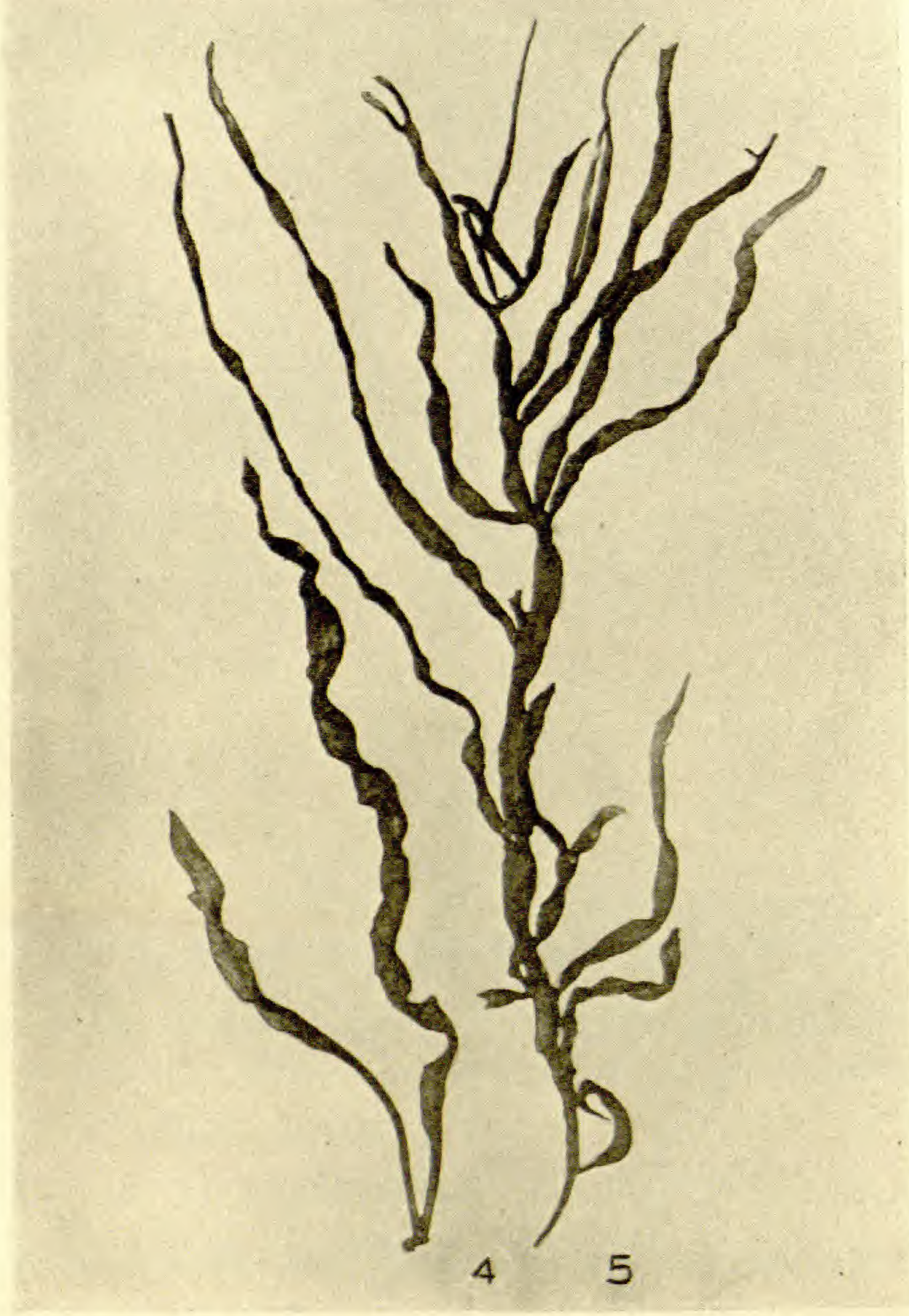
apparently no regular or constant system of branching, and the number of branches present is not related to the height of the plant (figs. 1–7). The plants shown in figs. 1 and 2 have almost



FIGS. 1–3.—Mature female plants showing cystocarps imbedded in thallus

the same number of branches, and their respective heights are 7 cm. and 19 cm. All the cystocarpic plants found were branched. Tetrasporic plants were found which were 12 cm. in height and

were unbranched. The female plants evidently attain practically their maximum size before the carpogonial branches are initiated. The average size of the female plants collected on April 12 was the



FIGS. 4, 5.—Mature tetrasporic plants branched and unbranched showing fraying out of thallus at apices of branches and main axis; ×0.6.

same as that of the mature cystocarpic plants collected in June. Some of these plants collected in April bore only young carpogonial branches, while others bore mature branches of this type and

auxiliary cell apparatuses in the upper portion of their thalli. Carpogonial branches therefore were probably initiated on these plants only a few days before they were collected. The average

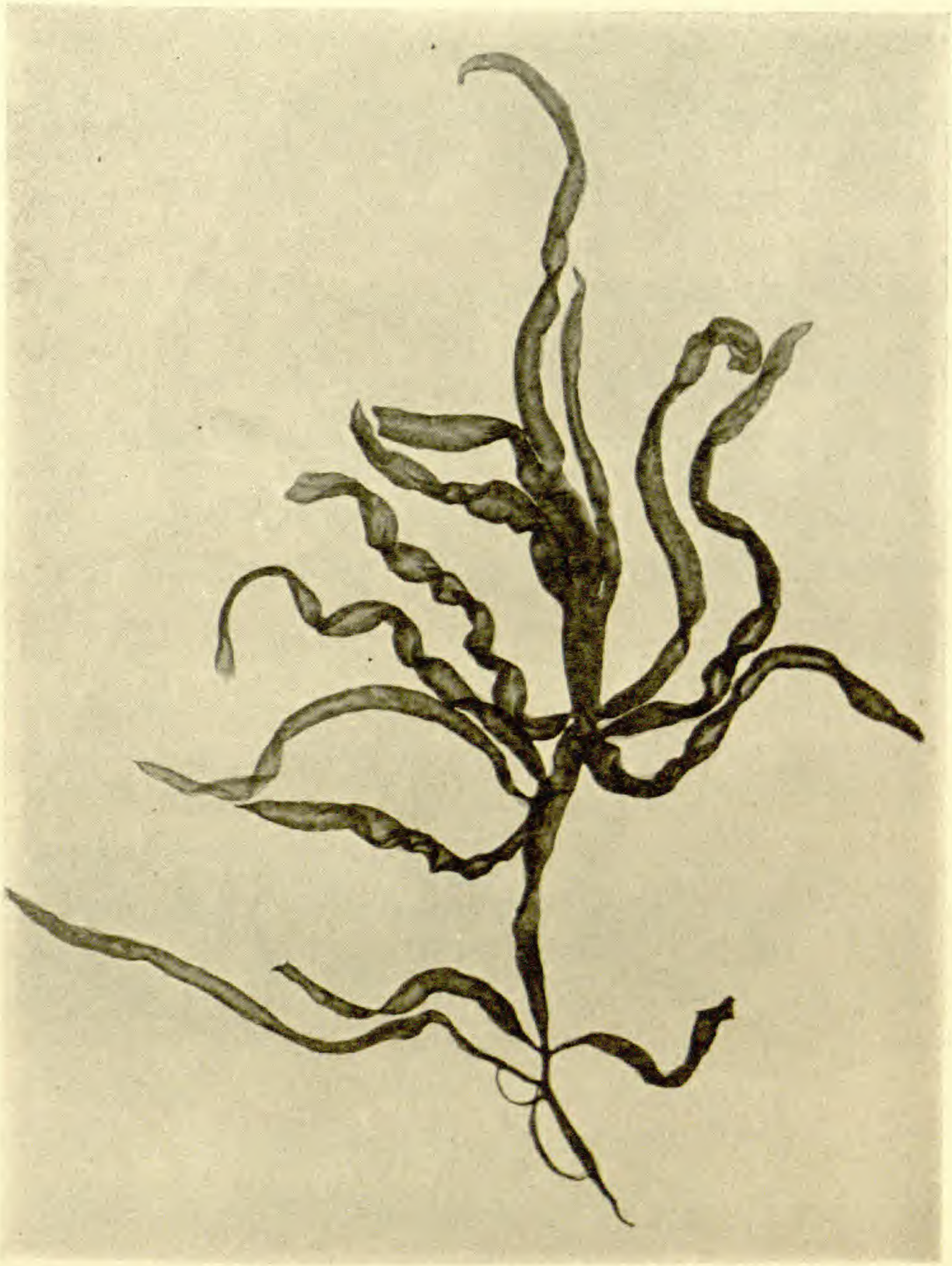


FIG. 6.—Tetrasporic plant showing much inflated main axis and branches; ×0.5

size of the male is less than that of the female plants. The maximum height of the male plants examined was 20 cm. They could be distinguished from the young female plants only by microscopical

examination. Female plants bearing mature cystocarps can readily be distinguished from the male and tetrasporic plants because the cystocarps form protrusions in the wall of the thallus (figs. 1–3).

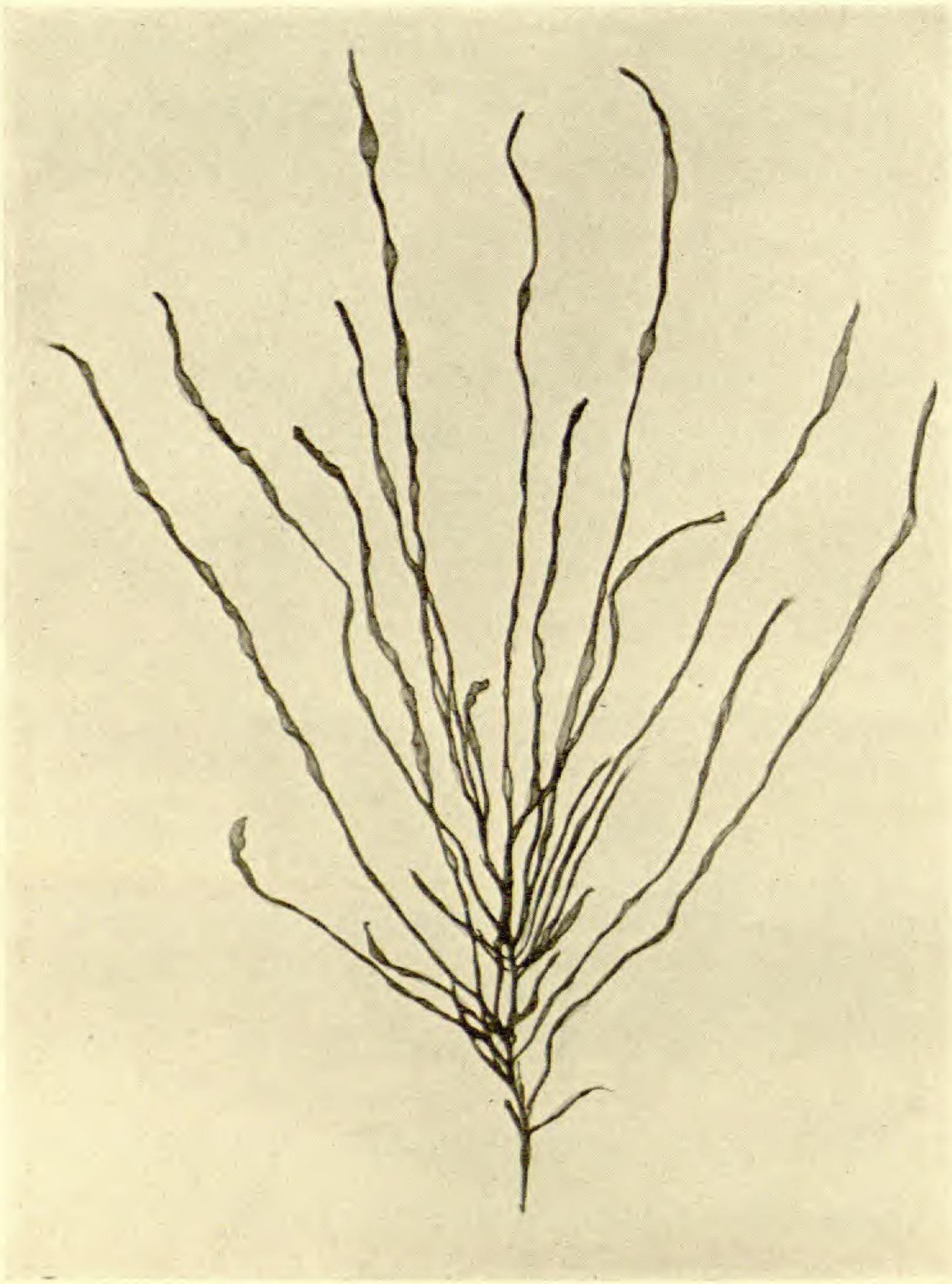


FIG. 7.—Tetrasporic plant showing large number of much twisted branches; ×0.4

The ends of the majority of plants collected in June and July were considerably frayed out (figs. 1, 3, 4, 6). Since growth is apical, the branches cannot increase greatly in length after the fraying has begun.

LEWIS (8) has shown that the sexual and asexual generations in most of the Florideae at Woods Hole differ physiologically, but are identical in vegetative structure, chromosome number of course excepted. These two generations in many forms appear at different seasons and have a tendency to grow on different kinds of substrata. The plants shown in figs. 2 and 5 are fair examples of well developed cystocarpic and tetrasporic plants of *Dumontia filiformis*. It is evident from a comparison of these plants that in this species also the two generations are morphologically almost identical. The tetrasporic and cystocarpic plants of *Dumontia*, so far as substrata on which they grow are concerned, appear to be physiologically identical also. The two kinds of plants grow together on all the large rocks and in most of the tide pools. The tetrasporic plants were more abundant on the whole than the cystocarpic ones, and a few pools contained only the former. These tide pools, however, were in every instance within 3 or 4 feet of apparently similar pools on the same level, in which both kinds of plants grew. The temperature of the water was taken in a number of the pools. It was found that the temperature of pools in the same vicinity did not vary more than 2° C. This difference existed between pools which contained tetrasporic plants only, as well as between these and those pools in which both kinds of plants were present.

SEASONAL OCCURRENCE

Male and young female plants of *Dumontia* were collected in the latter part of April 1914 and on April 12, 1915. An unsuccessful search was made for plants in January 1914. It is believed that the plants were not then present. The ratio of female to male plants in the collection made in April 1914 could not be determined, owing to the fact that the plants when examined were considerably broken up. The ratio of female to male plants in the collection of April 12, 1915, was 3 to 1. This ratio is based upon the examination of 24 plants collected from several different tide pools. Another collection of plants was made on April 26, 1915, and of these plants 24 were examined, all of which proved to be female. Of these 24 plants 10 bore only carpogonial and auxiliary cell branches, while 14 bore chiefly young cystocarps and auxiliary cell branches.

There were a few plants in the collection made on April 12 which bore no reproductive organs; these were probably female plants in which the carpogonial branches had not yet been initiated. All of the plants over 4 cm. in height, collected in June and July, with the exception of 2 or 3 individuals bearing carpogonial branches, bore either mature cystocarps or tetraspores. Hundreds of plants were collected and a careful but entirely unsuccessful search was made for individuals bearing spermatia. It seems evident from these facts that the male plants are present for only 2 or 3 weeks in April. It is possible, of course, that a few solitary individuals were present in June and July. This view is supported by the fact that it was possible to find on the female plants collected during this time all stages from the 1-celled carpogonial branch to the mature cystocarps. The cystocarpic plants reach their maximum development in the early part of June and have completely disappeared by the middle of July. The tetrasporic plants attain their maximum development in the latter part of June, although plants 19 cm. in height were very abundant as early as June 12. A few tetrasporic plants persist until late in August, but they are rare even in the latter part of July. The tetrasporic and female plants in all the red algae seem to be more numerous than the male plants. The experiments of LEWIS (7) with *Griffithsia Bornetiana* and *Dasya elegans* indicate that in both of these species the tetraspores form equal numbers of male and female plants. This is probably true of *Dumontia* and other members of the Florideae. The apparent scarcity of male plants may be due to the fact that in some forms they are exceedingly small and therefore are easily overlooked. This would not apply to such forms as *Dumontia*, however, in which the difference in the average height of the male and female plants is not more than 4–5 cm. SVEDELIUS (14, 16) reports that the male plants of *Martensia* and *Delesseria* die shortly after they have discharged their spermatia. This is probably true of the *Dumontia* plants also. LEWIS (8) has found that the tetrasporic plants of most of the red algae at Woods Hole are very abundant in July. The tetraspores germinate to form cystocarpic plants from which carpospores are released in September. The holdfasts of young sporelings formed from these

carpospores persist through the winter. These holdfasts, in the following June, produce adventitious shoots which develop into tetrasporic plants. LEWIS believes that this is in general the seasonal cycle of many of the Florideae, but states that "there are also exceptions to the separation in point of time of the two generations. This separation is never of a perfectly sharp and definite character, as the generations always overlap to a certain extent in midsummer."

The seasonal cycle of *Dumontia* at South Harpswell is evidently not similar to that of the algae just mentioned. The carpospores discharged in May and June apparently develop immediately into the tetrasporic plants which are present in June and July. This seems clear from the fact that the carpospores sometimes germinate even before escaping from the cystocarp, also that young tetrasporic plants 3–7 cm. in height were often found growing beside the stumps of the frayed off cystocarpic plants. Germination of the tetraspores has not been seen, but the occurrence of only male and female plants in the spring would indicate that this species must persist through the winter in the form of sporelings derived from the tetraspores discharged in June and July.

VEGETATIVE STRUCTURE

The holdfast of *Dumontia* is a platelike body composed of a single layer of horizontal filaments, each cell of which produces an ascending, vertical branch (figs. 8, 9). These vertical branches are closely packed together, averaging about 12 cells in length, and are very regular in form and arrangement. They are generally dichotomously branched. The cells of the horizontal filaments usually form no descending branches. The few branches of this character observed consisted of only one cell (fig. 8). It is evident from the size and arrangement of the cells in the ascending branches that they develop by apical growth.

A group of vertical branches in the holdfast elongates to form the upright portion of this alga (fig. 9). In such longitudinal filaments there is a gradual increase in the length of the cells. They are closely packed together at the base of the main axis, forming a solid tissue (fig. 9). At about 0.1 mm. above the holdfast these

longitudinal filaments or medullary hyphae separate, forming a cavity which extends nearly to the tip of the plant. The thallus of the plant is thus tubular in structure. The wall consists of 3 tissue layers. The inner layer is composed of 3 or 4 vertical rows of medullary hyphae. Each cell of a medullary hypha produces a radial branch. These radial rows of cells by repeated dichotomous branching, in planes parallel and perpendicular to the surface of the thallus, form the subcortex and the cortex (fig. 10). A branch arising from a cell of a medullary hypha terminates in 64–128 cortical cells. The cells in the inner subcortex are not closely packed together. The number of cells in a given area increases as a result of the repeated branching, and thus a compact cortex is formed. The 4 figures for each cell type in the following table indicate the two diameters of the cell as seen in a longitudinal section of the thallus, also the diameters of the nucleus and nucleolus.

Medullary hyphae.....	52.2 μ	8.7 μ	2.7 μ	1.0 μ
Larger subcortical cells.....	31.5 "	21.5 "	2.7 "	1.0 "
Smaller subcortical cells....	11.2 "	9.1 "	1.8 "	0.8 "
Cortical cells.....	8.4 "	7.0 "	2.8 "	1.4 "

In addition to the radial branches forming the subcortex and cortex, the medullary hyphae may give rise to other branches which remain axial and thus form longitudinal filaments. The medullary hyphae at the tips of the branches and the main axis terminate in a number of short branches composed of small cells (fig. 11). No single initial cell could be recognized at the apex of any branch in *Dumontia*. Branches varying in length from 2 mm. to 4 cm. were examined. The structure of the apex of a branch of *Dumontia* appears to be similar to that of *Furcellaria* (WILLE 18). *Furcellaria* is cited by both WILLE and OLTMANNS as a good illustration of the "Spring-brunnen" type of vegetative structure (OLTMANNS 9). The holdfast of *Dumontia* also resembles that of *Furcellaria*. Each of the medullary hyphae in *Dumontia*, as well as each of the lateral branches arising from these hyphae, has its own initial cell. Practically all the vegetative cells of *Dumontia* are uninucleate. All the chromatin in the resting nucleus is in the nucleolus. All the vegetative cells in the thallus, with the possible exception of a

few cells in the lower layers of the holdfast, contain but one chromatophore. The chromatophore is a clathrate hollow ellipsoid lying just inside the cell wall (fig. 12). It is similar to the peripheral portion of the chromatophore figured by WOLFE (19) in *Nemalion*. The chromatophore in some cells was seen to be enveloped by a thin layer of cytoplasm in the form of a coarse net. This cytoplasmic envelope, although not always visible, was undoubtedly present in all the cells.

Intercellular connections, such as are characteristic of the Florideae, are present between all the vegetative cells and all the sexual reproductive cells, including the carpospores, until they are almost mature. At each intercellular connection there are two similar disks joined by an apparently homogeneous strand of cytoplasm. The cytoplasm appears to penetrate these disks, but the matter has not been thoroughly investigated. The disks stain readily with hematoxylin, and in some cells appear to be composed of granules (fig. 13). One case was seen in which a strand of cytoplasm $4\,\mu$ wide connects two carpospores $22\,\mu$ in diameter (fig. 14). In this strand of cytoplasm are several granules having an average length of $0.7\,\mu$. These granules stain with the same intensity as the disks. It is probable that these granules would collect together to form the two disks when the strand of cytoplasm has assumed its normal size. Trichomes are found on all parts of the surface of the thallus. They seem to be most numerous at the base of the frond. They are present on the male, female, and tetrasporic plants. The trichomes are very abundant on the young plants collected in April.

SPERMATIA

Definition.—If the following discussion is to be intelligible, it will be necessary to define the terms which will be used in the description of the male reproductive organs. Those cells which are analogous to the sperms of the green and brown algae will be designated as spermatia. YAMANOUCHI (20), writing of *Polysiphonia*, calls these cells sperms, but "spermatia" is the term which has been most widely used by workers on the red algae and is therefore to be preferred.

SCHMITZ (13), WOLFE (19), and some other workers on the red algae have found that the spermatium is sometimes discharged as a naked protoplast. SVEDELIUS (14) therefore maintains that a distinction should be made between the free spermatium, the naked protoplast, and this same protoplast inclosed in a cell wall as it is when attached to the parent plant. He refers to the protoplast inclosed in the cell wall as the "spermatangium." The cell which SVEDELIUS refers to as the "spermatangium mother cell" is analogous to the "spermatium mother cell" of *Nemalion* (WOLFE 19).

Spermatium mother cells.—The spermatium mother cells of *Dumontia filiformis* are homologous to the outer cortical cells of the tetrasporic and cystocarpic plants. In a mature male plant of *Dumontia* almost all the outer layer of cells of all the branches and of the entire main axis from about 1 cm. above the holdfast consists of spermatia and their mother cells. The outer cortical cells of the main axis just above the holdfast are similar to those of the tetrasporic and cystocarpic plants. Although the distribution and position of the spermatium mother cells on the individuals of the different genera varies considerably, no other form has been reported in which they form a continuous layer over almost the entire thallus as they do in *Dumontia*. Each stalk cell in *Dumontia* bears at least two and probably more spermatium mother cells (fig. 15). The spermatium mother cell may bear two spermatia, just as it does in *Polysiphonia* (YAMANOUCHI 20), *Martensia* (SVEDELIUS 14), and *Delesseria* (SVEDELIUS 16).

A distinct chromatophore is certainly present in the stalk cell of the spermatium mother cell of *Dumontia filiformis* (fig. 15). A chromatophore is occasionally seen at the base of a spermatium mother cell borne on one of these stalk cells. The upper part of such a mother cell contains only granular cytoplasm (fig. 19). Many of the mother cells contain only cytoplasm and no chromatophores (fig. 15, first cell to right). Although it was not visible, a net of cytoplasm is undoubtedly present in the stalk cells as it is in all the vegetative cells of *Dumontia*. When the spermatium mother cell was first formed, it must have contained a chromatophore which had been cut off from that in the stalk cell. A large portion of the granular cytoplasm in the spermatium mother cell

was probably present originally in the chromatophore. The presence or absence of chromatophores in these cells could have been more readily determined if living plants had been available for examination. However, even in the preserved material it should be possible to distinguish the chromatophores from the cytoplasm. The protoplasm of the chromatophores is apparently homogeneous; they contain no visible vacuoles and have a definite outline. Many spermatium mother cells were seen which showed intermediate stages in the disappearance of the chromatophore and the formation of the granular cytoplasm (fig. 19).

OSTERHOUT (11) states that a reduced chromatophore is present in the young spermatium of *Batrachospermum*. This chromatophore disappears when the young spermatium matures. WOLFE (19) observed the division of the chromatophore in the spermatium mother cell of *Nemalion* in preparation for the formation of the spermatium. The chromatophore is for a time visible in the young spermatium and then disappears. Immediately after its disappearance a mass of deep staining cytoplasm is seen at one end of the spermatium. WOLFE believes that at least a portion of this cytoplasm has been derived from the protoplasm of the chromatophore. No other workers, with the possible exception of YAMANOUCHI, have seen chromatophores in the spermatia or in their mother cells. YAMANOUCHI (20) states that the sperm mother cells contain fine granular cytoplasm and generally no plastids. Chromatophores are present in all the genera, either in the immediate or somewhat remote ancestors of the spermatium mother cells. SVEDELIUS (14) states that he did not actually observe the disappearance of chromatophores in *Martensia*, but he believes that the protoplasm in the chromatophores of certain cells is used in forming the granular cytoplasm of their daughters which do not contain any chromatophores.

Spermatia.—No stages were seen showing a uninucleate spermatium mother cell. This cell in the earliest stages observed is binucleate (fig. 16). The first spermatium is cut off obliquely (figs. 15, 17, 19). The mother cell than elongates, again becomes binucleate (fig. 17), and a second spermatium is cut off. Many spermatia were seen in the swollen gelatinous sheath enveloping

the thallus and some which had actually reached the exterior (fig. 18). Every spermatium seen outside the parent plant is inclosed in a cell wall (fig. 41). No empty cell walls were seen attached to the spermatium mother cell. Many spermatia were seen lying close to the mother cells, but not attached to them (fig. 19). The spermatia in *Dumontia* are apparently cut off from the mother cell in the same manner as they are in *Polysiphonia* (YAMANOUCHI 20). The wall of the spermatium in both of these genera is a portion of the wall of the spermatium mother cell, and no body is formed which would be homologous to the spermatangium of *Delesseria* (SVEDELIUS 16). SVEDELIUS (14) believes that the spermatia in *Martensia* are set free in the same way as they are in *Polysiphonia*. LEWIS (6) states that in *Griffithsia* the spermatia are cut off from the mother cells. This form can hardly be compared with those previously mentioned, because in *Griffithsia* none of the cells of the antheridial filament form cellulose walls, but all are imbedded in the swollen wall of the mother cell of the branch.

The spermatia of *Dumontia*, as far as their contents are concerned, are similar to most of those which have been described in the other genera. The cytoplasm is much vacuolated at the proximal end of the spermatium and is very dense at the distal end. It is difficult to determine the structure of the nucleus, because it is situated at the distal end of the spermatium, imbedded in the dense, deep-staining cytoplasm. All the chromatin appears to be in the nucleolus or in several chromatin granules collected in the center of the nucleus (figs. 17, 19).

CARPOGONIAL BRANCHES

Nearly all the carpogonial branches found in the mature female plants were between the levels of 7.5 and 17.5 mm. from the holdfast. At levels higher up in the thallus, where mature cystocarps occur, a few carpogonial branches are occasionally present. These are not confined to one side of the thallus, but are scattered indiscriminately among the cystocarps. Young cystocarpic plants about 3 cm. in height were occasionally found even as late as July 5. The few cystocarps which were present on these plants were at the tips of the branches. Carpogonial branches were found in the

lower portions of the branches and in the main axis. The carpogonial branches in *Dumontia* evidently are not formed in acropetal succession. The carpogonial branches arise from the lateral branches of the medullary hyphae. They arise either from the basal cells of these subcortical branches or from cells intermediate in position between the medullary hyphae and the surface of the thallus. On the young plants every second or third large subcortical cell or occasionally each successive cell produces a carpogonial branch. Radial branches arise from the intervening cells. In mature plants, where carpogonial branches occur only at the base of the thallus, they develop from every fourth, fifth, or sixth cell. Sometimes the same cell will produce two carpogonial branches or one carpogonial branch and one radial branch (fig. 20).

A mature carpogonial branch consists of 6 or 7 cells and a trichogyne. If there are only 6 cells, they are all in a row. When the carpogonial branch is composed of 7 cells, one cell may be formed as a lateral outgrowth of the basal cell. For convenience and clearness the cells of the carpogonial branch will be numbered. The basal cell which is attached to the vegetative cell will be numbered 1, the cell above it 2, etc. The first cell of the carpogonial branches arises as a conical protrusion of the subcortical cell (fig. 21). A portion of the peripheral chromatophore of the latter is cut off in this protrusion. This first cell is uninucleate (fig. 22), and divides by a wall parallel to its base (fig. 23). The chromatophore in each of these young cells of the carpogonial branches is always peripheral, as it is in all the vegetative cells. The second cell next divides transversely, thus forming a 3-celled carpogonial branch (fig. 24). No data were obtained concerning the details of nuclear division in these earlier stages. This is due to the fact that these stages persist for only a short time, and that the cells are small and almost completely lined by the chromatophores. Considering the size and position of the cells in these young carpogonial branches, it is evident that it must be the terminal cell which divides each time. The cell wall separating the second and third cells was barely visible in some carpogonial branches which had just reached the 3-celled stage (fig. 24). These cells are separated considerably at a slightly later stage (fig. 25). In the 4-celled stage

also the 2 terminal cells are at first in close contact, but later become separated (fig. 26).

The cells of the carpogonial branch in the 4-celled stage may lie in a straight line, or the axis of the 3 terminal cells may form more or less of a right angle with that of the basal cell (figs. 27, 28). The nuclei here furnish evidence to support the assumption that the carpogonial branch develops by the repeated division of the terminal cell. In several cases the nucleus of this cell is considerably enlarged and is evidently just preparing for division (fig. 28). The chromatophores are much more openly clathrate in the 5-celled carpogonial branch than in the younger branches (fig. 29). One branch was observed in which the fifth cell, the terminal cell, was binucleate (fig. 30). Each cell in a carpogonial branch until it has reached the 5- or 6-celled stage generally contains one chromatophore. The one chromatophore then divides into a number of small parts which are connected by strands of cytoplasm (fig. 31). The structure and the arrangement of the cytoplasm and chromatophores at this stage appear to be very similar to those in the tetrasporangium and the tetraspores. The fate of most of the chromatophores in the cells of the carpogonial branch appears to be the same as that of those in the spermatium mother cells. The chromatophores disappear and at the same time the granular cytoplasmic contents of the cells increase. The protoplasm in the chromatophores is apparently used to form a part of the granular cytoplasm. There are generally present 2 or 3 chromatophores in each of the 3 or 4 basal cells, even after fertilization, when the sporogenous filaments are being formed (fig. 42). These chromatophores are hollow ellipsoids, like those in the tetraspores, but unlike the latter generally show no sign of being clathrate.

A large number of carpogonial branches were observed which bore short stumps or fairly long pieces of trichogynes (figs. 32–38). These trichogynes could often be traced almost to the surface of the thallus (figs. 35, 37). Other trichogynes were found which projected beyond the surface of the thallus and which could be traced back toward carpogonial branches (figs. 39, 40). Although no carpogonial branch was found in which the trichogyne could be traced from the carpogonium out beyond the surface of the thallus,

it is evident that this is actually its course. The failure to obtain a satisfactory section was due to the varying and indirect course of the trichogyne. Though most of the sections examined were 12 μ thick, the trichogyne nearly always passed out of the section and it was very difficult to locate it in the adjoining sections. The trichogyne, just beyond its point of attachment to the carpogonium, is often much coiled (figs. 34, 37, 40). The trichogyne is always surrounded by a fairly thick gelatinous wall which is a continuation of that of the carpogonium (figs. 34, 37). The granular cytoplasmic content of the trichogyne stains with the same intensity as does that of the terminal cells of the carpogonial branch. No structure was seen in any trichogyne which could positively be identified as a nucleus. In a few cases a body was seen which appeared to be similar to a nucleolus (fig. 39). This body is surrounded by a light area, but not by a definite membrane, and is therefore not thought to be a nucleus. There are present in some of the trichogynes (fig. 37) 2 or 3 masses which, with hematoxylin, stain like chromatin. The question of the presence of a nucleus in the trichogyne of the Florideae is still unsettled. SVEDELIUS reports that the trichogyne nucleus in *Delesseria sanguinea* disintegrates before fertilization and the chromatin granules pass out into the cytoplasm. It is possible that some of the granules seen in *Dumontia* and other forms are chromatin granules of similar origin.

There are two types of mature carpogonial branches. A cell is sometimes formed as a lateral outgrowth of the basal cell of the carpogonial branch (fig. 35). The cell thus formed is a supernumerary cell and will not be numbered, as it is not always present and has no special function. This supernumerary cell has never been observed in a carpogonial branch which is not mature. The basal cell of the carpogonial branch is often found to be binucleate (figs. 35, 38, 40) and sometimes contains as many as 3 nuclei (fig. 36). The basal cell is sometimes binucleate after having cut off the supernumerary cell (fig. 35). It thus appears that there is a tendency of the basal cell to form a lateral branch. Also the third cell of some of the carpogonial branches appears to be binucleate (figs. 33, 38). It is difficult to determine whether the nucleus in

these cells has actually divided or has merely elongated. The nuclei of cells 1, 2, and 3 of the carpogonial branch are often not in the resting condition, that is, all the chromatin is not in the nucleolus. The chromatin in these nuclei may be in one body surrounded by a number of small granules (fig. 32, cells 1, 2; fig. 38, cell 1), or in several small bodies (fig. 32, cell 3; fig. 36, cell 3; fig. 37, cell 3). The 3 terminal cells (4–6) are smaller than the first 3 or 4 cells and their nuclei are generally in the resting state. In many of the Delesseriaceae and Ceramiaceae some of the cells of the carpogonial branches contain two or more nuclei. The vegetative cells in these forms are multinucleate, and it is not surprising that this nuclear condition should occur also in cells of the carpogonial branches. In a form like *Dumontia*, where nearly all the vegetative cells are uninucleate, it is surprising that any cells of the carpogonial branch should contain more than one nucleus. However, the cytoplasmic contents of the carpogonial cells are much greater than those of the adjoining vegetative cells in proportion to their size, and the presence of an extra amount of chromatin in the larger cells of the carpogonial branches is quite in accord with the current belief of a definite relation in volume between cell and nucleus. The mature carpogonium lies close to or in contact with the third cell (figs. 32–38).

Only 4 trichogynes with spermatia attached to them were found in all the material examined. These were found in the material collected in April 1915. Although this number is small, it is not less than would be expected, since only a very few trichogynes were found projecting beyond the surface of the thallus. This may have been due to the fact that the mature trichogynes persist for only a short time, or that they are easily broken off. It is to be regretted that none of these trichogynes with the spermatia attached to them could be traced back to the carpogonium. In none of these cases was it possible to find even the carpogonium. In one case one spermatium had fused with the tip of a trichogyne, while 7 others were merely adhering to its sides (fig. 41). Judging from the way it stained, the cytoplasm in this one spermatium and in the tip of the trichogyne had begun to disintegrate. The other spermatia stained very lightly, and it was not possible to

distinguish the structure of their contents. The cytoplasm in all the trichogynes with the spermatia attached to them appeared to be disintegrated, and no trace of a male nucleus was seen in any of them. Disintegrating cytoplasm stains very deeply in vegetative cells which have been injured, in trichogynes which have functioned, in carpogonial branches which have not been fertilized but are destined soon to disappear (fig. 36), and in those cells of the auxiliary cell apparatuses which are terminal and will also soon disappear. The cytoplasm of the trichogyne would not disintegrate as soon as the male nucleus had entered it, so that this nucleus in all these cases had probably passed into the carpogonium. Only one spermatium was attached to each of the other 3 trichogynes.

It has always been extremely difficult to obtain clear evidence concerning the phenomenon of fertilization in the Florideae. A few workers, as OLTMANNS (9), OSTERHOUT (11), HASSENCAMP (3), WOLFE (19), YAMANOUCHI (20), and SVEDELIUS (16) have succeeded in finding consecutive stages showing the fusion of the spermatium to the trichogyne, the passage of the male nucleus down the latter, and the fusion of the male and female nuclei in the carpogonium. The only two members of the Dumontiaceae in which the structure of the female reproductive organs has been carefully worked out are *Dudresnaya purpurifera* and *D. coccinea* (OLTMANNS 9). OLTMANNS in *D. purpurifera* observed the entrance of the male nucleus into the trichogyne. The nucleus of the carpogonium at this time has moved out into the coiled portion of the trichogyne. No nucleus is present in the trichogyne in the next stage which he observed, but in the carpogonium there is a nucleus which he assumes to be the fusion nucleus. OLTMANNS states that he was not able to secure satisfactory evidence concerning the fusion of the male and female nuclei. He does not describe or picture fertilization in *Dudresnaya coccinea*, but states that it is in no way unusual. In *Dumontia* less evidence has been obtained concerning fertilization than OLTMANNS presented in the discussion of the two species of *Dudresnaya*. Nevertheless, there is really no reason to doubt the occurrence of fertilization in these forms.

As previously stated, the mature carpogonial branch is always bent around so that the carpogonium is close to or in actual contact

with the third cell. In many cases it lies very close to the second cell also. Thus the structure of the carpogonial branch suggests that the fusion nucleus passes from the carpogonium into the second or third cell. This evidently does occur, although satisfactory stages showing the process have not been found. Such figures as 42 and 43 show that the sporogenous filaments originate from either the second or third cells of the carpogonial branch. Since the actual passage of the fusion nucleus into the cell producing the sporogenous filaments has not been observed, there will naturally arise a question concerning the origin of the nuclei in these filaments. It cannot positively be stated that the nuclei in the sporogenous filaments are descended from the fusion nucleus of the carpogonium, but most of the evidence leads to this conclusion. Hundreds of carpogonial branches which have not been fertilized have been examined, and in only two or three cases is there any evidence that the third cell is binucleate. The second cell has never been observed to contain more than one nucleus. OLTMANNS (9) states that in *Dudresnaya coccinea* the cell of the carpogonial branch with which the sporogenous filament fuses is often binucleate, but that these nuclei never move out into the sporogenous filaments. Spermatia are found fused to trichogynes projecting beyond the surface of the thallus. Sporogenous filaments are found arising from cells of carpogonial branches whose trichogynes probably had projected beyond the surface of the thallus (fig. 42). The cells which produce the sporogenous filaments are those which in other carpogonial branches are always close to or in contact with the carpogonium. Considering these facts it seems highly probable in *Dumontia filiformis*, as in *Dudresnaya purpurifera* and *D. coccinea*, that the nuclei in the sporogenous filaments are derived from the fusion nucleus in the carpogonium. All the cells in the carpogonial branches stain very faintly at the time of the formation of the sporogenous filaments. The cytoplasm in all the cells, particularly the terminal ones, becomes very thin (fig. 42) and in some cases practically nothing but the cell walls is visible. The cytoplasm in these cells is disintegrating, but not in the same manner as it does in the trichogynes and some of the other cells. The failure to find the carpogonium may be due to the fact that it disintegrates

after it has discharged its nucleus. The carpogonium is much smaller than the cell which produces the sporogenous filaments, so that it might still be present, although not recognizable, after the fusion of the two cells.

The sporogenous filaments in *Dumontia*, according to SCHMITZ (13), grow out from the carpogonium and do not fuse with any cell in the carpogonial branch. This statement obviously is not correct. One cell of the carpogonial branch in *Dumontia* may produce three sporogenous filaments (fig. 42). A mass of fairly dense cytoplasm which always contains a nucleus and sometimes a chromatophore is present at the tip of each filament (fig. 42). The remainder of the filament appears to be entirely empty. The sporogenous filaments in *Dudresnaya purpurifera* (OLTMANNS 9) arise from the carpogonium and do not fuse with any cell in the carpogonial branch. A carpogonial branch in *D. purpurifera* and *D. coccinea* produces 2 or 3 sporogenous filaments. Each of these filaments in *D. coccinea* fuses with a cell of the carpogonial branch before growing out into the tissue of the thallus. All the cytoplasm in the 3 sporogenous filaments in *D. purpurifera* is derived from the carpogonium, in *D. coccinea* from the carpogonium and 3 other cells of the carpogonial branch, and in *Dumontia* from either the second or third cell of the carpogonial branch.

AUXILIARY CELL BRANCHES

The auxiliary cell branches of *Dumontia* have the same origin and the same distribution as the carpogonial branches, but they are not so numerous as the latter. The ratio of carpogonial to auxiliary cell branches, considering the average number of branches initiated on a plant, is approximately 7 to 1. The carpogonial branches are very numerous in certain regions, as at the base of the mature cystocarpic plant. The auxiliary cell branches are found to predominate over the carpogonial branches at slightly higher level on this same plant.

The mature auxiliary cell branches vary in length from 4 to 6 cells (figs. 44, 45). The basal cell, just as in the carpogonial branch, may cut off a supernumerary cell (figs. 43, 45, 46). The size of the cells and the mode of development of the auxiliary cell branch

are essentially similar to those of the carpogonial branch. The youngest auxiliary cell apparatus which could be distinguished from a carpogonial branch consists of 3 cells (fig. 47). The similarity of the two branches is very apparent. The terminal cell of the auxiliary cell apparatus as a rule is not as pointed as that of the carpogonial branch (compare figs. 44–53 with figs. 24–28). There are, however, exceptions to this rule (figs. 48, 54). This cell may not be pointed even when it is about to divide (figs. 52, 53). There is also some difference in the way in which the cytoplasm of the cells of the two branches stains. This difference is so slight that it can be used as a criterion in distinguishing the two kinds of branches only when they are in one section or in sections which have been similarly fixed and stained. The basal cell of the auxiliary cell apparatus is often binucleate (fig. 50) and sometimes contains 3 nuclei (fig. 48), as does the similar cell in the carpogonial branch (fig. 36). None of the cells except the terminal one was ever observed to be binucleate in a carpogonial branch which was not mature. The auxiliary cell branch shown in fig. 49 is not mature, and the second cell is binucleate. Fig. 50 shows an immature branch in which 3 cells are binucleate. Some of the cells in the auxiliary cell branches contain chromatophores similar to those in the cells of the mature carpogonial branches and in the sporogenous filaments (figs. 44, 55). The auxiliary cell is either the second or third cell of the branch (figs. 43, 45, 57–60, 63). The sporogenous filament with the nucleus in its end grows toward the auxiliary cell branch (fig. 54).

The sporogenous filament fuses with the auxiliary cell (figs. 43, 45, 56, 59). Some of the cytoplasm of the sporogenous filament undoubtedly fuses with that of the auxiliary cell. This appears evident from the fact that the end of the sporogenous filament always contains cytoplasm and in some cases terminates in the auxiliary cell (figs. 45, 56, 59). After the fusion of the sporogenous filament with the auxiliary cell, the original nucleus of the latter maintains its former position (figs. 45, 60, 63) or withdraws to one side (fig. 58) as in *Dudresnaya purpurifera* and *D. coccinea*. It has been stated that cells 2 and 3 of the auxiliary cell branch are occasionally binucleate. This binucleate condition in the auxiliary

cell is apparently of no significance, because the nucleus from the sporogenous filament enters here just as it does in the uninucleate auxiliary cell (fig. 57). OLTMANNS reports that the sporogenous filaments in *Dudresnaya purpurifera* and *D. coccinea* branch freely. These filaments in *Dumontia* apparently branch only occasionally (figs. 42, 43). In both species of *Dudresnaya* no septa are formed in the filaments except when they fuse with the auxiliary cells. When the septa do occur, they are formed in the filament on both sides of its point of fusion with the auxiliary cell. The tip of the filament may then grow on to fuse with 2 or 3 more auxiliary cells. In *Dumontia* only one case was observed in which a filament has actually fused with an auxiliary cell and does not also terminate in the cell (fig. 43). No septa were seen in this filament. A few filaments growing over auxiliary cells were observed, but in these cases there was no indication of any fusion (figs. 44, 53). The sporogenous filament in fig. 43 branches just before it terminates in the auxiliary cell.

CYSTOCARPS

Carpospore development is initiated by the formation of 3 or 4 gonimoblast filaments, of about 3 cells each, which arise successively from the lateral protrusion of the auxiliary cell. These filaments branch once, often twice, and every cell forms a spore (figs. 56, 59). The cells at first are uninucleate (figs. 56, 58, 59, 63). At a little later stage they become binucleate and divide (fig. 62). No sterile cells are present at the base of the gonimoblast filaments. The carpospores when first formed are rounded or subangular and about 11 μ in diameter. They are well filled with a spongy cytoplasm which contains many small vacuoles (fig. 63). No chromatophores are visible, but often a number of small dark staining granules are present. When the nucleus is in the resting state, all the chromatin is in the nucleolus. In the young cystocarp there are generally present 3 or 4 cells of the auxiliary cell branch (figs. 56, 58, 63), and sometimes as many as 5 (fig. 62). A portion of the auxiliary cell branch is often present even in the mature cystocarp (fig. 60). The wall of the cystocarp is formed by branches which grow out from these subcortical cells that have been displaced inward by the enlargement of the group of carpospores (fig. 63).

The growth of these branches which form the pericarp is similar to that of the ordinary subcortical branches.

On an average 9 carpospores are present in a median transverse section of a mature cystocarp. Often 3 or 4 cystocarps will crowd together, so that in a section they appear as one. The average diameter of the mature carpospores is 38 μ. When the carpospores are actually mature, they are well filled with cytoplasm, contain a large amount of Floridean starch, and a number of protein granules. These granules respond to the stain and to the protein test in the same way as those in the tetrasporangia. These protein granules when they first appear are small and very numerous. In one carpospore in a median section 12 μ thick there are 170 of these granules (fig. 64). The ringlike chromatophores, about 2.5 μ in diameter, first appear in the carpospore just before it is mature. They are not peripheral but are scattered throughout the entire protoplast. Chromatophores are often present in the sporogenous filaments and in the auxiliary cells, but have never been seen in the latter at the time the carpospores are formed. It is possible that chromatophores which do not take the stain are present in these cells, although it seems hardly probable that they could be completely overlooked, since the cytoplasm in the auxiliary cell is very thin and much vacuolated. It is generally believed that chromatophores never arise *de novo*, and SCHMITZ (12) has stated that they are always present in the spores of the Florideae. In other Florideae besides *Dumontia* the chromatophores are evidently not readily seen at this stage, since their presence in the young carpospores is rarely mentioned.

The protein granules in the mature carpospores often disappear just before the spores are discharged, and are never present in the germinating spores. Certain of the chromatophores increase greatly in their staining power coincident with the disappearance of these granules (fig. 65). There has evidently been some modification in the substance of these chromatophores, and it seems quite possible that the substance of the protein body is concerned with this change. The chromatophores in the mature carpospores which have thus become differentiated stain with the same intensity as those in the germinating carpospores and appear to have the same

structure as those in the mature tetraspores. The majority of carpospores in a mature cystocarp contain one large nucleus each (fig. 66). Occasionally a spore which is just about to escape contains 2 nuclei (fig. 67). The spore shown in fig. 67 was directly behind a spore which was just passing through the pore in the wall of the cystocarp. The fact that the nucleus divides in some of these carpospores just as they are escaping indicates that the spores germinate as soon as they are discharged. In fact, the spores sometimes germinate while still inclosed in the cystocarp. In most of the female plants collected, the tips of the main axis of the thallus and branches were frayed out. The mature carpospores are present at these points, and it is therefore evident that the disintegration of the cells surrounding them furnishes one possible means of escape. As the carpospores enlarge, they compress the surrounding vegetative cells on all sides, and also cause the wall of the thallus to bulge out. The layers of cortical and subcortical cells gradually become thinner on the bulging side of the pericarp, until finally they are ruptured and the naked carpospores escape through the pore thus formed (fig. 68, 1).

Groups of multinucleate cells, which are of the same size and have the same position as the normal cystocarps, occasionally occur in the wall of the thallus. In group 1, fig. 68, a section of a normal cystocarp, 16 spores appear to be present, but probably not all of these are in this one cystocarp. Similar sections of two groups of multinucleate cells on the other side of the thallus (2 and 3) contain respectively 30 and 70 cells. Some of the cells in the groups of multinucleate cells are uninucleate and of the same size as the mature carpospores (fig. 66), while others of approximately the same size or smaller contain 2 or 3 nuclei (figs. 67, 69, 71). In some cases nuclear division is followed by cell division (fig. 70). Evidently after one of these larger spores divides, the daughters may in turn become multinucleate (fig. 71). From the arrangement of some of the cells it appears as though the larger cells have divided to form the smaller ones. The number of nuclei in the cells of a cystocarp similar to group 3, fig. 86, does not seem to be determined by the size of the cells. Some of the smaller cells may contain as many as 11 nuclei and the larger ones only

1 or 2. These are certainly nuclei and not pyrenoids, since they were clearly distinguished by hematoxylin, safranin, or methyl green. Many of these cells are somewhat vacuolated, and none of them contains protein granules or visible chromatophores. No cases have been observed in which any of these cells are escaping from the cavity. It seems probable from all the evidence available that such a group as 3 is formed by division of the spores of a normal cystocarp, and 2 is an intermediate stage between 1 and 3. Each of these groups of multinucleate cells, therefore, is the product of an abnormal cystocarp.

Germination of the carpospores may begin long before they escape from the thallus. The first step in germination is the formation of a gelatinous wall 2 μ thick (fig. 72). The chromatophores in these spores were 3 μ in diameter and stained darkly. They are similar in structure to those in the tetraspore, but are larger and more openly clathrate (figs. 73, 74). The chromatophores are not merely peripheral, but, as in the younger carpospores, are scattered throughout the whole protoplast. The next step in germination is the elongation of the spore until it becomes somewhat pear-shaped (fig. 74). The nucleus then divides and the first cell wall is formed perpendicular to the longitudinal axis of the carpospore (fig. 75). The narrow cell, as in the germinating spores of *Fucus*, is destined to form the basal part of the young plant. Neither growth nor cell division takes place as rapidly here as in the upper cells. The next wall formed apparently divides the upper cell obliquely (fig. 76). In a longitudinal section of an older sporeling these two upper cells were divided into 9 cells and the lower cell into 3 cells. All the cells of these germinating carpospores are rich in cytoplasm and contain chromatophores. The maximum size of the sporelings examined was 235 μ by 123 μ. In cavities containing germinating carpospores traces of disintegrating cytoplasm and nuclei have been observed, showing that some of the unicellular carpospores have degenerated.

CYTOLOGY

The nuclei in the auxiliary cell and the carpogonial branches are the most satisfactory ones in the cystocarpic plants for the study of

mitosis, since they are considerably larger and divide more actively than the vegetative nuclei. The cell history of these branches is also of some aid in identifying stages in nuclear division. All the chromatin in the nuclei of most of the young carpogonial branches is in the nucleolus (figs. 22–30). This is true of the nuclei also in cells 4, 5, and 6 of the mature branches (figs. 32–34, 37). The nuclei in cells 1, 2, and 3 of the mature carpogonial branches have a tendency to divide. The failure to secure any stages of mitosis in the nuclei of the cells of the young carpogonial branches is probably due to the fact that these cells divide very rapidly. The chromatin in the nuclei in most of the uninucleate cells of the mature auxiliary cell branches is not in the nucleolus but in a number of small granules (figs. 48, 51–55). The nuclei in the cells of these branches divide often (figs. 46, 48–50). The frequency of division of these nuclei is probably due, as in the basal cells of the carpogonial branch, to the fact that these cells are usually completely filled with dense cytoplasm (fig. 51). Thus in the resting nuclei of the cells of the carpogonial branches, as in the tetraspores and vegetative cells, all of the chromatin is in the nucleoli.

The following changes are observed in the nuclei in preparation for division. Radial fibrillae appear running from the nucleolus to the nuclear membrane (cell 2, fig. 55). Small chromatin granules pass out from the nucleolus, along the fibrillae, to the nuclear membrane. When the granules first appear on the linin strands, there is no appreciable decrease in the size of the nucleolus. The position of these granules when they first appear indicates that they have come from the nucleolus. Of the 6 granules present in fig. 77, 3 are in contact with the nucleolus, and only 1 has yet reached the periphery of the nucleolus. Nearly all of the granules at a slightly later stage are present only at the points where the fibrillae terminate in the nuclear membrane (fig. 78). More linin strands are formed which connect the radial fibrillae already present (fig. 79). All of the chromatin evidently passes out of the nucleolus and becomes distributed along the linin net. The net disappears just before the nucleus divides (fig. 80). Practically all of the chromatin in the nuclei which have just divided is in 7 fairly uniform granules (cell 4, fig. 52). The nucleus of cell 4,

fig. 53, is evidently just dividing. All of the chromatin in the nucleus of this cell is in 14 granules of approximately the same size. Nuclei which are probably preparing for division often contain 7 similar chromatin bodies (cell 3, fig. 51; cell 4, fig. 55; fig. 81). It is thought that the chromatin bodies in these nuclei may be chromosomes. Nuclei in the earlier stages of division contain 16–24 granules (cells 1 and 3, fig. 52; figs. 79, 80). These granules must become grouped together to form the chromosomes. Thus possibly the haploid number of chromosomes in *Dumontia* is 7. However, it is evident that not enough data have been accumulated to determine with any degree of certainty the number of chromosomes. These larger chromatin bodies in some of the nuclei are vacuolated (cell 3, fig. 51). All the chromatin in the resting nucleus is in the nucleolus, hence the chromosomes must fuse together after division. It is quite possible that all the chromosomes do not fuse at one time. Thus in fig. 82 each of the 2 large chromatin bodies in the nucleus which contains 5 may have been formed by 2 chromosomes fusing together. If the fusing continued, the nucleus would appear quite similar to that in the adjoining cell. When the nucleus is being organized after division, the nucleolus appears granular, and a few small chromatin bodies may, for a time, remain outside of it (fig. 62). In the nucleus of the mature carpospore the linin net is well developed, and all the chromatin is in the nucleolus, which always contains at least one vacuole (fig. 66). Our knowledge of the details of mitosis in this species of *Dumontia* is as yet very fragmentary. The stage represented in cell 3, fig. 52, is similar to the prophase of *Delesseria* as described by SVEDELIUS (15). This cannot be the prophase in *Dumontia* because the granules present at this stage collect together to form larger units, probably chromosomes.

In *Polysiphonia* (YAMANOUCHI 20) and *Delesseria* (SVEDELIUS 15) the chromatin from which the chromosomes are formed is never contained in the nucleolus. It is distributed in fine granules along the linin threads. The granules are in groups or short rows, each one of which represents a prochromosome. A chromosome is then formed by the fusion of several granules. Mitosis in *Dumontia* up to the time of chromosome formation seems to be

exactly similar to that in *Nemalion* (WOLFE 19). Nearly all the chromatin in the resting nucleus of *Dumontia* is in the nucleolus, and, as in *Nemalion* and *Griffithsia* (LEWIS 6), this chromatin passes out along the fibrillae to the periphery of the nucleus. The number of granules present in *Griffithsia* and *Nemalion* seems to be about twice the number of chromosomes formed. This may be the case in *Dumontia* also, although in this form the number of granules seems proportionately larger. There is no indication of any chromatin being expelled from the nucleus of *Dumontia* as it is in *Griffithsia*.

Discussion and results

The auxiliary cell branch and carpogonial branch of *Dumontia filiformis* resemble each other very closely in origin, mode of development, and structure. This similarity is so great that in some cases it is almost impossible to determine the character of a branch. The number, arrangement, and contents of the cells may be the same in these two kinds of branches. The trichogyne persists for only a short time after it has functioned. Hence the absence of this structure is not a safe criterion for distinguishing the auxiliary cell branches. The carpogonial and auxiliary cell branches differ greatly from the vegetative branches in the size and contents of their cells. It seems quite possible that the auxiliary cell branches in *Dumontia* once bore trichogynes and functioned as carpogonial branches. This similarity in structure of the auxiliary cell and carpogonial branches is almost as marked in *Dumontia* as in *Dudresnaya coccinea*. The auxiliary cell branch of *D. coccinea* consists of 12 cells and the carpogonial branch of 7 cells (OLTMANNS 9); otherwise the two kinds of branches appear similar in origin and structure and differ greatly from the vegetative branches.

It has been stated that the auxiliary cell branches and carpogonial branches of *Dumontia* are probably homologous structures. If this is true, the sporogenous filaments were probably developed at the time when certain carpogonial branches ceased to be capable of fertilization. The male plants in this species of *Dumontia* are present for only 2 or 3 weeks during each spring. The ratio of the number of female to male plants at the time when the latter are supposed to be at the height of their development is 3 to 1.

An examination of scores of young female plants has shown that the trichogynes must persist for only a short time after they have reached the surface of the thallus. Under these conditions an arrangement whereby the fertilization of one carpogonium would make possible the development of 3–6 cystocarps would evidently be of considerable advantage to the plant. If the auxiliary cell branches are carpogonial branches which have ceased to function, *Dumontia* presents a case quite parallel to that of *Corallina*. In *Corallina* (OLTMANNS 10) only those carpogonia in the center of the conceptacle which bear long trichogynes are capable of being fertilized. The carpogonia at the periphery of the conceptacle cannot be fertilized because they bear no trichogynes. In each conceptacle often only one carpogonium is fertilized. Descendants of the fusion nucleus in this one carpogonium pass to the auxiliary cells of many procarps, and several cystocarps develop in one conceptacle.

Cell 2 or 3 of the carpogonial branch of *Dumontia* probably functioned as the auxiliary cell before the plant had acquired the habit of forming sporogenous filaments. It is cell 2 or 3 of the auxiliary cell branch which forms the carpospores. One of the 3 cells in the carpogonial branch of *Dudresnaya coccinea* with which the sporogenous filaments fuse, before passing to the auxiliary cell branches, at one time probably functioned as the auxiliary cell. The sporogenous filament often fuses with the fifth cell of the carpogonial branch and with the fifth cell of the auxiliary cell branch. In both *Dumontia* and *Dudresnaya coccinea*, therefore, that which is supposed to have been the original auxiliary cell and that which now functions as such occupy similar places in their respective branches. The families of the Cryptonemiales show a considerable variation in the distribution and structure of their auxiliary cell and carpogonial branches. Even the species in one genus as *Dudresnaya* (OLTMANNS 9) may vary greatly in this respect. It would then be rather surprising to find that the history of the development of the auxiliary cells in all the Cryptonemiales is similar. OLTMANNS (9) suggests that the sporogenous filaments of the Cryptonemiales have been developed from the gonimoblast filaments of such forms as *Wrangelia* and *Naccaria*. He considers

Nemastoma the transitional form between the Nemalionales and the Cryptonemiales. The auxiliary cells of *Nemastoma* do not occur in special branches, but are modified cells of the cortical hyphae. Thus in *Nemastoma* there are no auxiliary cell branches which can be considered as homologous with the carpogonial branches. But there is also the evidence which has been presented that the auxiliary cell branches in some forms as in *Dumontia* are not vegetative hyphae which have by chance become highly specialized in the same manner as the carpogonial branches. Thus the sporogenous filaments, structures which are peculiar to this one order, the Cryptonemiales, have probably been developed along two independent lines.

The female reproductive organs of the other red algae are relatively simple when compared with those of the Cryptonemiales. It is not surprising that the history of the nuclei in the sporogenous filaments and auxiliary cells of the members of this order was an especially puzzling problem to the earlier students. It has been stated in the introduction to this paper that the origin of the nucleus functioning in the auxiliary cells at the time of the formation of the carpospores proved to be a stumbling block to most of these students. The results of the work of SCHMITZ on certain genera of the Cryptonemiales, including *Dudresnaya*, *Dumontia*, and *Gloeosiphonia*, were conflicting in regard to the occurrence of a fusion between the sporogenous and auxiliary cell nuclei. OLTMANNS' (9) investigation established practically beyond doubt the two following facts: the sporogenous and auxiliary cell nuclei in *Dudresnaya* and *Gloeosiphonia* do not fuse, and the nuclei in the carpospores are descended from the sporogenous nucleus and not from the original auxiliary cell nucleus.

No member of the Cryptonemiales has been carefully investigated since 1898 in regard to the occurrence of a nuclear fusion in the auxiliary cell. A considerable amount of excellent work, however, has been done on other red algae during the last 17 years. The fusion nucleus or one of its daughters, in many genera, has been traced from the carpogonium into the auxiliary cell. The fusion nucleus in all these forms appears to take charge of the cytoplasm in the auxiliary cell and becomes the ancestor of the nuclei

in the carpospores. In none of these forms is the nucleus in the auxiliary cell reported to fuse with the nucleus entering it from the carpogonium. Thus HASSENCAMP (3), YAMANOUCHI (20), SVEDELIUS (14), and LEWIS (6) have found, in the forms studied by them, that only one nuclear fusion occurs during fertilization and the formation of the carpospores, and this is the fusion between the nucleus of the spermatium and that of the carpogonium. It would seem that the evidence is overwhelming against the occurrence of a second fusion in the auxiliary cell. Undoubtedly one reason why OLTMANNS' work has been questioned is the fact that certain morphologists have for years cherished the theory that a relationship could be established between the Ascomycetes and the Florideae. The ascogonium of certain genera is remarkably similar in structure to the carpogonium of some of the Florideae. No other plants except those belonging to these two classes have this kind of a female reproductive organ. According to some workers, certain genera of the Ascomycetes are distinguished from all other plants by the fact that two distinct nuclear fusions occur during fertilization and the formation of the spores. If it could be shown that a second nuclear fusion does actually occur in the auxiliary cell of such a form as *Dudresnaya* or *Dumontia*, the carpogonium with its trichogyne and long sporogenous filaments with the carpospores at their extremities might be proved to be homologous with the ascogonium of some form like *Pyronema* with its trichogyne and long ascogenous hyphae bearing ascospores.

It has been stated that in all probability the nuclei in the sporogenous filaments of *Dumontia* are descended from the fusion nucleus in the carpogonium. However, more evidence is to be desired in regard to the origin of these nuclei. The sporogenous nuclei in *Dudresnaya purpurifera*, *D. coccinea*, and *Gloeosiphonia* (OLTMANNS 9) are unquestionably derived from the fusion nucleus in the carpogonium. In *Dumontia*, as in the 3 species of the Cryptonemiales studied by OLTMANNS, there can be no doubt in regard to the passage of a nucleus from a sporogenous filament into an auxiliary cell. A nucleus is always present in *Dumontia* at the tip of each filament. Tips of filaments are found lying quite near the auxiliary cells. A similar filament is found fused to the auxiliary

cell, and no nucleus is then present in the filament. There are, however, two widely separated nuclei in the auxiliary cell itself, and one of these lies quite near the point of fusion of the filament and the cell. The sporogenous nucleus in *Dudresnaya purpurifera*, *D. coccinea*, and *Dumontia filiformis* at no time even closely approaches the original auxiliary cell nucleus. The 2 nuclei in all 3 species lie at almost opposite ends of the cell. The carpospores are budded off from that end of the auxiliary cell which contains the sporogenous nucleus. In *Dumontia* the original auxiliary cell nucleus and the descendant of the sporogenous nucleus could be identified in nearly all the auxiliary cells which bore carpospores. OLTMANNS (9) observed in *Gloeosiphonia* and *Dudresnaya* several cases of "blind fusion" where, although a sporogenous filament had fused with an auxiliary cell, no nucleus had passed over and the auxiliary cell contained only its own nucleus. No examples of "blind fusion" were found in *Dumontia*. It would seem that OLTMANNS' statement that it is a daughter of the sporogenous nucleus in *Gloeosiphonia* which moves into the pericentral cell might be questioned. The two daughters of the auxiliary cell nucleus and the sporogenous nucleus always lie close together. One of these 3 nuclei divides and one of the daughters moves into the pericentral cell. In *Dudresnaya purpurifera*, *D. coccinea*, and *Dumontia* there can be no question as to the origin of the nuclei in the carpospore. They are derived from the sporogenous nucleus.

Summary

Dumontia filiformis, during May, June, and the first half of July, grows in abundance in the tide pools and on the bed rock at South Harpswell, Maine. This alga became established on the coast at South Harpswell between 1905 and 1913. Antheridial, cystocarpic, and tetrasporic plants may have essentially identical size and vegetative structure. The average size of the antheridial plants is a little less than that of the other plants. Cystocarpic and tetrasporic plants are found growing together on the same rock and in the same tide pools. Female plants which bear mature cystocarps are easily recognized by the protrusions which these form in the wall of the thallus. The type of branching varies

considerably. All male and female plants collected were branched. Tetrasporic plants, simple and branched, were found. The maximum number of branches observed on any individual plant was 30. The color of the plants varies from dark red to pale reddish yellow. Male plants bearing mature spermatia are present in the early part of April and two weeks later have almost completely disappeared. Young female plants were found on April 12, and these reach their maximum stage of development about the middle of May. Tetrasporic plants are most abundant in the latter part of June and have almost entirely disappeared by the first of August. *Dumontia* at South Harpswell must persist through the winter in the form of sporelings developed from the tetraspores.

The vegetative structure is of a type occurring in many families of the Florideae. The disk-shaped holdfast is composed of a single layer of horizontal filaments, each cell of which produces a vertical ascending branch. Certain of these branches elongate to form the medullary hyphae of the tubular thallus. Each medullary hypha has its own initial cell. Every cell of each medullary hypha produces a radial branch. These radial branches, by repeated dichotomous forking, form the subcortex and the cortex. Growth is apical throughout the entire thallus. All the other vegetative cells, except the trichomes, are uninucleate and contain one chromatophore each. All the chromatin in the resting nucleus is in the nucleolus. The chromatophore is a clathrate hollow ellipsoid, lying just inside the cell wall.

The tetraspores are imbedded in the wall of the thallus, and are distributed evenly throughout practically the entire length and circumference of the branches and main axis. Younger tetrasporangia are found toward the base of the plant. The larger subcortical cells become modified to form the tetrasporangia. The chromatophore becomes constricted at intervals, so that it appears to consist of rows of small irregular plates. These bodies persist through all stages of the tetrasporangium, and their number is increased in the tetraspores. No spindle or spireme was seen. The chromatin in some of the nuclei is in several small bodies, but these do not resemble chromosomes. The first cleavage furrow completely divides the tetrasporangium, is perpendicular

to its longitudinal axis, and is parallel to the surface of the thallus. The chromatophores in the tetraspores are hollow, oval bodies with perforated walls. The mature tetraspores do not round off while imbedded in the thallus. They escape either by the disintegration of the cells surrounding them or by a pore formed in the wall of the thallus.

The spermatia form a continuous layer over nearly the entire surface of the thallus. The spermatium mother cells terminate the branches forming the cortex and subcortex. The chromatophore which is present in the young spermatium mother cell partially or completely disappears as this cell matures. The protoplasm which was in the chromatophore is used in forming the granular cytoplasm of the mature cell. The youngest spermatium mother cells observed were binucleate. The first spermatium is cut off diagonally. The mother cell may again become binucleate and cut off a second spermatium in a similar manner on the side opposite to that on which the first was formed. The second spermatium may be formed while the first one is still attached to the mother cell. No chromatophore is present in the spermatium. The nucleus is situated at the distal end of the spermatium in a dense mass of cytoplasm. The proximal end is vacuolated. The spermatium is cut off from the mother cell as a cell and not as a naked protoplast.

The distribution of carpogonial branches in the young female plants is general, as in the case of the tetrasporangia in the tetrasporic plants. The carpogonial branch develops by apical growth and arises as a lateral outgrowth of a large subcortical cell. A mature carpogonial branch consists of 6 or 7 cells and a trichogyne (6 cells always lie in a row). The basal cell ("cell 1") sometimes divides to form a lateral cell. The carpogonium in a mature branch is always close to or in contact with "cell 2" or "cell 3." The sporogenous filaments always arise from one of these two cells. Only a few trichogynes were found projecting beyond the surface of the thallus. Spermatia were found fused to 4 trichogynes.

Each carpogonial branch which has been fertilized produces 2 or 3 sporogenous filaments, all of which arise from one cell. It is thought that the nuclei in these filaments are descended from

the fusion nucleus in the carpogonium. The sporogenous filaments grow out toward the auxiliary cell branches. The auxiliary cell branches in origin, distribution, structure, and mode of development are very similar to the carpogonial branches. Only about 1 auxiliary cell branch is initiated to every 7 carpogonial branches. The time of initiation of the former is a little later than that of the latter. The mature auxiliary cell branch consists of 4–7 cells. The second or third cell of the branch is the auxiliary cell, the cell with which the sporogenous filament fuses and the one which forms the carpospore. The original nucleus in the auxiliary cell takes no part in the formation of the carpospores. The nuclei in the carpospores are descended from the nucleus which enters the auxiliary cell from the sporogenous filament.

In the development of the carpospores and cystocarps 3 or 4 gonimoblast filaments arise from the auxiliary cell. Every cell of these filaments forms a spore. There are about 20 carpospores in each cystocarp. The pericarp is formed by radial branches similar to those which form the subcortex and cortex of the wall of the thallus. Mature carpospores are usually uninucleate, well filled with a cytoplasm, and contain chromatophores. The chromatophores are similar to those of the vegetative cells. The nucleus sometimes divides just as the carpospore is about to escape. Naked carpospores escape through a pore formed in the pericarp. Carpospores sometime germinate while in the cystocarp. The ends of branches of mature plants fray, and this disintegration of cells surrounding the cystocarp furnishes one means of escape for the carpospores and sporelings.

In the resting nucleus of *Dumontia* all the chromatin is in the nucleolus. The nucleolus often contains a vacuole. The chromatin in preparation for mitosis passes out of the nucleolus and in the form of small granules becomes distributed along the linin net. The net disappears and the granules become massed together to form larger units, chromosomes. The number of chromosomes was not definitely determined, but was apparently about 7. No spireme or spindle was seen. After division, the chromatin is again found massed together in the nucleolus.

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LITERATURE CITED

1. GREVILLE, R. H., Algae Britannicae. Edinburgh. 165. 1830.
2. HARVEY, W. H., Phycologia Britannica. London. 1846.
3. HASSENCAMP, A., Über die Entwickelung der Cystocarpien bei einigen Florideen. Bot. Zeit. **60**:65–86. 1902.
4. HAUPTFLEISCH, P., Die Fruchtentwickelung der Gattungen *Chylocladia*, *Champia*, und *Lomentaria*. Flora **50**:307–367. 1892.
5. KÜTZING, F. T., Phycologia Generalis. Leipzig. 1843.
6. LEWIS, I. F., The life history of *Griffithsia Bornetiana*. Ann. Botany **23**:639–690. 1909.
7. ———, Alternation of generations in certain Florideae. BOT. GAZ. **53**:233–246. 1912.
8. ———, The seasonal life-cycle of some red algae at Woods Hole. Plant World **17**:1–35. 1914.
9. OLTMANNS, F., Zur Entwickelungsgeschichte der Florideen. Bot. Zeit. **56**:99–141. 1898.
10. ———, Morphologie und Biologie der Algen. Jena **1**:538–599. 1904.
11. OSTERHOUT, W. J. V., Befruchtung bei *Batrachospermum*. Flora **87**:109–115. 1900.
12. SCHMITZ, F., Die Chromatophoren der Algen. Bonn. 1882.
13. ———, Untersuchungen über die Befruchtung der Florideen. Sitzungsb. d. Königl. Akad. Berlin 215–259. 1883.
14. SVEDELIUS, NILS., Über den Bau und die Entwicklung der Florideen-Gattung *Martensia*. Königl. Svensk. Vet. Akad. Handl. **43**: pp. 101. 1908.
15. ———, Über den Generationswechsel bei *Delesseria sanguinea*. Svensk. Bot. Tidsk. **5**:260–324. 1911.
16. ———, Über die Spermatienbildung bei *Delesseria sanguinea*. Svensk. Bot. Tidsk. **6**:239–265. 1912.
17. THURET, G., Études phycologiques analyses d'Algues marines. Paris. 1878.
18. WILLE, N., Beiträge zur Entwickelungsgeschichte der physiologischen Gewebesyteme bei einigen Florideen. Nov. Act. Leopold. Carolin. Akad. Nat. **52**:51–100. 1888.
19. WOLFE, J. J., Cytological studies on *Nemalion*. Ann. Botany **18**:608–628. 1904.
20. YAMANOUCHI, S., The life history of *Polysiphonia violacea*. BOT. GAZ. **17**:401–449. 1906.

EXPLANATION OF PLATES XIX–XXII

Lettering of figures.—*aux.n.*, auxiliary cell nucleus; *chr.*, chromatophore; *cps.*, carpospores; *m.h.*, medullary hyphae; *p.g.*, protein granule; *sbc.c.*, subcortical cell; *s.c.*, supernumerary cell; *sp.fil.*, sporogenous filament; *sp.n.*,

sporogenous nucleus; *spm.m.c.*, spermatium mother cell; *st.c.*, stalk cell; *tr.*, trichogyne.

PLATE XIX

FIG. 8.—Vertical section through holdfast showing horizontal hypha and vertical ascending, dichotomously branched hyphae; ×355.

FIG. 9.—Slightly diagrammatic; vertical section through base of plant and holdfast; ×170.

FIG. 10.—Longitudinal section through wall of thallus showing radial hyphae arising as branches of longitudinal, medullary hyphae; ×700.

FIG. 11.—Longitudinal section through apex of young branch; ×340.

FIG. 12.—Surface view of subcortical cell showing structure of chromatophore; ×1000.

FIG. 13.—Longitudinal section through an intercellular connection showing granules of which disk is composed; ×700.

FIG. 14.—Longitudinal section through an intercellular connection, between 2 carpospores, in which granules have not yet become grouped together to form disks; ×1000.

FIG. 15.—Transverse section through wall of thallus of male plant showing stalk cells, spermatium mother cells, and spermatia; ×1000.

FIG. 16.—Longitudinal section through binucleate spermatium mother cell; ×1400.

FIG. 17.—Similar section; spermatium mother cell which has budded off one spermatium; ×1400.

FIG. 18.—Transverse section through wall of male plant showing free spermatia imbedded in gelatinous sheath of thallus; ×1000.

FIG. 19.—Longitudinal section through spermatium mother cell and spermatium; chromatophore at base of spermatium mother cell; ×1400.

FIG. 20.—Longitudinal section through wall of thallus showing origin of carpogonial branches; ×355.

FIGS. 21, 22.—Longitudinal section of first cell of carpogonial branch, showing mode of origin from subcortical cell; chromatophore is similar to those of vegetative cells; ×1000.

FIG. 23.—Longitudinal section of a 2-celled carpogonial branch; ×1000.

FIG. 24.—Similar section of a 3-celled carpogonial branch; ×1000.

FIG. 25.—Longitudinal section of a 3-celled carpogonial branch; ×1000.

FIG. 26.—Similar section of a 4-celled carpogonial branch showing that number of cells is increased by division of terminal cell; ×1000.

FIG. 27.—Similar section of a 4-celled carpogonial branch showing that branches at this stage may be bent to form a right angle; ×1000.

FIG. 28.—Four-celled carpogonial branch; nucleus in terminal cell contains 7 chromosomes (?); ×1000.

FIG. 29.—Five-celled carpogonial branch showing position and structure of chromatophores; ×1000.

PLATE XX

FIG. 30.—Similar carpogonial branch; terminal cells binucleate; ×1000.

FIG. 31.—Section of fifth cell of a 6-celled carpogonial branch; 26 chromatophores distributed on the cytoplasmic net; ×1400.

FIG. 32.—Six-celled mature carpogonial branch showing carpogonium lying near cell 3; ×1000.

FIG. 33.—Similar to fig. 32; cell 3 is binucleate; ×1000.

FIG. 34.—Six-celled carpogonial branch showing coiled trichogyne; ×1000.

FIG. 35.—Seven-celled carpogonial branch showing trichogyne reaching almost to surface of thallus; ×1000.

FIG. 36.—Six-celled carpogonial branch showing carpogonium in contact with cell 3; cell 1 contains 3 nuclei; cytoplasm in cells 4, 5, and 6 is beginning to disintegrate; ×1000.

FIG. 37.—Six-celled carpogonial branch showing much coiled trichogyne reaching almost to surface of thallus; ×1000.

FIG. 38.—Six-celled carpogonial branch showing carpogonium in contact with cell 3 which is much enlarged, nucleus of which is preparing to divide; ×1000.

FIGS. 39, 40.—Base of carpogonial branch, and trichogyne projecting beyond surface of thallus; ×1000.

FIG. 41.—Base of carpogonial branch (cells 1–4), and trichogyne projecting beyond surface of thallus; 8 spermatia attached to trichogyne; ×1000.

FIG. 42.—Carpogonial branch; 3 sporogenous filaments arising from cell 2; ×1000.

FIG. 43.—Carpogonial branch and auxiliary cell branch connected by sporogenous filaments; auxiliary cell branch consists of 6 cells; ×700.

PLATE XXI

FIG. 44.—Auxiliary cell branch; sporogenous filaments lying over cell 4; ×1000.

FIG. 45.—Auxiliary cell branch; sporogenous filament fused with cell 3; ×1000.

FIG. 46.—Auxiliary cell branch; cells 2 and 3 binucleate and a supernumerary cell present; ×1000.

FIG. 47.—Three-celled auxiliary cell branch; ×1000.

FIG. 48.—Five-celled auxiliary cell branch; nucleus of cell 4 preparing to divide; cell 1 contains 3 nuclei; ×1000.

FIG. 49.—Auxiliary cell branch consists of 4 cells; cell 2 binucleate; ×1000.

FIG. 50.—Four-celled auxiliary cell branch; cells 1, 3, and 4 binucleate; ×1000.

FIG. 51.—Four-celled auxiliary cell branch showing dense cytoplasmic contents of cells; 7 chromosomes and colorless nucleolus in nucleus of cell 3; ×1000.

FIG. 52.—Four-celled auxiliary cell branch; part of chromatin in nuclei of cells 1 and 3 in granules distributed on linin net; cell 4 contains 2 nuclei; ×1000.

FIG. 53.—Four-celled auxiliary cell branch; sporogenous filaments lying under cell 3; ×1000.

FIG. 54.—Sporogenous filament lying near 4-celled auxiliary cell branch; ×550.

FIG. 55.—Auxiliary cell branch showing structure of chromatophores; ×1000.

FIG. 56.—Auxiliary cell branch; 5 carpospores budded off from auxiliary cell; sporogenous filament fused with auxiliary cell; ×1000.

FIG. 57.—Auxiliary cell branch; auxiliary nucleus has divided; sporogenous nucleus has just entered auxiliary cell; ×1000.

FIG. 58.—Section through auxiliary cell branch and 8 young carpospores; auxiliary nucleus and sporogenous nucleus at opposite ends of auxiliary cells; ×1000.

FIG. 59.—Section through auxiliary cell branch and group of young carpospores; auxiliary cell and one carpospore shaded; sporogenous filament fused with auxiliary cell; ×1000.

FIG. 60.—Section through auxiliary cell branch and group of mature carpospores; auxiliary nucleus and sporogenous nucleus at opposite ends of auxiliary cell; ×550.

PLATE XXII

FIG. 61.—Same sporogenous filament as in fig. 75 showing cytoplasm nucleus and chromatophore at tip; ×1000.

FIG. 62.—Section through young cystocarp; 2 of the 18 carpospores which have arisen from auxiliary cells binucleate; ×1000.

FIG. 63.—Section through young cystocarp showing origin of radial branches which will form pericarp; ×350.

FIGS. 64, 65.—Partly shaded; sections of mature carpospores showing structure of nuclei, distribution of chromatophores, and protein granules; ×1000.

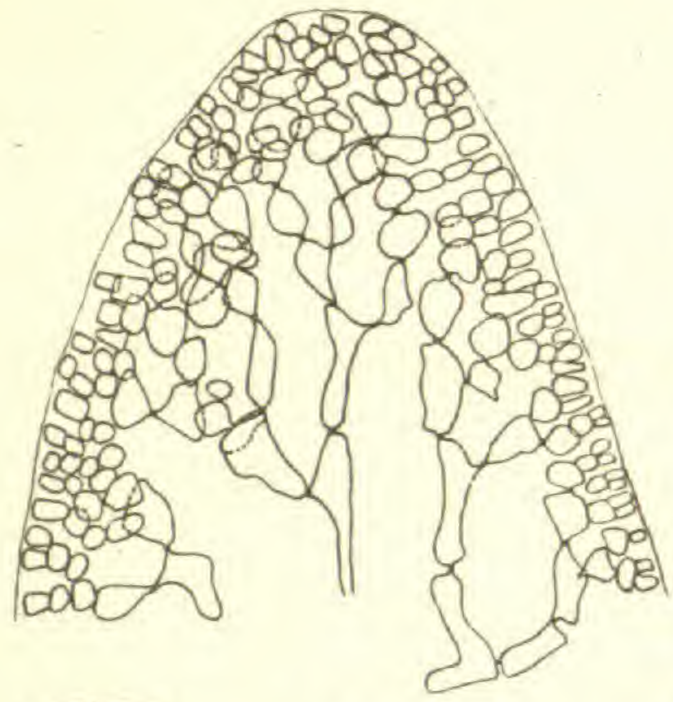
FIGS. 66, 67.—Sections of carpospores in a mature cystocarp; spores of same size and exactly similar to those which occur in abnormal cystocarps; ×700.

FIG. 68.—Transverse section of thallus showing 3 normal and 2 abnormal cystocarps; ×350.

FIGS. 69, 70, 71.—Sections through carpospores in abnormal cystocarps showing that nuclear division is not always directly followed by cell division; ×700.

FIG. 72.—Section of germinating carpospore showing cell wall; ×170.

FIG. 73.—Chromatophores of germinating carpospores; ×1400.



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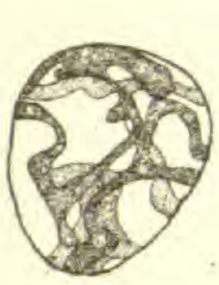

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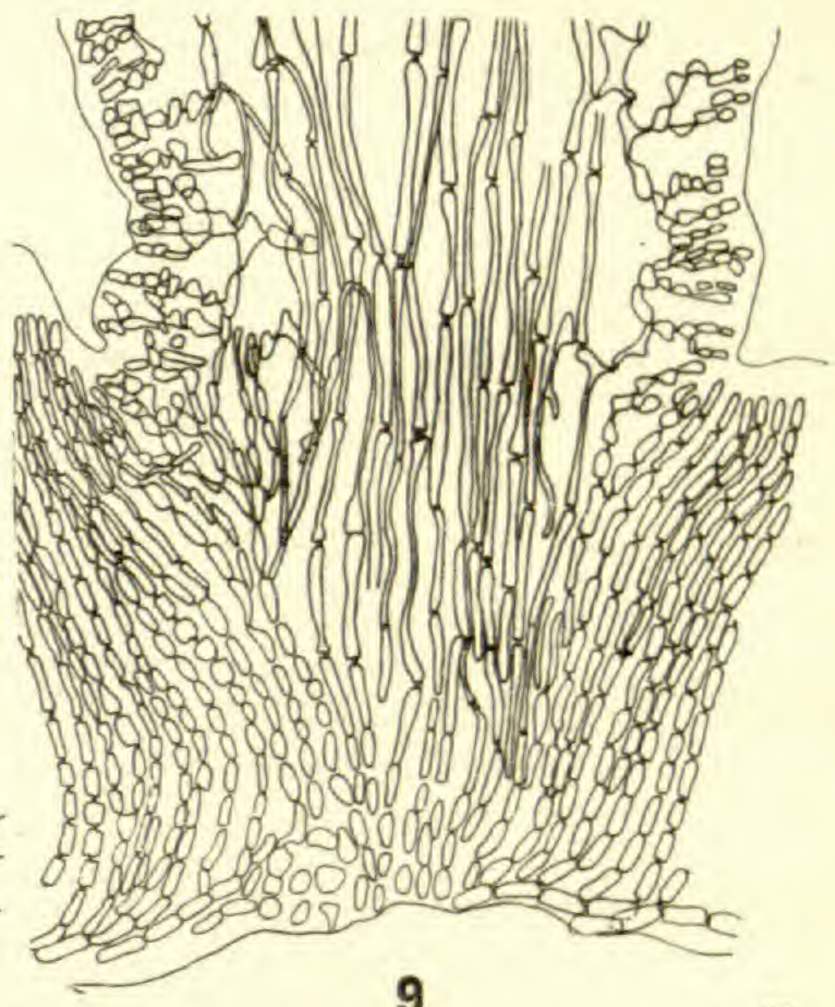

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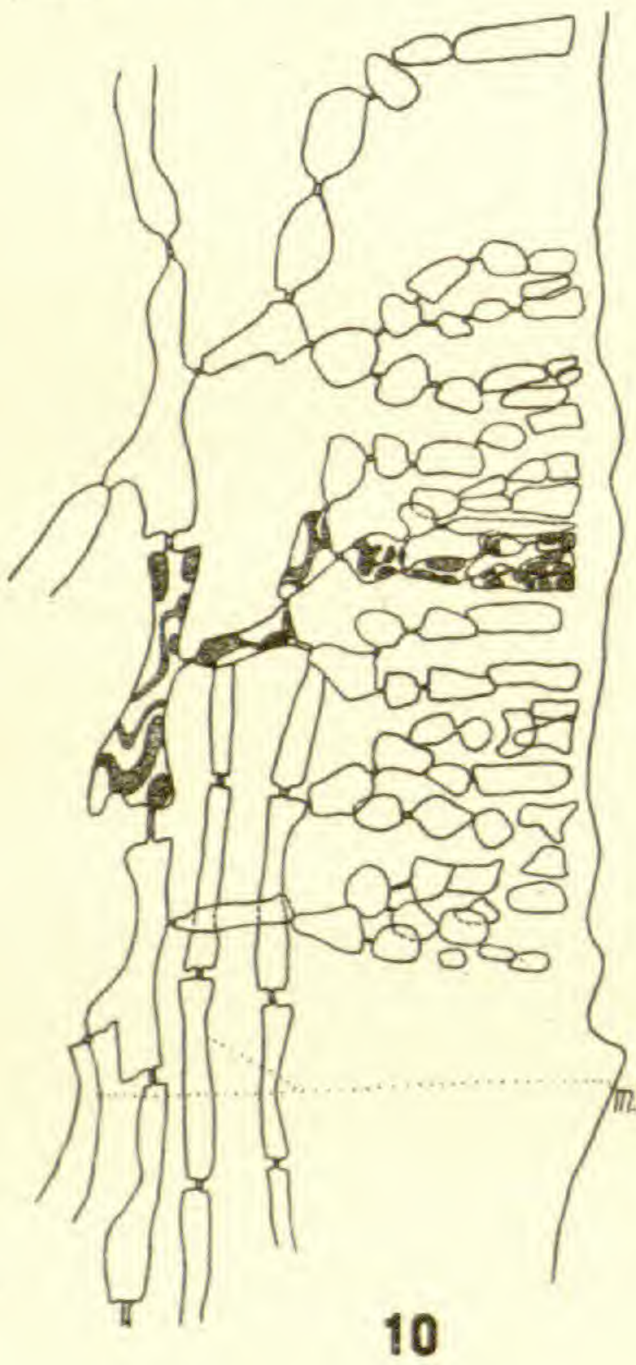

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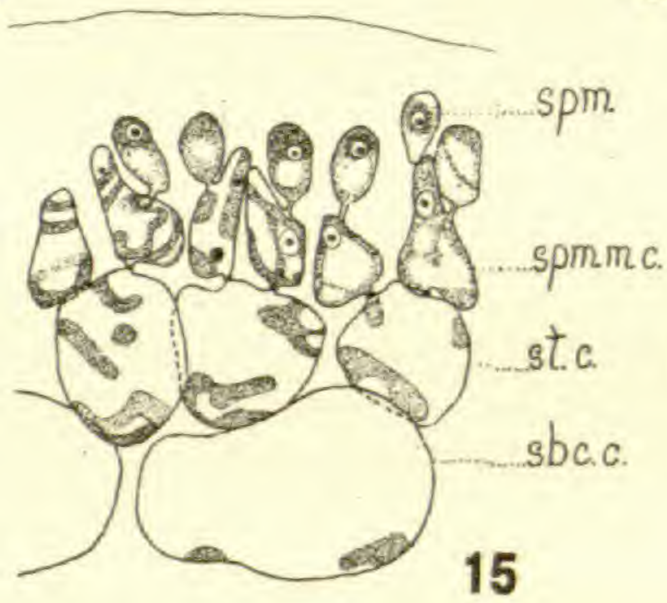

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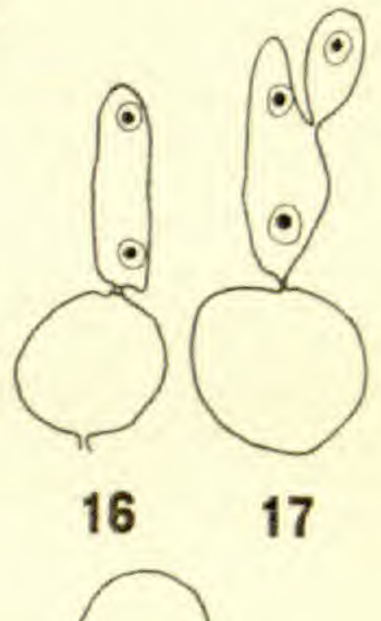

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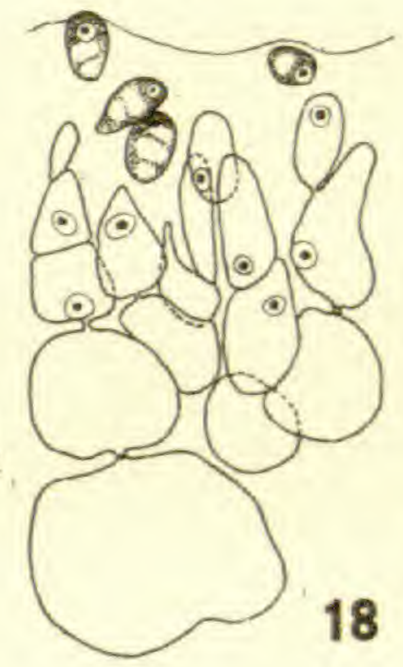

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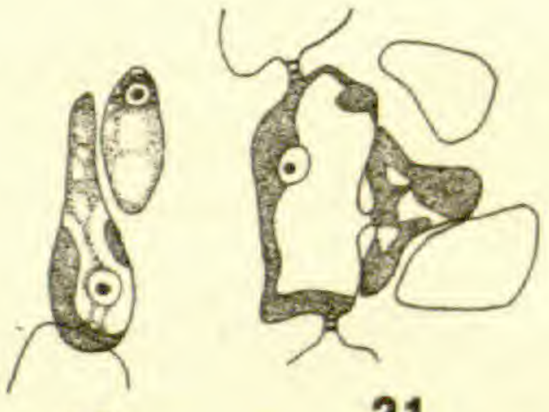

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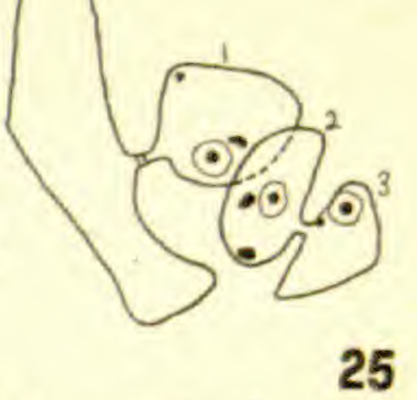

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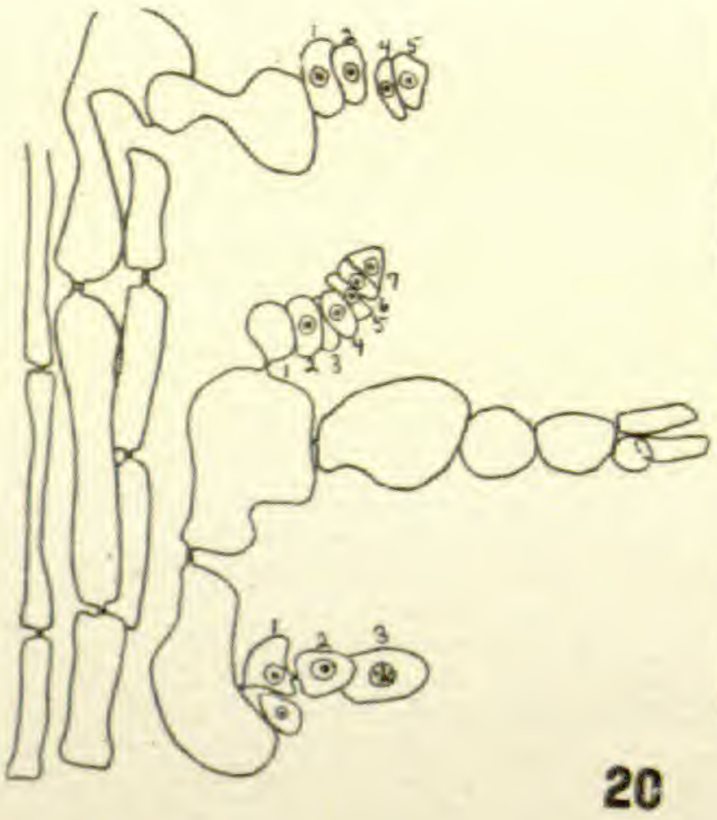

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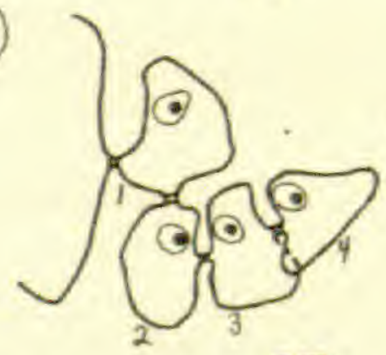

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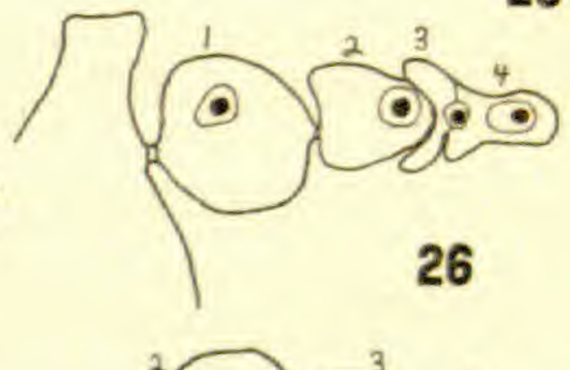

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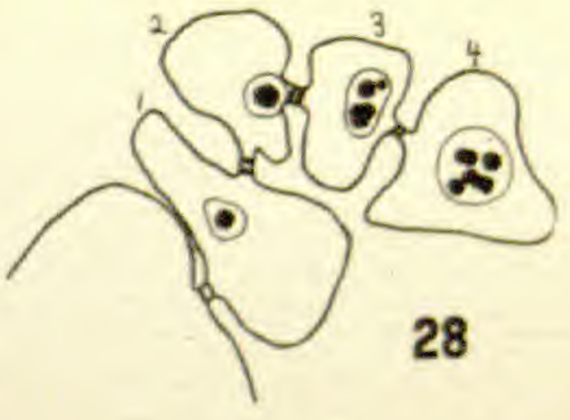

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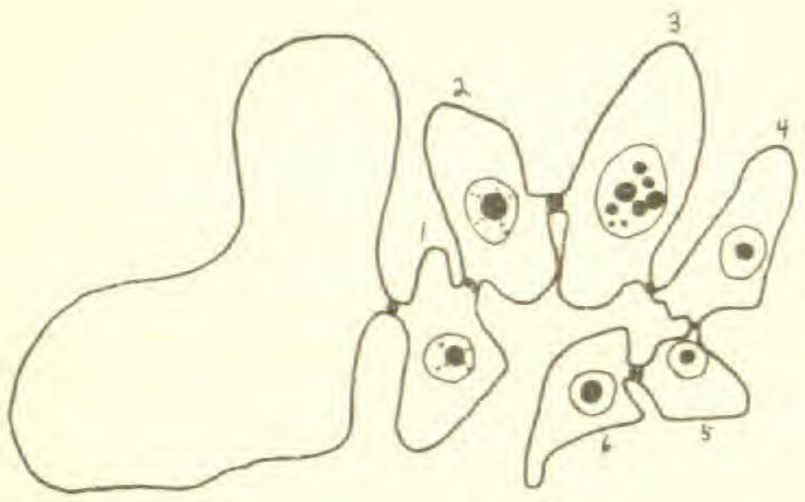

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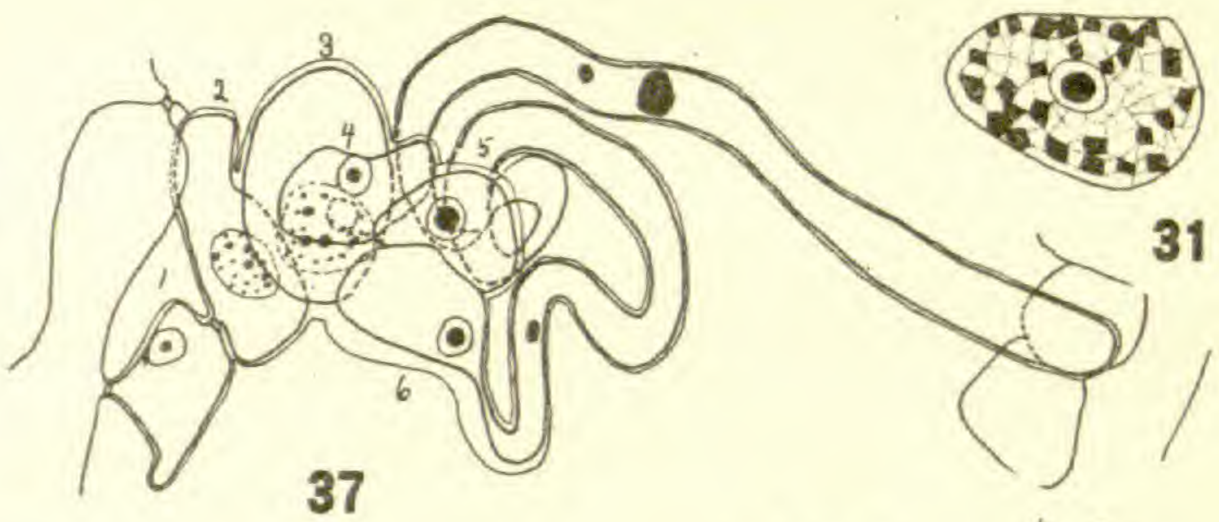

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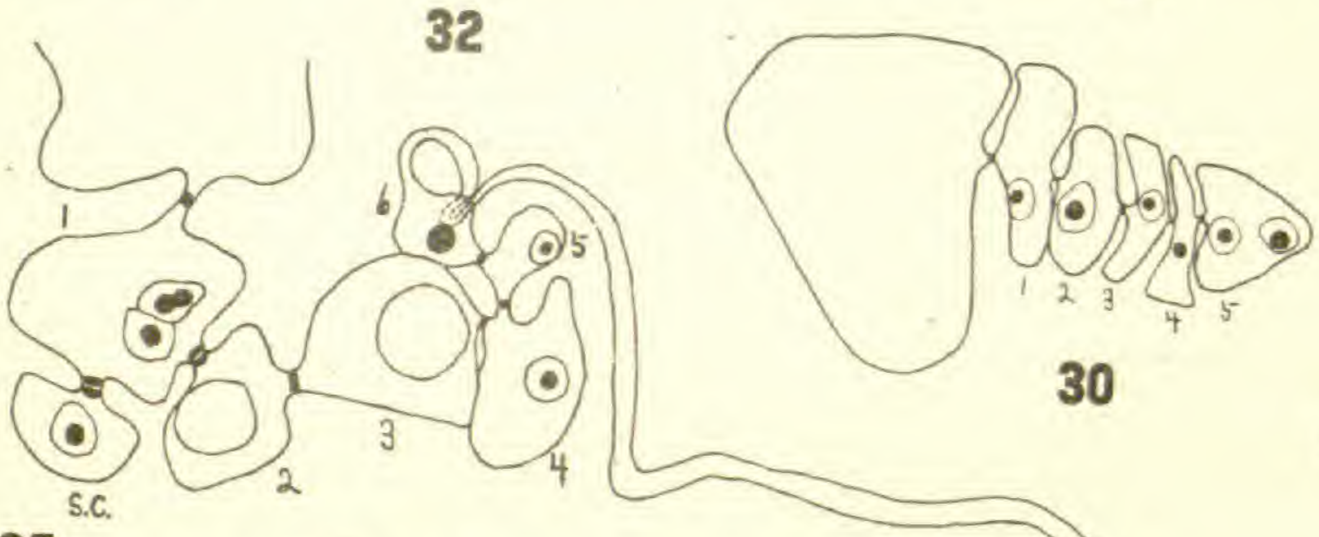

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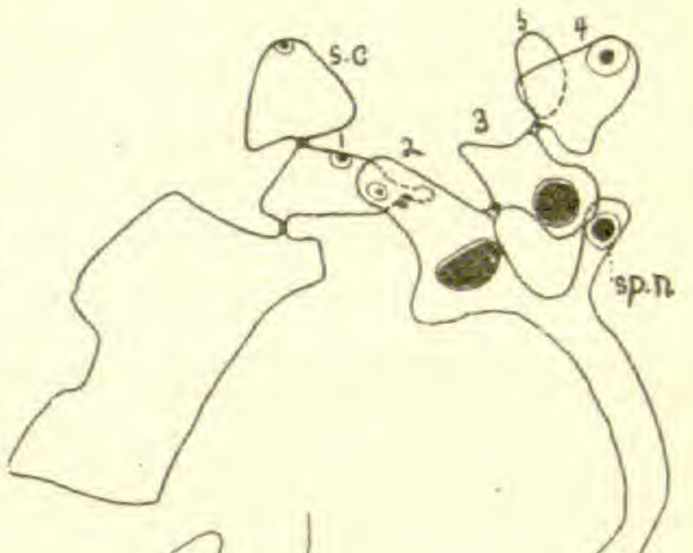

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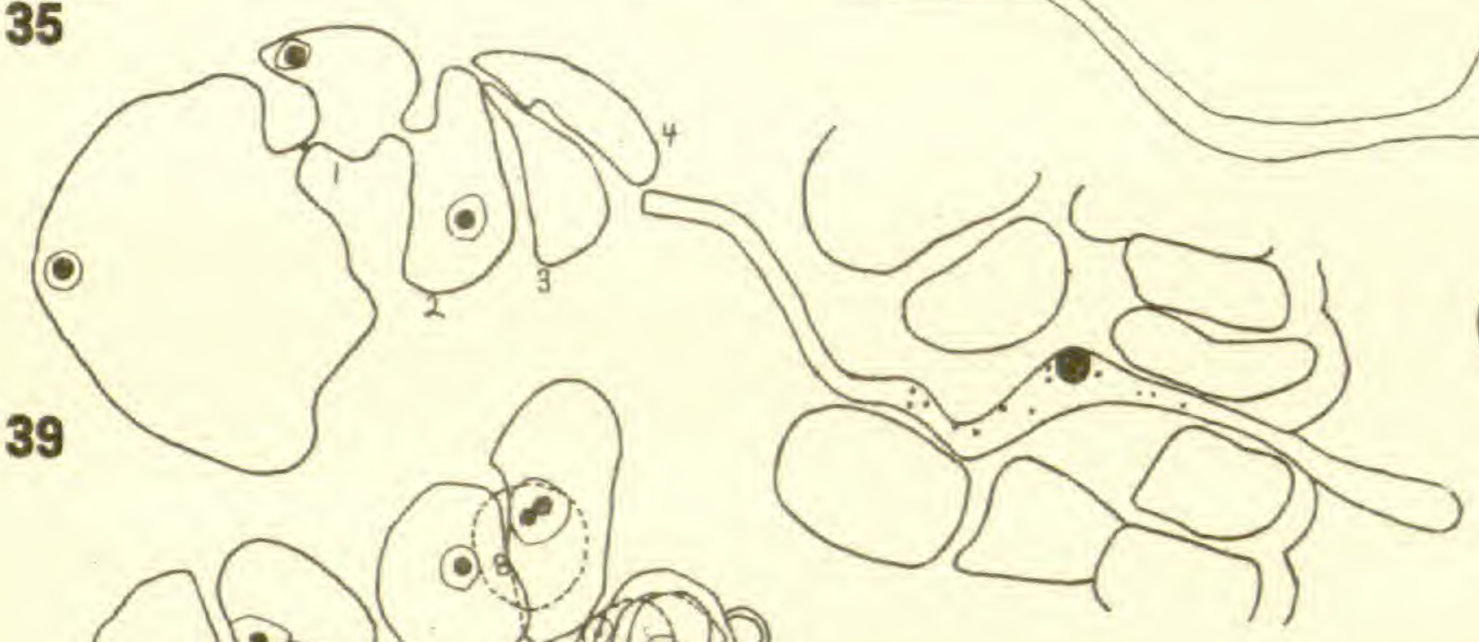

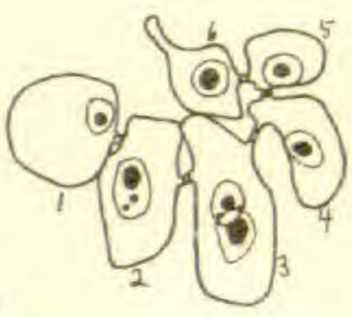

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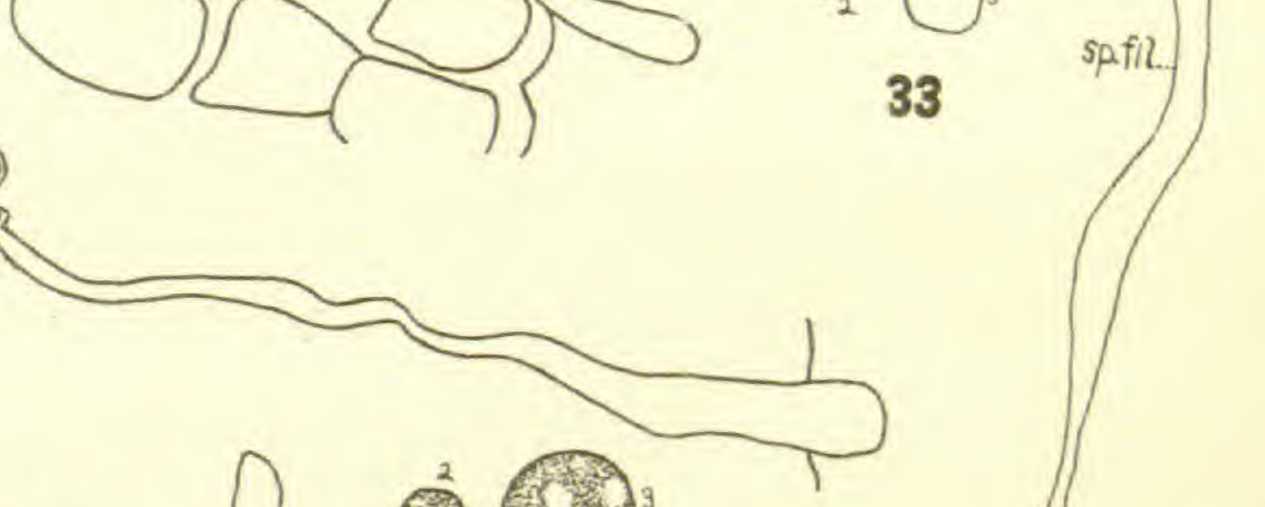

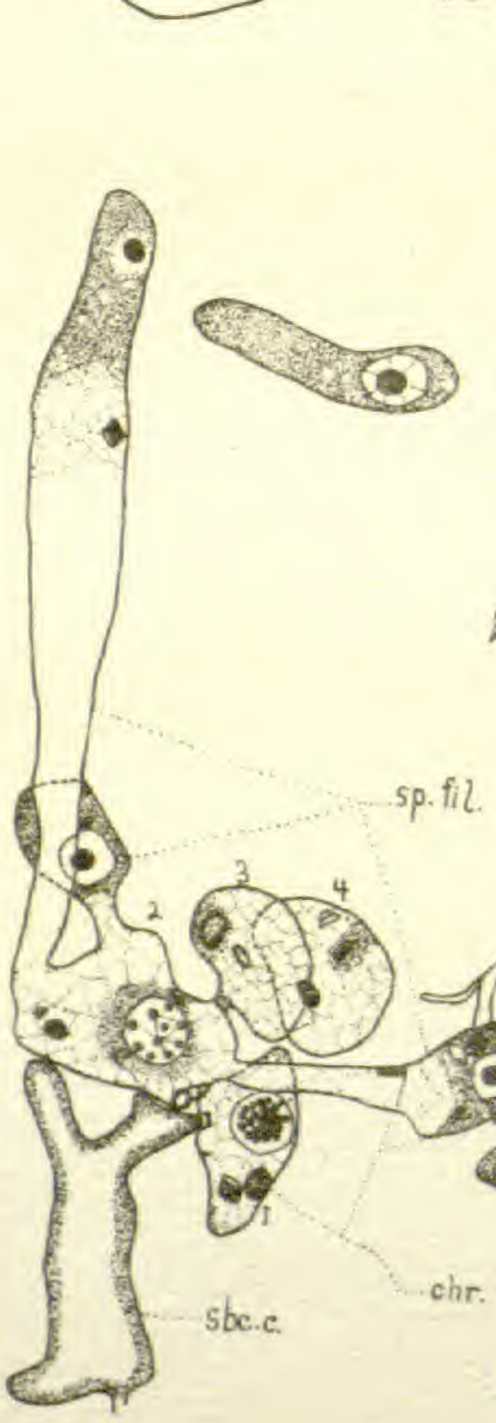

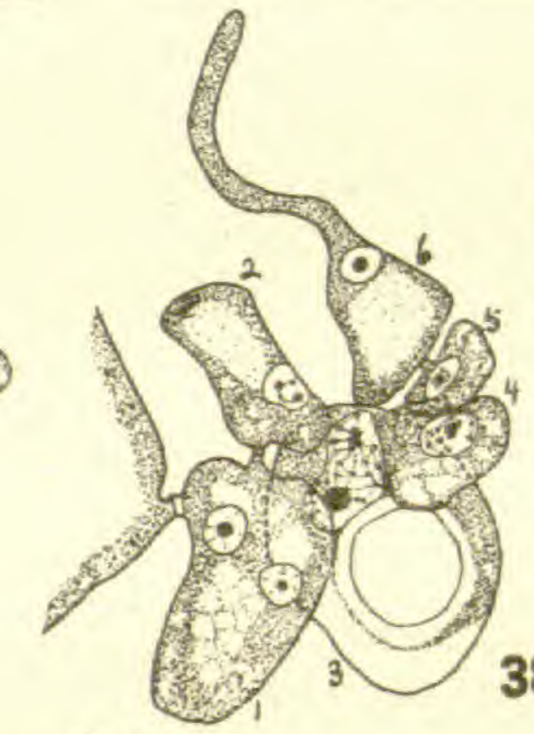

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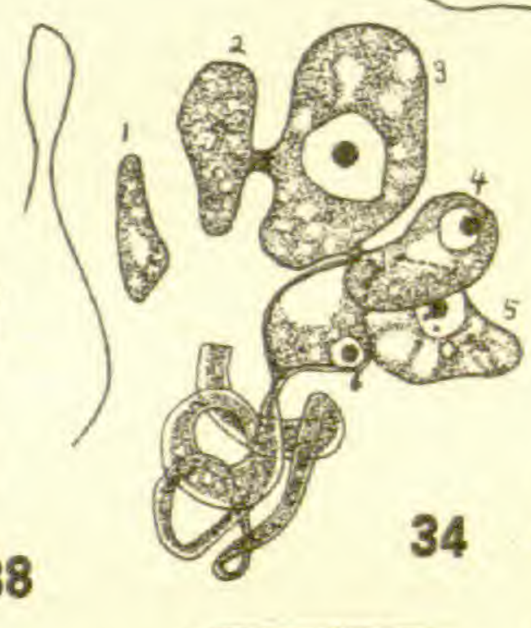

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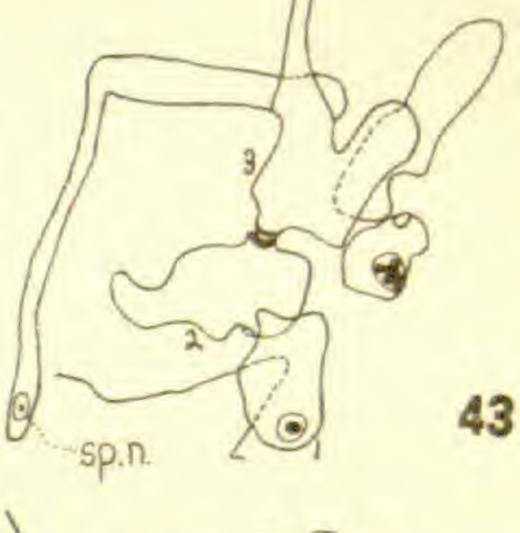

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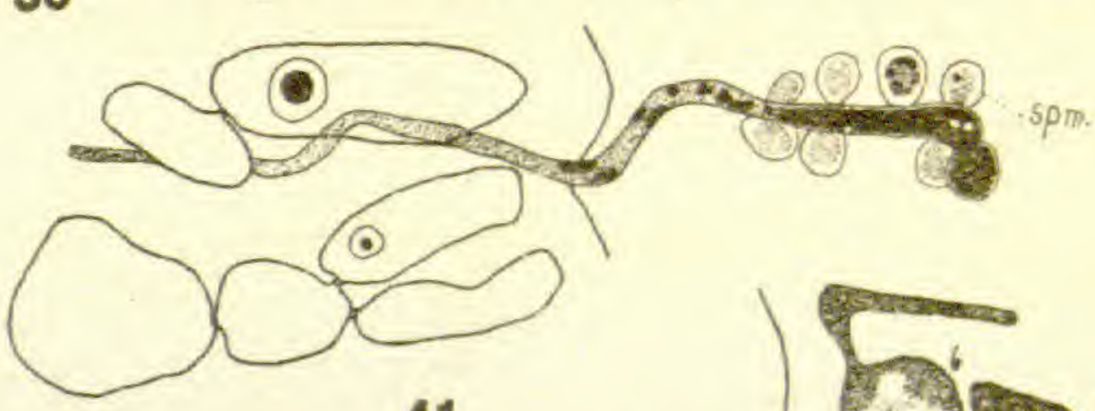

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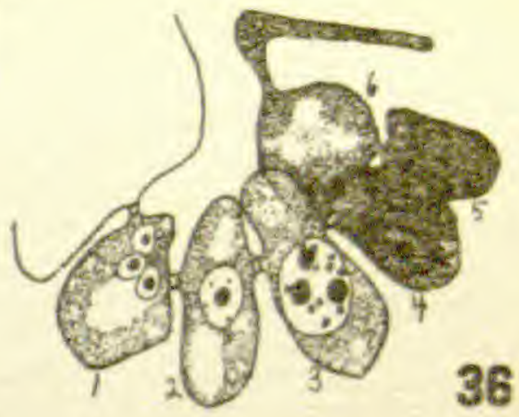

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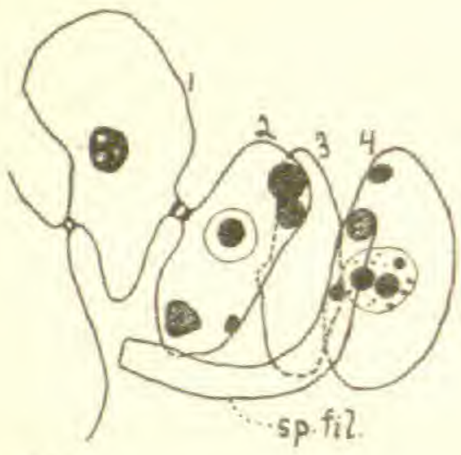

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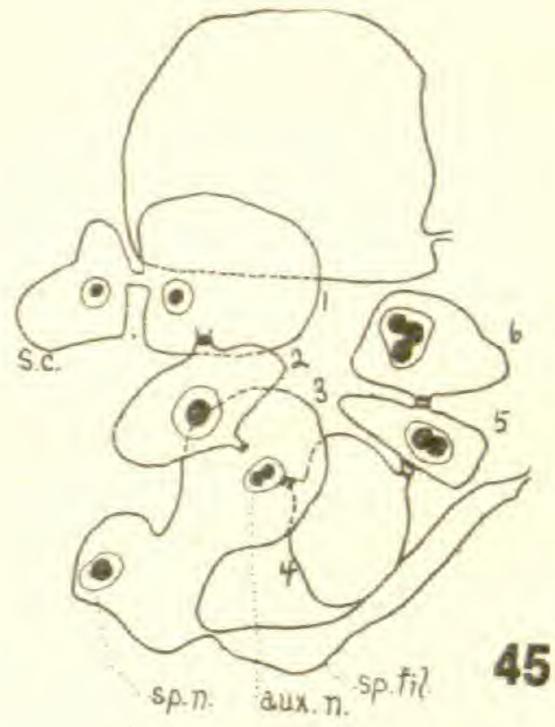

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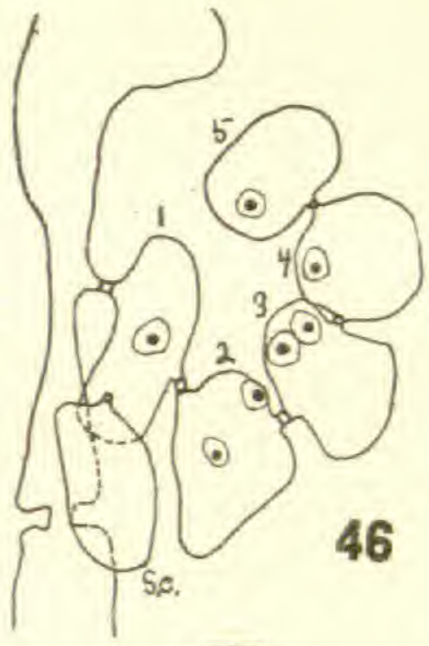

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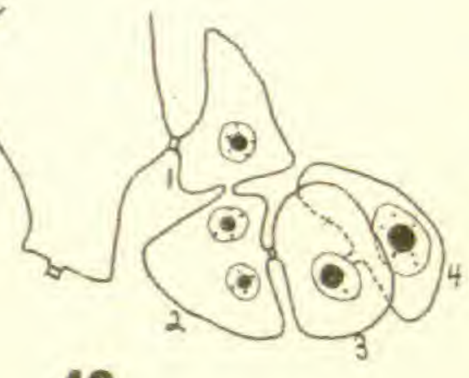

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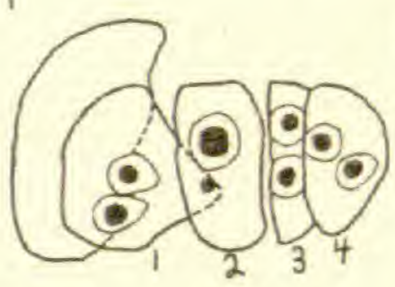

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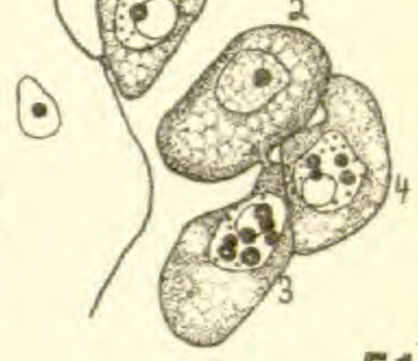

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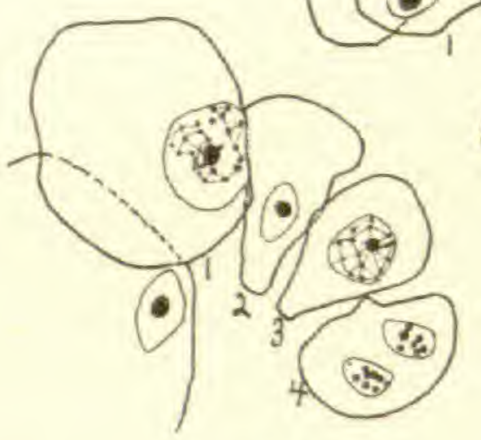

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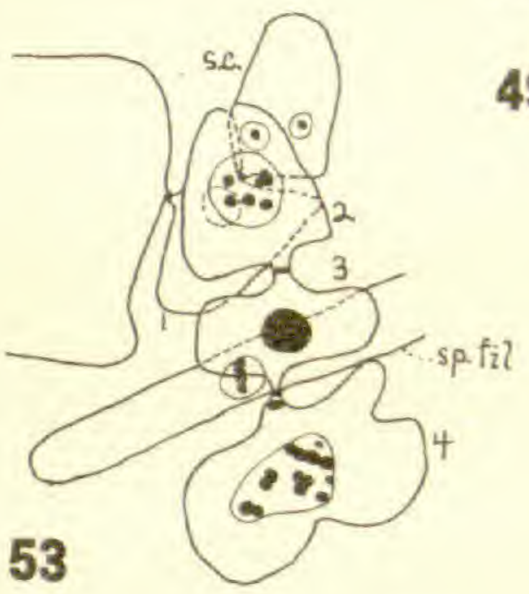

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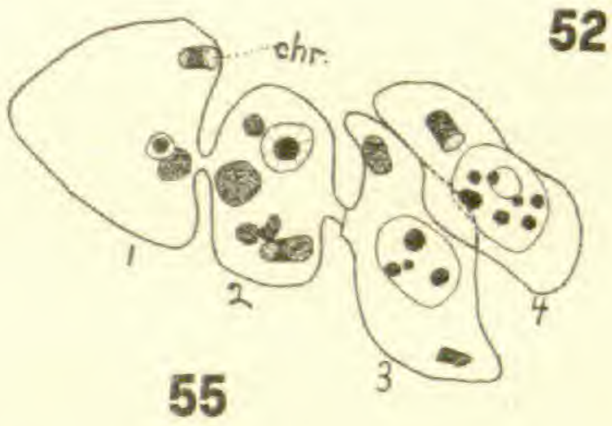

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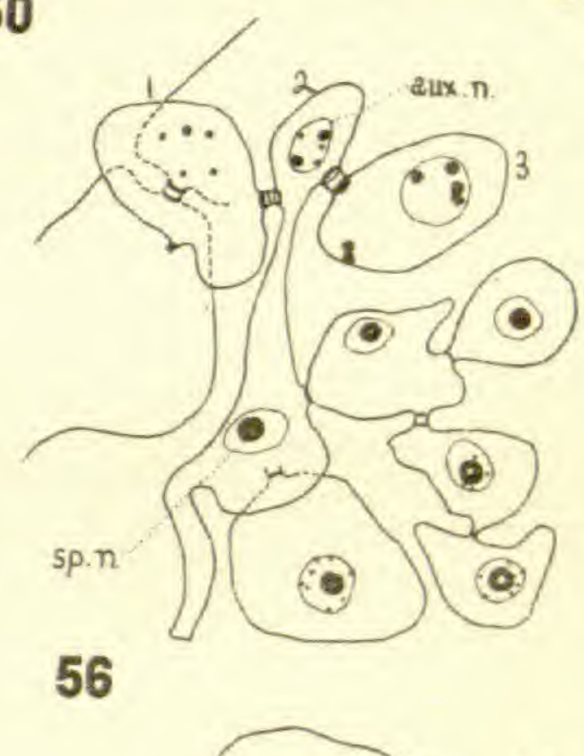

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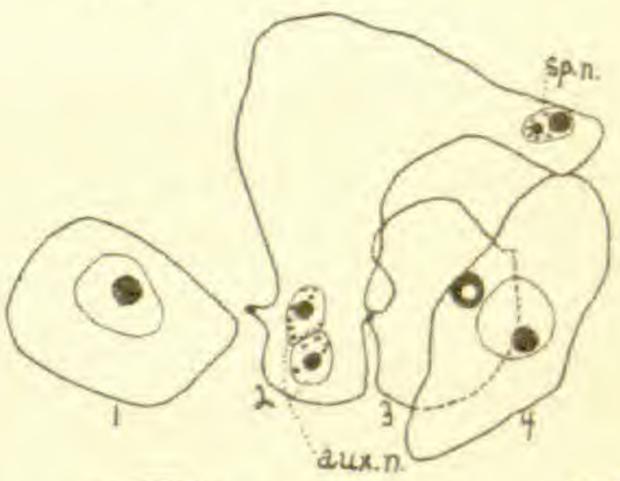

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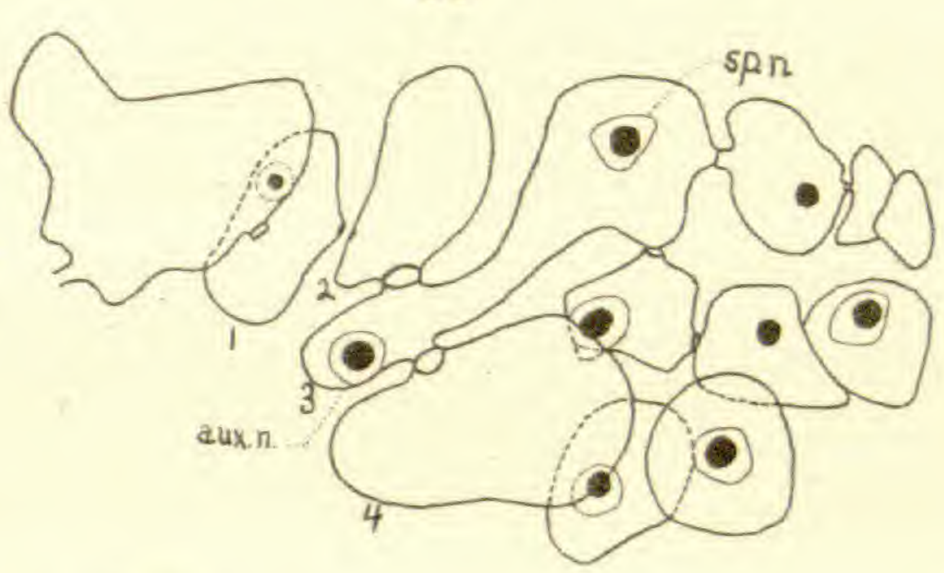

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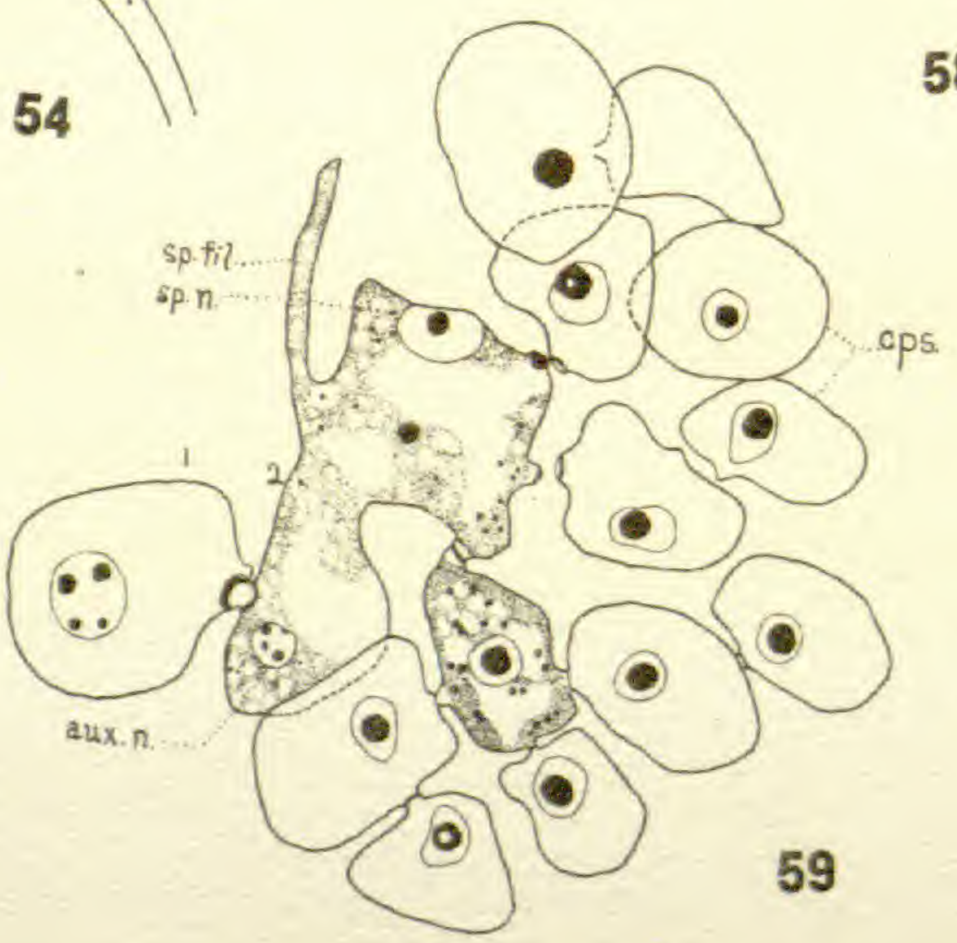

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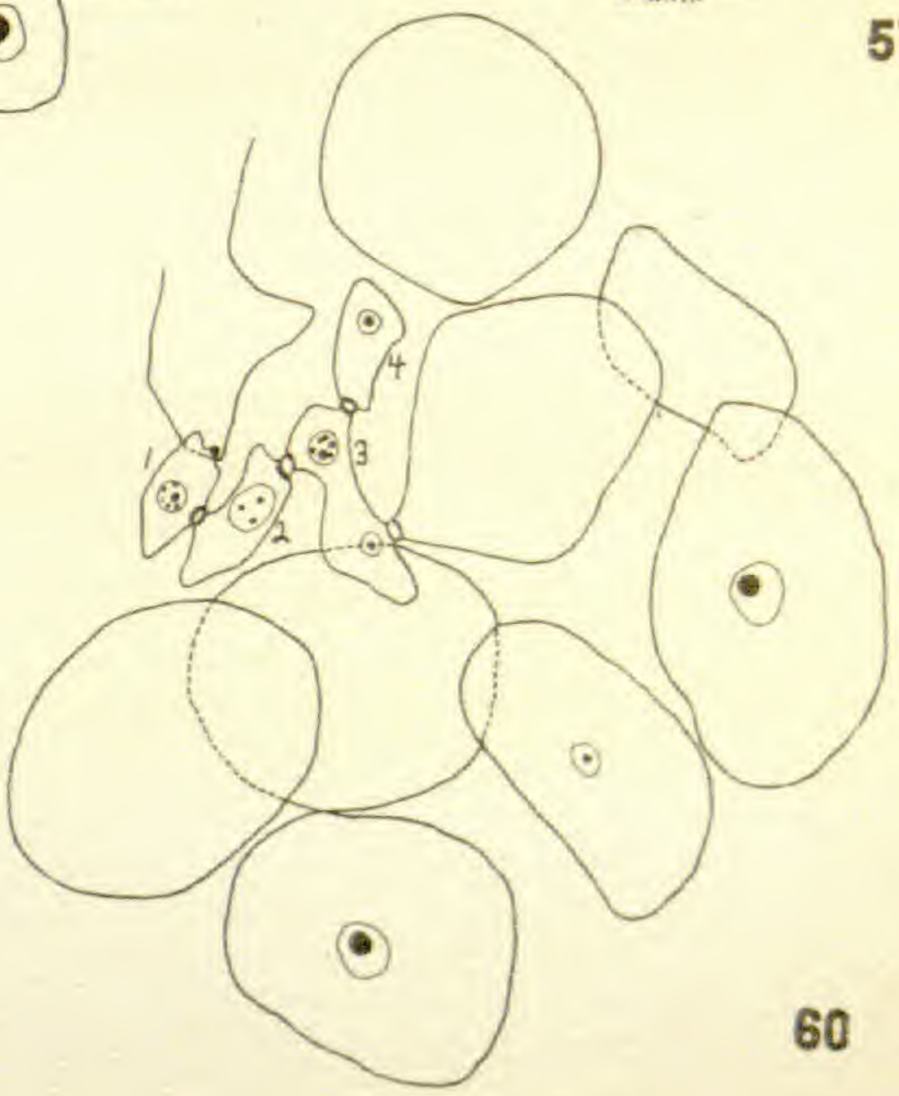

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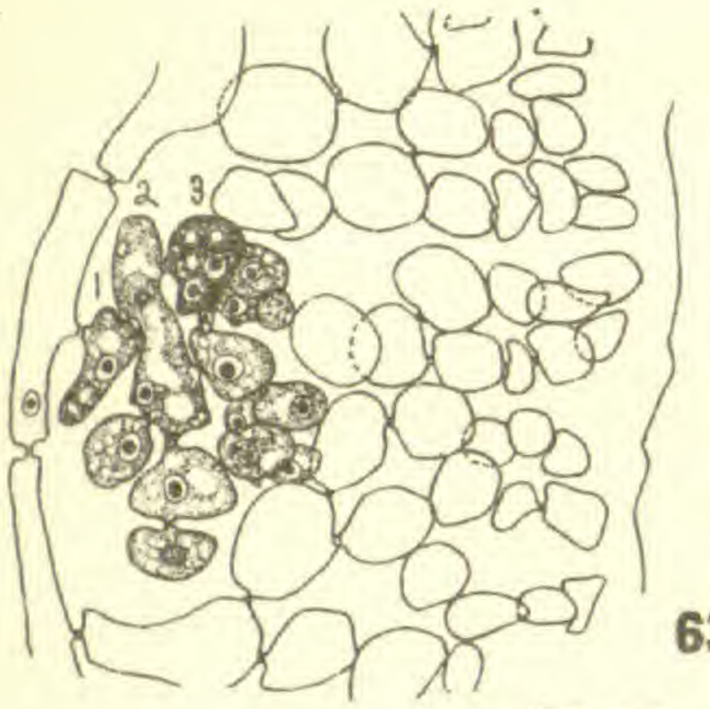

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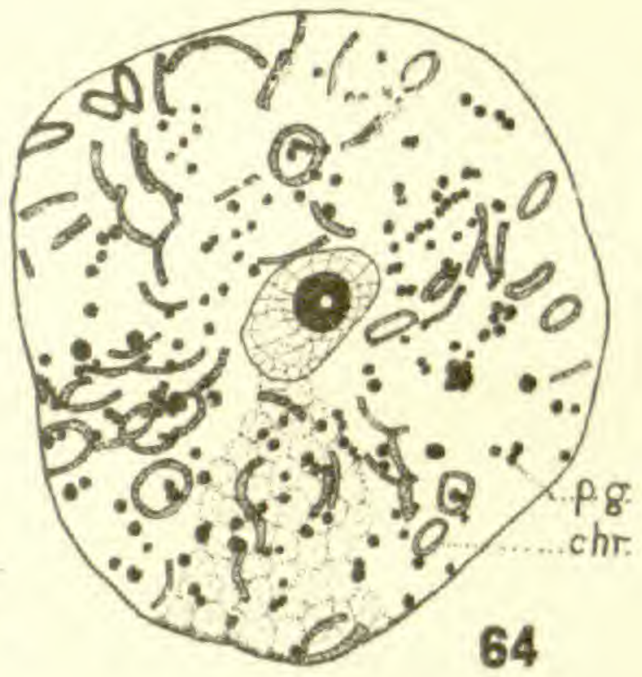

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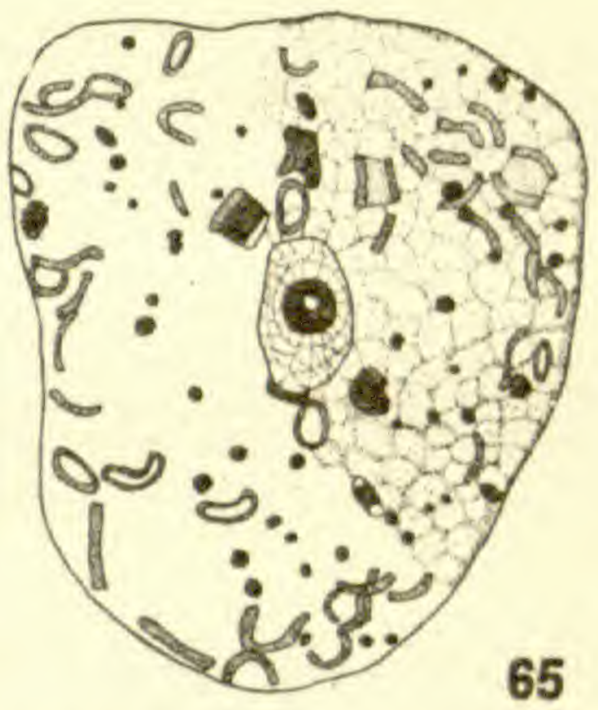

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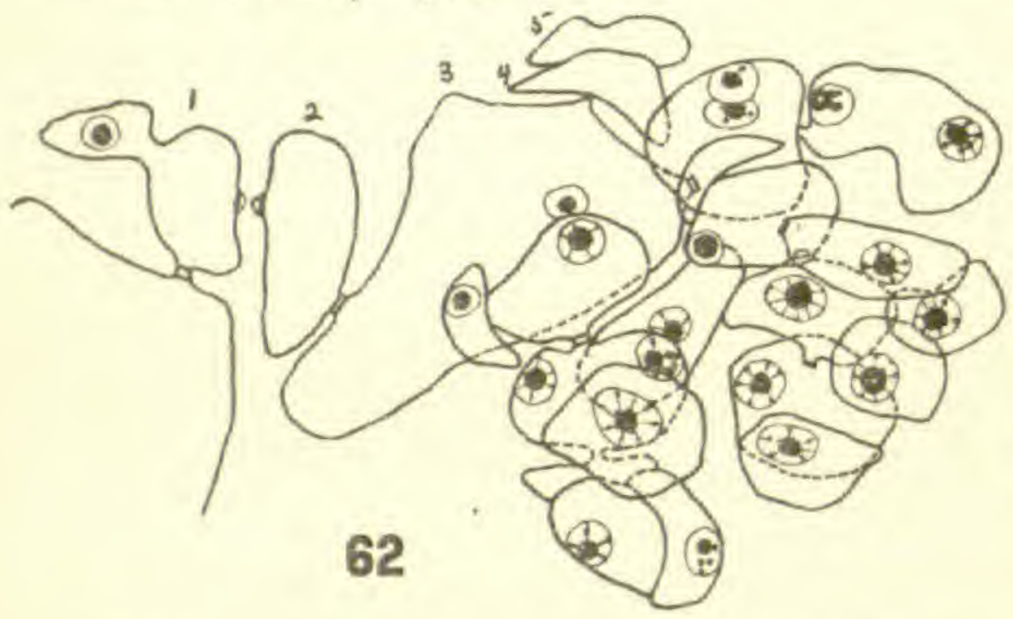

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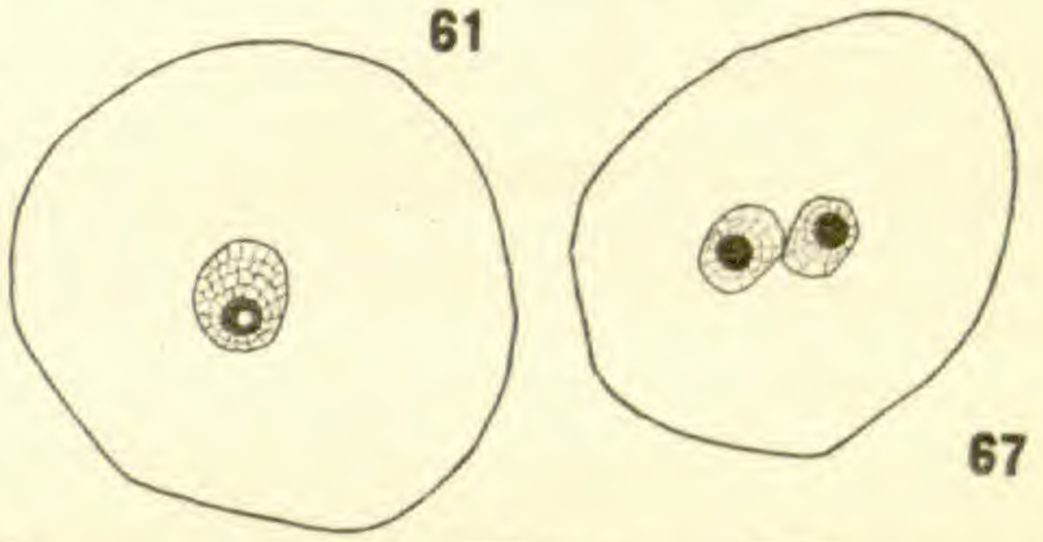

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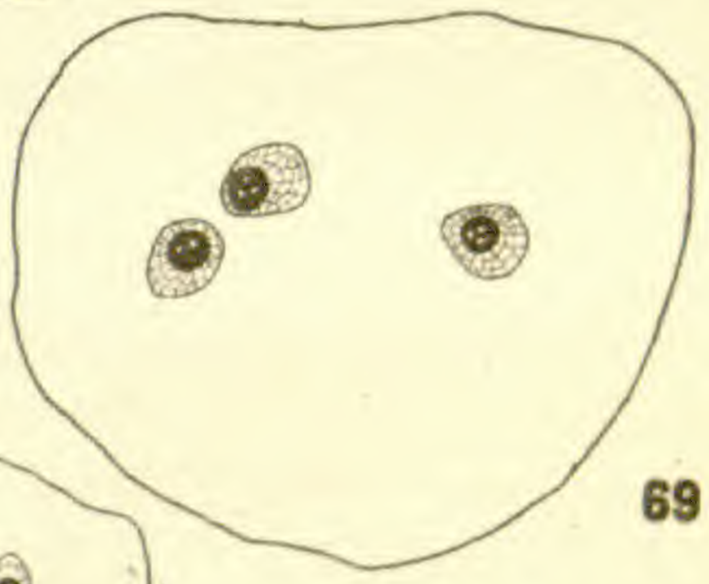

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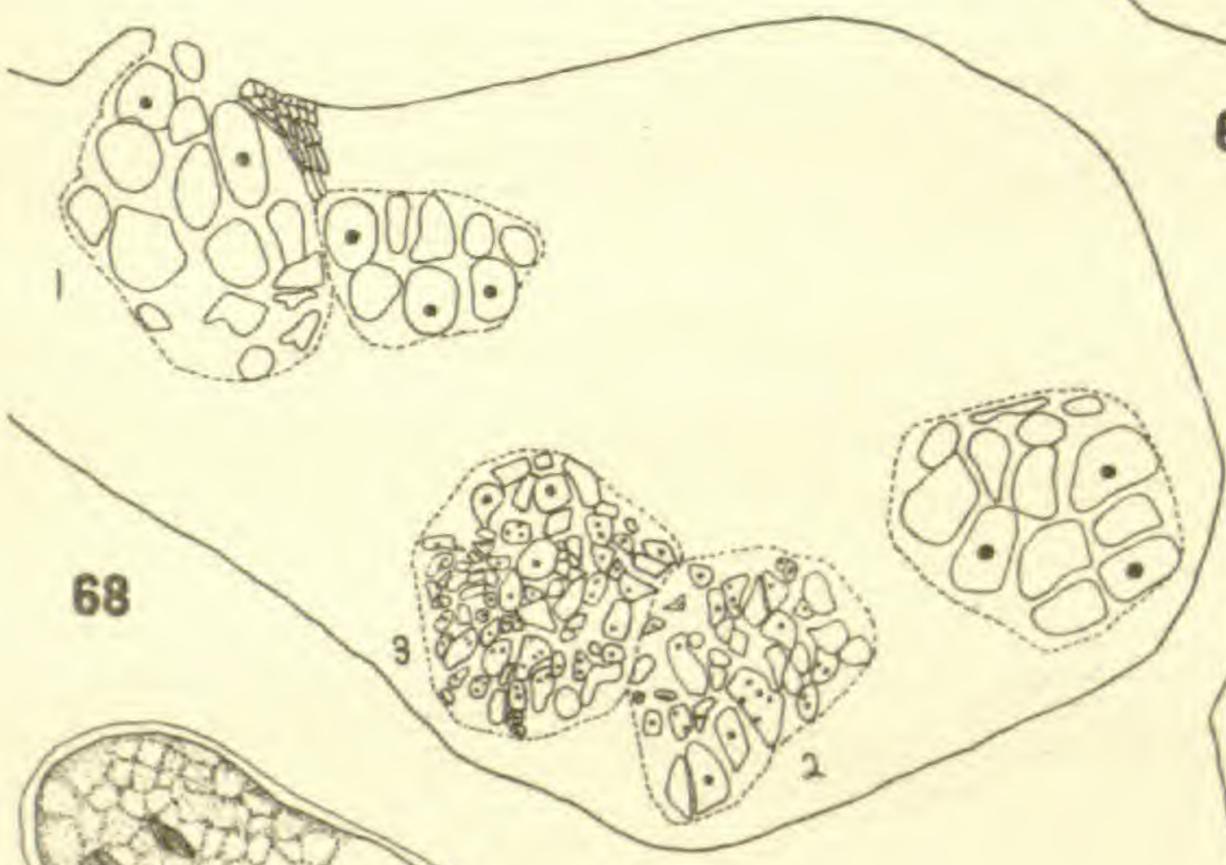

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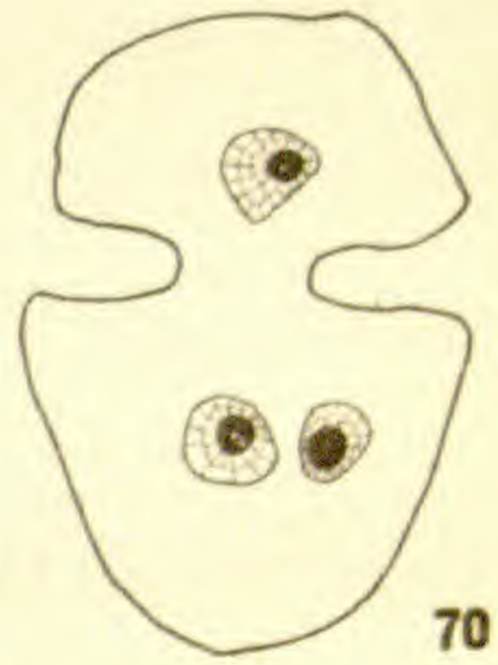

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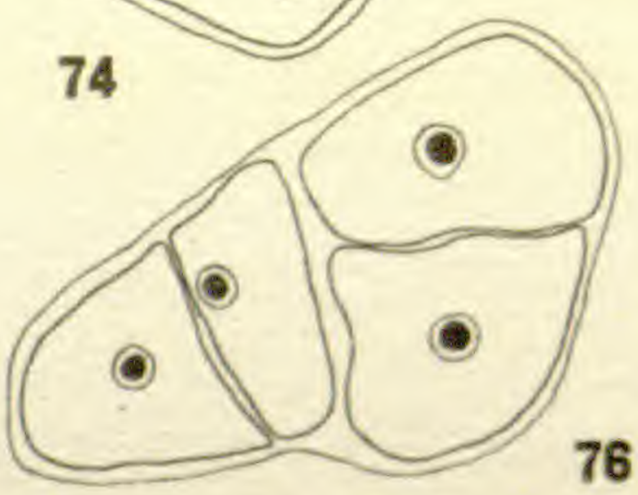

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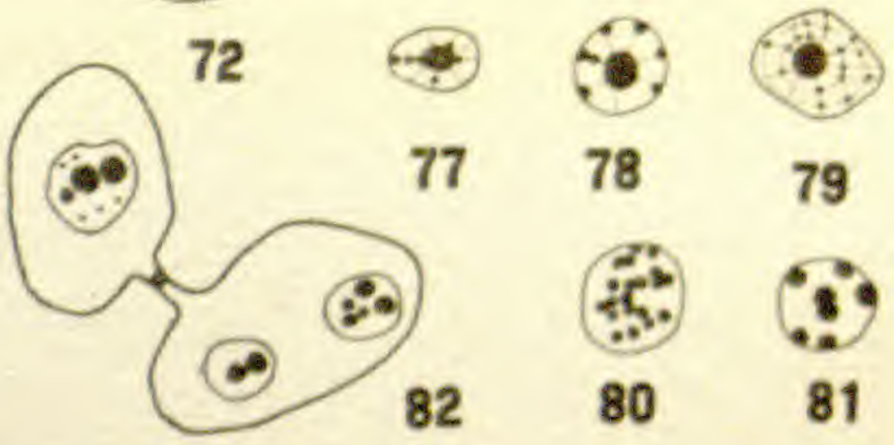

77 78 79

82 80 81

FIG. 74.—Partly shaded; longitudinal section of germinating carpospore showing structure and distribution of chromatophores and structure of cytoplasm; ×1000.

FIGS. 75, 76.—Sections of germinating carpospores showing sequence of cell walls during germination; ×170.

FIG. 77.—Nucleus of cell 3 of auxiliary cell branch showing chromatin granules passing from nucleolus to periphery of nucleus; ×1400.

FIG. 78.—Nucleus of cell 3 of auxiliary cell branch showing chromatin granules at periphery of nucleus; ×1400.

FIG. 79.—Nucleus of cell 3 of a carpogonial branch; small chromatin granules present on linin net; ×1400.

FIG. 80.—Nucleus of cell of an auxiliary cell branch; chromatin in small granules; no nucleolus or linin net present; ×1400.

FIG. 81.—Nucleus of cell 1 of a carpogonial branch showing 7 bodies which may be chromosomes; ×1400.

FIG. 82.—Section of 2 cells of an auxiliary cell branch showing chromatin granules in nuclei; ×1000.

PERMEABILITY OF MEMBRANES AS RELATED TO THEIR COMPOSITION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 231

F. E. Denny

(WITH SIX FIGURES)

Introduction

In a previous paper (1) measurements were made of the rate at which water passes through known areas of certain membranes. Different species of plants and different membranes of the same species showed large differences in the rate of penetration. From these facts the following questions arise. What substances in the membrane are determining the rate at which water can pass through it? Of what relative importance in this process are lipoids, proteins, tannoids, suberin, pectic substances, etc.? An attempt was made to answer these questions by quantitative measurements, and this paper records the results.

The rôle of different substances in the membrane in regulating its permeability to water was determined by comparing the permeability of a membrane before and after extracting it with the solvent of the material studied. Thus, for example, to determine the effect of lipoid materials upon the permeability of the membrane, its permeability in the normal condition was first measured; it was then extracted with a lipoid solvent (for example, acetone), and its permeability again measured. These two measurements were then compared. To accompany these measurements, microchemical tests were made to give information as to the nature of the materials composing the membrane, and the effect of these extractions upon its composition; and for comparison chemical analyses were made of the membrane and the extracted materials.

Methods

The osmometer used has been described and figured in a previous paper (1). The apparatus was immersed in a Freas constant temperature water bath and the temperature kept constant to

approximately 0°.1 C. In all experiments reported in this paper the temperature was 25° C. Sodium chloride solutions were used to induce the osmotic movement of water through the membrane. With the peanut membrane (*Arachis hypogaea*), which is rather highly permeable, concentrations of 0.5 M or 0.6 M were found to be suitable; but for squash, cocklebur, etc., which have fairly high resistance to water penetration, strong solutions of sodium chloride were necessary; for use with such membranes a saturated solution was used, that is, saturated at room temperature.

As noted in the previous paper, different membranes of the same species differ in their permeability to water. This difficulty was overcome by using the same membrane under the various conditions of the experiment. In the tables of results each line of a table represents data obtained from a single membrane; it is not possible to compare one membrane with another; the data given by an individual membrane under different conditions must be compared.

Effect of extracting membranes with boiling water

It was noted that heating the seed coat of *Arachis hypogaea* in boiling water increased the rate at which water passed through. Measurements were made to determine the extent of this increase; and other membranes were tested for similar behavior. The method employed was as follows. A membrane was removed from a soaked seed, fitted into the osmometer, and a measurement made of its permeability. It was then removed from the apparatus, placed in cold distilled water, and heated to boiling point and allowed to remain in boiling water for 5 minutes. It was then placed in the osmometer and a reading made of its permeability. This process was then repeated with other membranes. Table I shows the results, which are expressed in milligrams of water passing through a membrane per hour; the same unit is also used in subsequent tables.

The data show that heating the membrane in boiling water increased the permeability of the peanut and almond membranes, but not that of the grapefruit and squash coats. However, the last two membranes are so slightly permeable that a considerable

increase in permeability might escape notice. With more delicate methods an increase under these conditions might appear. In heating the peanut seed coat a brownish extract was obtained, and it is thought that the increase was due in a large measure to the removal of the soluble substances. The nature of these substances will be treated in that portion of the paper dealing with microchemical and chemical tests.

TABLE I

EFFECT OF EXTRACTING MEMBRANES WITH BOILING WATER

Membrane	Area in sq. mm.	Concentration of solution	Rate before extraction	Rate after extraction	Percentage of increase
Peanut	19.635	0.5 M	28.31	66.73	135.7
"	"	"	32.35	82.90	156.3
"	"	0.6 M	35.05	115.59	229.8
"	50.265	0.5 M	89.54	212.31	137.2
"	19.635	0.6 M	54.67	150.00	174.4
"	50.265	0.5 M	34.67	114.30	229.7
"	"	"	88.56	210.00	137.1
"	19.635	0.5 M	40.44	101.10	150.0
Almond	"	Saturated	17.52	105.14	500.1
Grapefruit	"	"	0	0	.....
"	"	"	0	0	.....
Squash	"	"	0	0	.....
"	"	"	0	11.76	.....

TABLE II

EFFECT OF HEATING DRY MEMBRANES

Membrane	Area in sq. mm.	Concentration of solution	Rate before heating	Rate after heating	Percentage of increase
Peanut	19.635	0.5 M	28.31	42.47	50.03
"	50.265	0.8 M	78.86	101.10	28.2

To gain further information on this point, the effect of heating the dry membrane was compared with the effect of heating the membrane in water. The permeability of a membrane was first measured; it was air dried, placed in a glass tube, and the latter heated in water as described. The membrane was then soaked in water, placed in the osmometer, and a reading again made of its permeability. Table II expresses these results.

The results indicated in table II show that part of the increase in permeability after heating in water is due to the heating effect itself, and that not all the increase is due to the extraction of materials from the membrane.

Effect of extracting membranes with hot lipoid solvents

The rôle of lipoid substances present in the membrane in limiting the rate of penetration of water was investigated by determining the permeability of a membrane before and after extraction of the membrane with a lipoid solvent. The solvents used were alcohol, acetone, and ether. After 4 hours' treatment with the solvent, the solvent was removed from the membrane and the latter soaked in water before being placed in the osmometer. Table III shows the results obtained. In this series all membranes were previously heated in boiling water. It will be noted that extracting the

TABLE III

EFFECT OF EXTRACTING MEMBRANES WITH HOT LIPOID SOLVENTS

Membrane	Area in sq. mm.	Concentration of solution	Rate before extraction	Rate after extraction with hot alcohol	Percentage of increase	Rate after extraction with hot acetone	Percentage of increase	Rate after extraction with hot ether	Percentage of increase
Peanut	50.265	0.5 M	92.67	144.45	55.9	.....	.....	169.84	83.3
"	19.635	0.6 M	52.23	92.34	76.8	.....	.....	92.47	77.0
"	50.265	0.5 M	98.40	228.48	132.2	.....	.....	.....	.....
"	"	"	78.86	161.76	112.6	171.87	126.3	.....	.....
"	"	"	151.65	257.80	69.9	.....	.....	279.06	84.1
"	19.635	"	101.10	117.26	15.9	.....	.....	131.43	30.0
"	50.265	"	113.24	242.65	114.3	217.70	92.2	.....	.....
Squash	19.635	Saturated	11.12	99.42	793.9	101.10	809.2	.....	.....
"	"	"	15.51	82.90	434.5	98.07	532.6	.....	.....
"	"	"	11.45	111.22	871.3	119.08	939.8	.....	.....
Almond	50.265	"	60.66	232.55	283.4	234.89	287.2	.....	.....
"	"	"	50.55	279.71	452.3	279.06	452.0	.....	.....
Cocklebur	19.635	"	20.22	48.28	138.8	40.44	200.0	.....	.....
"	"	"	14.83	45.83	209.1	.....	.....	.....	.....
"	"	"	22.24	56.62	154.6	.....	.....	.....	.....
Grapefruit	"	"	0	0	.....	.....	.....	.....	.....
"	"	"	0	10.11	.....	.....	.....	.....	.....
"	"	"	0	0	.....	.....	.....	.....	.....

membrane with lipoid solvents greatly increased the permeability to water. Only in the case of the grapefruit seed did such treatment fail to show an increase. The different solvents did not

show large differences, but in general acetone and ether were more effective than alcohol in increasing the rate, possibly due to their better dissolving power. The effect of the lipoid solvents upon the permeability of the squash membrane is to be noted especially. Most squash membranes will not show any penetration by water under the conditions of these experiments until they are treated with a lipoid solvent. They then become rather permeable to water.

Effect of extracting membranes with cold lipoid solvent

The significance of lipoids in the membranes is well shown by the series of measurements made when the extraction was carried on at room temperature. The unheated membranes were merely placed in bottles of acetone and allowed to undergo extraction at room temperature for 18 hours. The permeability of the membranes was measured before and after such treatment. Table IV shows the results of this experiment.

TABLE IV

EFFECT OF EXTRACTING MEMBRANES WITH COLD LIPOID SOLVENT

Membrane	Area in sq. mm.	Concentration of solution	Rate before extraction	Rate after extraction with cold acetone	Percentage of increase
Peanut	19.635	0.5 M	14.49	60.66	318.6
"	50.265	"	65.71	246.68	275.4
"	19.635	"	36.00	88.29	145.3
"	"	"	24.27	76.84	216.6
Pumpkin	50.265	Saturated	0	251.77	.....
"	"	"	4.04	141.54	.....
Squash	19.635	"	0	118.64	.....
"	"	"	0	107.84	.....
Almond	"	"	17.52	34.37	96.18
"	50.265	"	101.10	153.67	53.21
Grapefruit	19.635	"	0	0	.....

Infiltration experiments

Experiments were made to infiltrate the extracted membrane with the extracted material. Membranes that had been extracted were soaked in the solvent containing the lipoid extract and then exposed to evaporation; by a continuance of this process it was hoped to impregnate the membrane again with the material that

had previously been removed. Table V shows these results. It is apparent that while the permeability is reduced by the infiltration, the decrease does not reach the low point exhibited by the membrane before the original extraction. Apparently the lipoid materials cannot be put back into the membrane in the condition and position in which they existed before extraction. This would indicate an organized distribution of these materials in the membranes in nature.

TABLE V

EFFECT OF INFILTRATING MEMBRANES

Membrane	Area in sq. mm.	Concentration of solution	Rate before extraction	Rate after extraction with hot acetone	Rate after extraction with cold acetone	Rate after infiltration with lipoid extract
Peanut	50.265	0.5 M	65.71	.....	246.68	181.98
"	19.635	"	24.27	.....	76.84	30.33
Pumpkin	"	Saturated	4.08	146.26	.....	84.92
Cocklebur	"	"	16.17	54.59	.....	42.46
Almond	50.265	"	101.10	298.26	.....	232.52
"	"	"	121.34	326.03	.....	258.81

Effect of extracting membranes with calcium chloride

The calcium ion has been shown by HANSTEEN-CRANNER (2) to reduce the rate of water intake by the cell walls of roots, and it was thought desirable to determine whether calcium had similar effects upon the permeability of seed coats to water. Membranes were selected and measurements made of their permeability to water; these membranes were then soaked for 24 hours in calcium chloride solutions of the concentrations described later; the membranes were then rinsed in distilled water, placed in the osmometer, and their permeability again measured. Table VI shows the results. It thus seems that calcium chloride at the concentrations used increased the permeability of the membrane to water. Whether more dilute concentrations would have similar or opposite effects is yet to be determined.

The fact that callose is soluble in calcium chloride solutions would suggest the hypothesis that the increase in permeability is due to the extraction of callose from the membrane. No microchemical differences were noted in membranes before and after

extraction in calcium chloride, however. The peanut seed coat became dark brown in color after treatment in saturated calcium chloride.

TABLE VI

EFFECT OF EXTRACTING MEMBRANES WITH CALCIUM CHLORIDE

Membrane	Area in sq. mm.	Concentration of solution	Rate before extraction	Rate after extraction	Percentage of increase	Concentration of CaCl
Peanut	50.265	0.5 M	89.17	117.96	32.3	Saturated
English walnut	19.635	Saturated	22.92	38.43	67.6	"
Almond	"	"	24.26	48.53	100.0	"
"	"	"	26.96	32.69	21.3	1 gm. in 259 cc. water
Peanut	50.265	0.5 M	205.58	220.06	7.0	1 gm. in 259 cc. water

Microchemical tests

The membranes were examined microchemically to determine the nature of the substances composing them, and to note the effect upon them of the various methods of treatment. Thin cross-sections of the seed coats were made with a freezing microtome. These sections were 12.5–25 μ thick. The substances for which tests were made are given, together with the tests applied in each case. The textbooks of TUNMANN (**6**) and of MOLISCH (**4**) were consulted for directions regarding these tests.

Tests for suberin.—Insoluble in 50 per cent chromic acid and concentrated sulphuric acid; soluble in 3 per cent alcoholic potash after heating; gives the ceric acid reaction; and stains red with Sudan III.

Tests for tannins.—Blue color with dilute ferric chloride, and a violet color with 0.1 per cent gold chloride.

Tests for lipoids.—Soluble in acetone, ether, and hot alcohol; stain red with Sudan III; true fats give crystals when saponified with potassium hydroxide.

Tests for pectic substances.—Stain red with ruthenium red, and dissolve by treatment with 2 per cent hydrochloric acid followed by 2 per cent potassium hydroxide.

Tests for proteins.—Biuret reaction; those containing tyrosine give a red color with Millon's reagent; those containing tryptophane give a violet color by Liebermann's reaction; other protein tests were not found to be suitable because the color of the membranes interfered with the tests.

Tests for cellulose.—Blue color with sulphuric acid and iodine, and dissolves in copper-oxide-ammonia.

Membrane of peanut (*Arachis hypogaea*)

The cells in the layer at *a* (fig. 1) have walls of cellulose and pectin, and their contents consist of granular lipoid substances. The layer at *b* contains coloring matters, of which tannin was shown to be one. A water extract of this seed coat also gives a positive reaction for tannin. The color in this layer is not completely removed either by treatment with hot water or with lipoid solvents. The layer at *c* consists of the old walls of a parenchymatous tissue that, in the mature seed coat, is much compressed. These walls are of cellulose and pectin. It requires a long period of heating in acid and dilute base to cause these cells to fall apart. The membrane at *d* has no visible contents. The outer walls are of cellulose and pectin, but of a type very resistant to chromic acid. When sections of this membrane are treated with chromic acid these walls are the last to be broken down. Furrows extend across the thick wall of this layer, but the remains of protoplasmic connections between the cells could not be detected. Fatty bodies could be seen

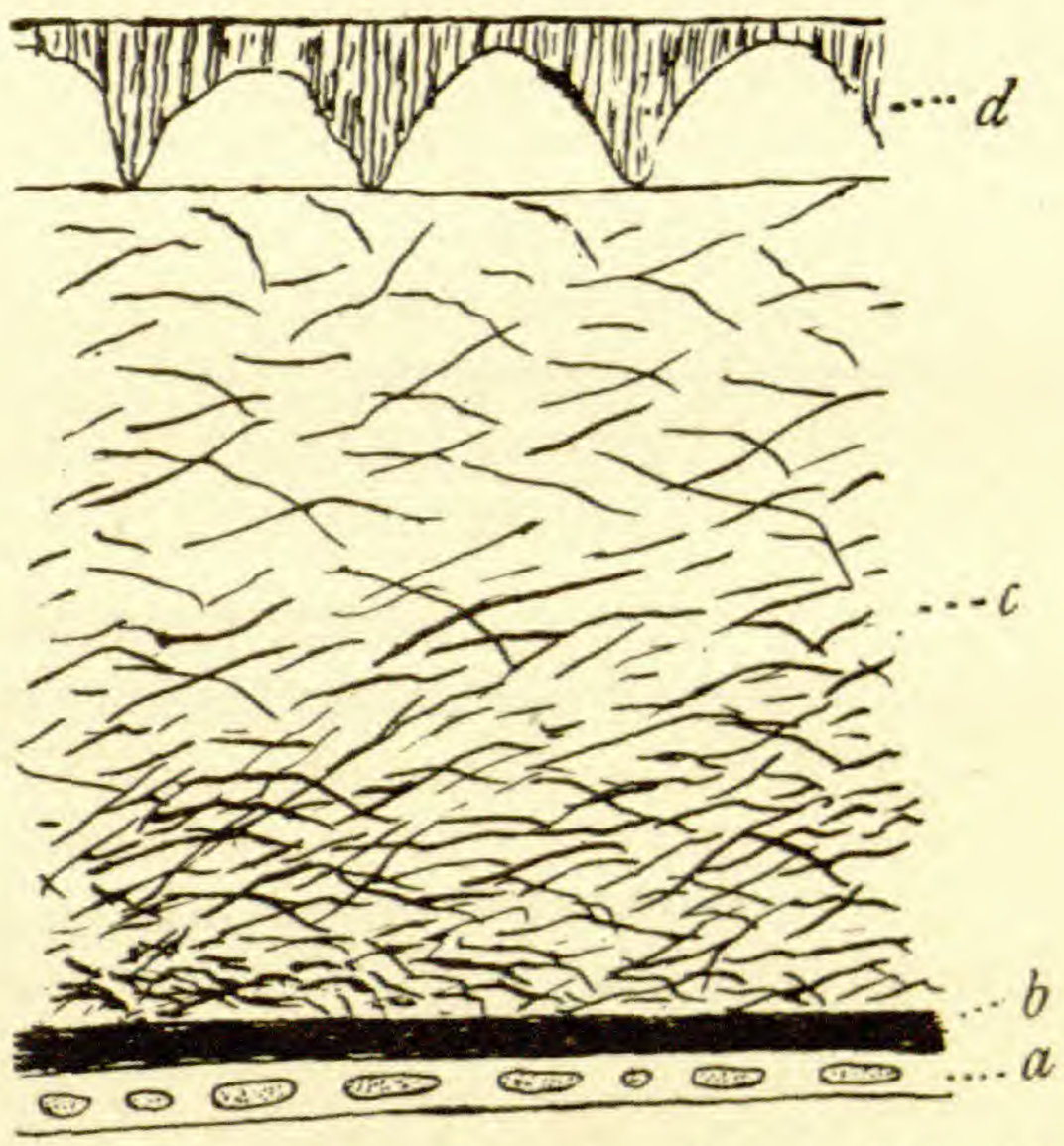

FIG. 1.—Cross-section of seed coat of *Arachis hypogaea*, ×450.

occasionally in the layers *c* and *d*, but saponification crystals were not obtained in place in the tissue.

That lipoid substances are present in the peanut membrane is shown by the analysis of the membrane given later in this paper. It is probable that the lipoids are present in a very fine state of dispersion. No distinctly suberized layers could be detected in the seed coat. All protein tests were likewise negative.

The hot water extraction removes most of the extractable tannins from the membranes, also part of the lipoid materials; the increase in permeability by heating the membrane in boiling water is probably due in large measure to the removal of these substances; and the increase in permeability after extraction with acetone, alcohol, etc., is due to the further removal of lipoid materials. The high permeability of the peanut membrane as compared with other membranes studied is related to its low lipoid content, and especially to the lack of the layers of cells filled with lipoid substances that were found in the other seed coats.

Membrane of cocklebur (*Xanthium pennsylvanicum*)

The walls at *a* (fig. 2) are thick and lignified; those at *b* are much compressed and are composed of cellulose and pectin; at *c* is a suberized layer containing tannins. Tannins also appear in *b*,

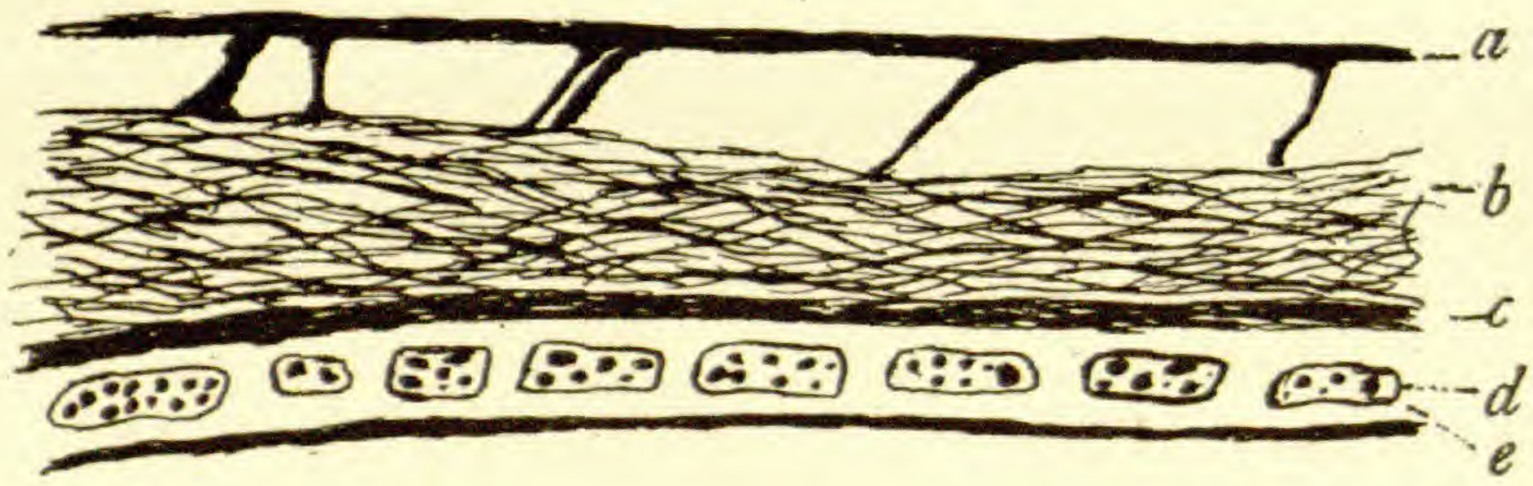

FIG. 2.—Cross-section of seed coat of *Xanthium pennsylvanicum*, ×450

but may have diffused there from *c*. The layer at *d* and *e* is an endosperm layer; the cell walls marked *e* are composed of cellulose and pectin, and the contents *d* are of lipoid substances. Extraction with lipoid solvents does not completely remove the fatty substances from this layer. Protein tests all gave negative results. Layers *a* and *c* are resistant to chromic acid, but layer *d*, *e* breaks up, and the fatty substances flow out in drops.

Membrane of squash (*Cucurbita maxima*)

This is the greenish membrane surrounding the cotyledons, and is obtained from soaked seeds by first removing the horny white spermoderm layer, and then removing the greenish coat from the cotyledons. At *d* (fig. 3) is the greenish layer; at *o* is a compressed layer of the cell walls composed of cellulose and pectin. The inner layer is the endosperm layer, and the cells of this layer are completely filled with fatty substances. The walls are thin and are of cellulose and pectin. In some cases protein reactions were positive in layer *d*, but most tests were negative. No tannins were found and suberized membranes were absent.

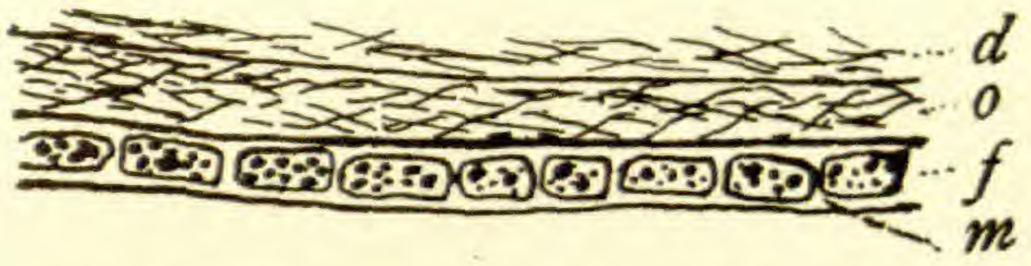

FIG. 3.—Cross-section of seed coat of *Cucurbita maxima*, ×450.

It is believed that the high resistence to the flow of water through this membrane is due to the high content of lipoid substances in layer *f*, *m*. These substances are almost entirely removed by treatment with lipoid solvents, and a large increase in permeability results. It is worthy of note that although this membrane is highly resistant to the passage of water through it as compared with other membranes, distinctly suberized layers do not enter into its composition.

Membrane of almond (*Prunus Amygdalus*)

Large cells *f* (fig. 4) were found to extend from the outer surface of the seed coat. These were in some places much distorted in shape and they also did not cover the entire surface. The walls were of cellulose and pectin, and protein tests gave positive results in this layer. At *d* is a thick layer of parenchymatous cell walls giving cellulose and pectin reactions. A suberized layer is found at *e* and tannin reactions were positive in this region. At *m* again is a layer of compressed parenchymatous cell walls. The endosperm layer is at *o*, *s*. The cells are filled with fatty substances (*o*), and the cell walls *s* are composed of cellulose and pectin. These walls are not thick as compared with either the *Citrus* or *Xanthium* coats. The cell contents also are not completely removed by extraction with lipoid solvents.

Membrane of grapefruit (*Citrus grandis*)

Layer *f* (fig. 5) is a mucilaginous mass of cell wall material giving both cellulose and pectin reactions. The slimy character of soaked seeds of this species is due to this layer. At *o* is a thick

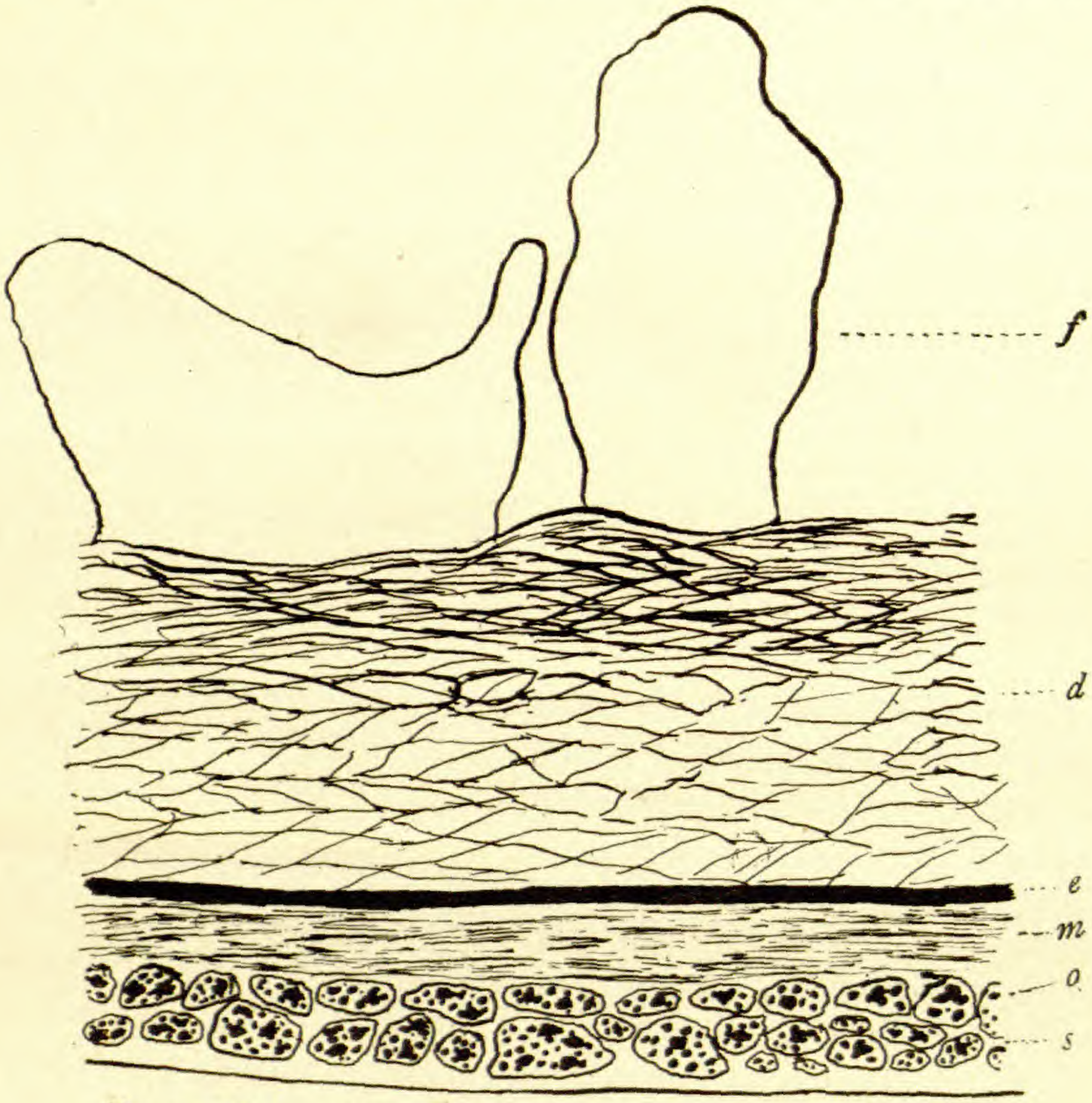

FIG. 4.—Cross-section of seed coat of *Amygdalus communis*, ×450

suberized layer impregnated with tannin and other coloring materials. The inner wall of this layer is especially well suberized. It is more resistant to chromic acid than any layer in any other membranes studied. The thick endosperm layer is marked at *d* and *w*. In some places this layer is 2 cells and in other places only 1 cell thick, the walls are very thick and are composed of cellulose impregnated with pectin. They break apart on treatment with

dilute acids and bases. The cells themselves are filled with fatty substances that are only partly removed by extraction with lipoid solvents. Even after 18 hours of extraction lipoids may still be detected in the coat.

In these various experiments the grapefruit seed coat differed from all others in failing to show increased permeability, or at least a readable rate of water movement after any of the methods of extraction. All tests of this membrane with the osmometer indicated a relatively high resistance of the coat to the passage of water.

A parallel experiment was carried out to give further evidence on this point. This was done by comparing the absorption of

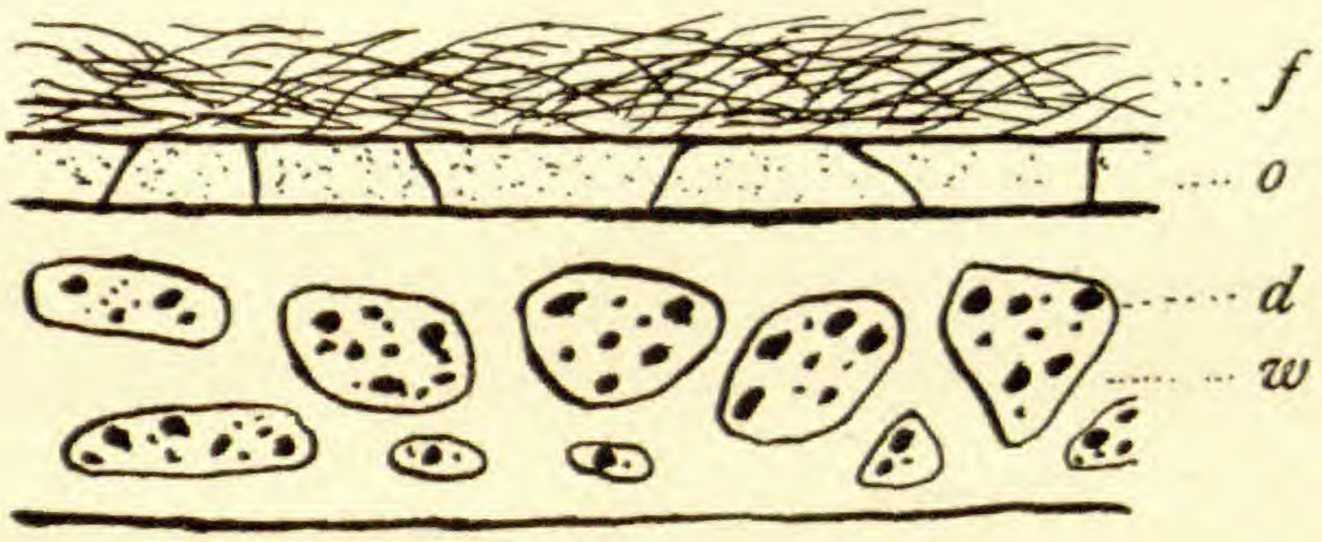

FIG. 5.—Cross-section of seed coat of *Citrus grandis*, ×450

water by the seeds, one lot with coats and the other lot without coats. More than one embryo is usually found in these seeds, so that when the coat is removed the embryos fall apart and accurate weighings are impossible. To overcome this difficulty, a narrow band of seed coat was left around the embryos. This band held the embryos together so that weighings could be made. Table VII shows the results obtained, and curves of these results are shown in fig. 6.

It was found that treating the grapefruit membrane with acids and bases increased its permeability to such an extent that a readable rate of water movement could be obtained. Thus, soaking a membrane in warm 2 per cent sodium hydroxide increased the rate from 0 to 24.26 mg. of water per hour; and in another test soaking the membrane in 2 per cent sodium hydroxide for 24 hours increased the rate from 0 to 72.12 mg. By such treatment, however, the tissue of the seed undergoes a disintegration, so that the

outer yellowish layer *o* (fig. 5) comes off. On using this as a membrane the rate was now found to be 210.27 mg. per hour. This indicates that the inner portion of the membrane is the one that

TABLE VII

RATE OF ABSORPTION OF WATER BY SEEDS OF *Citrus grandis*

Time	Coats on		Coats off	
	Weight in gm.	Percentage of water imbibed	Weight in gm.	Percentage of water imbibed
Commencing	2.423	6.13	2.087	5.67
After 12 hours	2.762	20.9	3.135	58.9
" 36 "	2.888	26.5	3.233	64.3
" 84 "	2.932	28.4	3.171	60.8
" 108 "	2.942	28.9	3.123	54.8
" 144 "	2.986	30.8	3.129	58.7
" 204 "	3.056	33.9	3.139	59.2
Oven dry	2.283		1.971	

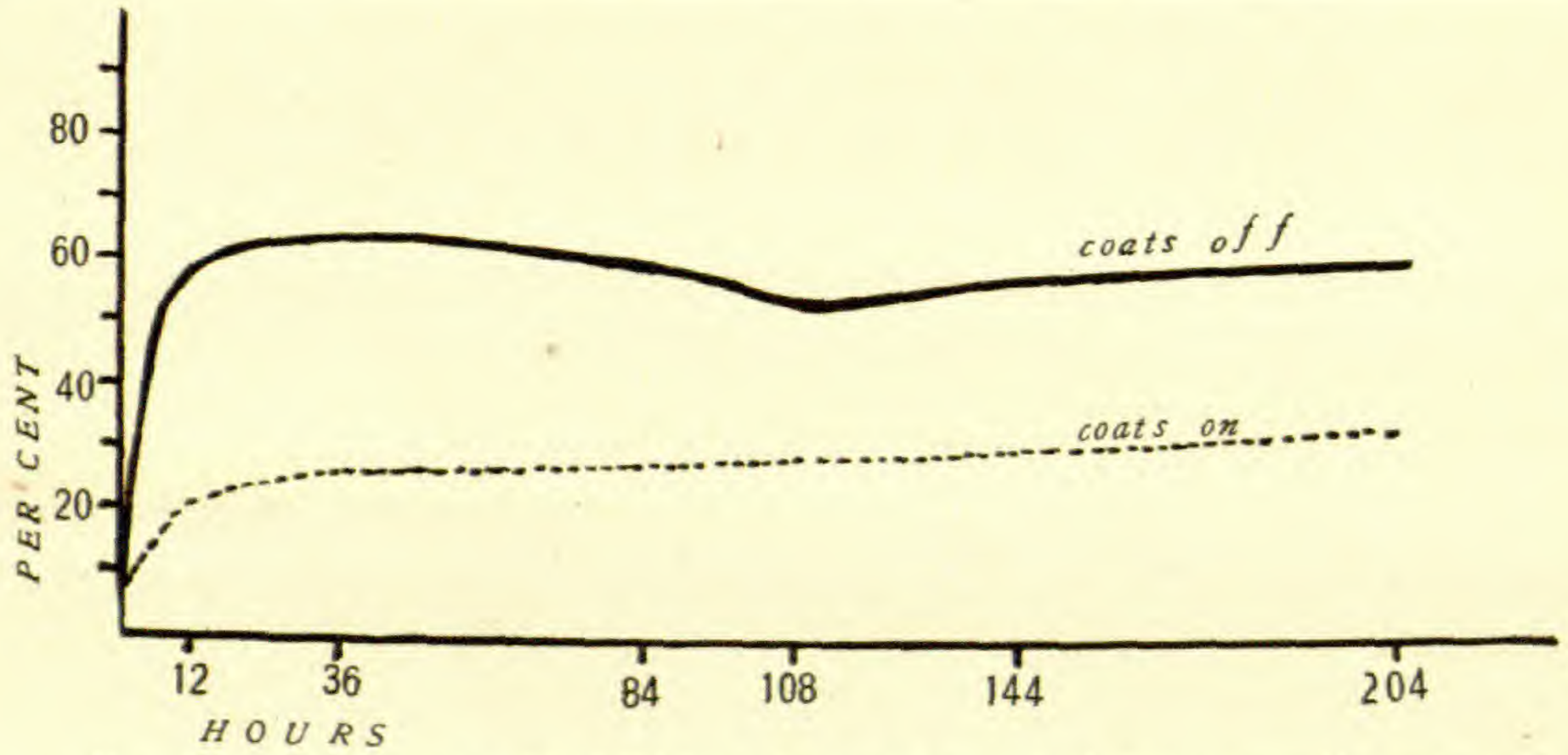

FIG. 6.—Showing rate of imbibition of water by seed of *Citrus grandis;* rate with coats compared with rate without coats; curves obtained by plotting data given in table VIII.

offers the resistance to the passage of water, and it is believed that this resistance is due to the presence of the large amount of fatty substances surrounded by thick pectinized walls.

Results of chemical analysis

An analysis was made of a quantity of the seed coats of *Arachis hypogaea*. A complete analysis for all constituents of the membrane was not attempted, but an examination was made only for

those substances that appeared to be significant as judged by the results of the extraction experiments and of the microchemical tests.

About 10 pounds of unroasted peanuts were shelled, the pods discarded, and the seeds put at once into cold water to soften the seed coats; as soon as the coats were moist enough, they were removed from the embryos, and dried on blotting paper in the air. The water in which the seeds had been soaking was brownish in color; it was made up to a volume of 1000 cc. with distilled water; this was called the "preliminary extract," and later its content of tannins was determined.

The air dried seed coats were divided into two lots, one lot to undergo extraction in a manner comparable to the extractions performed in the experimental work, and the other lot held as a check to determine the original composition of the membrane before extraction. The first lot was extracted with water, by putting the membranes into cold water, raising the temperature of the water to boiling point, and holding it at this temperature for 5 minutes; the water was then drained off through a filter, and the residue washed with cold distilled water until only a faint straw-colored liquid passed through the filter. The filtrate was made up to 1500 cc., and was called the "hot water extract." The residue was vacuum dried, placed in Schleicher and Schüll extraction cups, and extracted in a Soxhlet extractor for 4 hours with acetone. The extract was made up to a volume of 250 ccm. and was called the "acetone extract." The insoluble material was called "residue."

Methods

Tannins were estimated by Proctor's modification of Lowenthal's method (**7**, p. 150). The distribution of tannins in the preliminary extract, in the hot water extract, and in the acetone extract was determined.

The lipoids in the acetone extract were estimated by removing a sample, evaporating in a Jena glass dish, and drying to constant weight in a desiccator. To obtain the amount of lipoid in the hot water extract a sample was taken; strips of filter paper were dipped in the sample until the liquid was absorbed by the filter paper; the

latter was then dried in a vacuum desiccator, placed in extraction shells, and extracted with acetone in a Soxhlet extractor; the weight of the lipoid material was determined by evaporation. The nitrogen content was measured by the Kjeldahl method, and the protein content by the Van Slyke method, both of the latter methods as described by MATHEWS (3). An attempt was made to determine the phosphorus content of the various portions, but it was found to be very low, and insufficient material was left for conclusive analyses. The results of the analyses are summarized in table VIII.

TABLE VIII

RESULTS OF ANALYSIS OF SEED COATS OF *Arachis hypogaea;* DISTRIBUTION OF MATERIALS*

	Preliminary extract	Hot water extract (gm.)	Acetone extract (gm.)	In residue (gm.)	In sample (gm.)
Total solids	.....	3.8940	0.6170	.....	.....
Lipoids	.....	0.2646	0.6170	.....	0.8816
Nitrogen†	.....	0.0507	.....	0.6784	0.7615
Tannins, equivalent to cc. $KMnO_4$	1970.0 cc.	1245.0 cc.	0	.....	.....

* Weight of sample, vacuum dried, 30.1166 gm.
† Amino acid nitrogen not present in determinable amounts.

The following points with reference to table VIII are of interest. The water extract contains two substances that are believed to be of importance in the permeability of the membrane, i.e., tannins and lipoids. Tannins have been reported by REICHARD (5) to be of importance in the permeability of the barley grain to salts, and these results indicate that tannins may also take part in the permeability of the peanut seed coat to water. Remembering the effect of the acetone extractions in increasing the permeability of the membranes, it is interesting to note the low content of lipoid in this extract, only 2.04 per cent of the solid matter. A small amount seems to have great effect in reducing the rate of water movement. From the fact that lipoids were also found in the water extract we may conclude that a part of the increase after the water extraction was due to the removal of lipoids. The lack of proteins in the membrane will also be noted in the table.

Discussion

These experiments point to the important rôle of lipoids in permeability to water. The peanut seed coat, which is the most permeable of the membranes studied, is the one containing the least amount of lipoid substances, and when the lipoids are removed an increase in permeability results. Only in the case of the grapefruit did extraction with lipoid solvents fail to increase the permeability. Reasons for this behavior are suggested.

Suberized layers have usually been considered the main factors in restricting water movement through plant membranes. In these experiments there was no evidence that suberin was a dominant factor. The squash seed coat has no distinctly suberized layers, and yet it is resistant to the passage of water. These statements apply only to the membranes studied; in other membranes, more highly impermeable, suberized layers may be of first importance.

Other substances that appeared to be effective in reducing the permeability of these seed coats were tannins and pectic substances, the latter especially when deposited in thick cell walls.

Summary

1. The rôle of different substances in seed coats in regulating their permeability to water was studied.

2. Membranes were extracted with water, alcohol, acetone, ether, and calcium chloride, and their permeability measured before and after such treatments.

3. Cross-sections of the seed coats were made and tested microchemically to determine the nature of the substances present, and the effect upon them of the different methods of treatment employed in the experiment. A chemical analysis of the seed coat of the peanut and of the extracted materials was made to determine the content of tannins, lipoids, and proteins.

4. Extraction with hot water increased the permeability of the peanut and almond seed coats, the percentage increase ranging from 135 to 500 per cent. Such treatment removed from the peanut membrane the tannins and a part of the lipoid materials.

5. Extraction with hot water did not measurably increase the permeability of the grapefruit and squash seed coats; but these membranes before such treatment were so resistant to the passage of water that an increase could have resulted from the hot water extraction without being detected by the apparatus under the conditions of the experiment.

6. Extraction with hot lipoid solvents increased the permeability of all membranes studied except the seed coat of the grapefruit. The percentage of increase ranged from 15 to 871 per cent.

7. Extraction with acetone at room temperature also increased the permeability of all the seed coats except that of the grapefruit. The percentage increase ranged from 53 to 313 per cent.

8. After a membrane had its lipoid content removed, its permeability was decreased by impregnating it with the lipoid material that had been extracted; but in no case was the permeability reduced to the low point exhibited by it before the process of extraction.

9. Calcium chloride treatments increased the permeability of the membranes, but the cause of this increase could not be determined.

10. The substances found to be factors in determining the permeability of the membranes to water were lipoids, tannins, and pectic substances. Suberized layers were not found to be significant in the membranes studied, and the presence of soluble proteins could not be detected.

I wish to express my thanks to Dr. WILLIAM CROCKER and to Dr. S. H. ECKERSON for helpful advice and suggestions during the progress of this work.

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LITERATURE CITED

1. DENNY, F. E., Permeability of certain plant membranes to water. BOT. GAZ. **63**: 373–397. *figs. 2.* 1917.
2. HANSTEEN-CRANNER, B., Über das Verhalten des Kulturpflanzen zu der Boden Salzen. Jahrb. Wiss. Bot. **53**: 553–599. 1913–14.
3. MATHEWS, A. P., Physiological chemistry. New York. 1915.
4. MOLISCH, HANS, Mikrochemie der Pflanze. Jena. 1913.
5. REICHARD, ALBERT, Hat der Gerbstoffe des Gerstenkorns einen Anteil an der Halbdurchlässigkeit dieser Membran? Zeit. Gesamte. Brauw. **33**: 145–148, 157–169. 1909.
6. TUNMANN, O., Pflanzenmikrochemie. Berlin. 1913.
7. U.S. Dept. Agric., Bur. of Chem. Bull. 107 (revised). 1912.

SEXUALITY OF FILAMENT OF SPIROGYRA[1]

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(WITH PLATES XXIII–XXV)

Is the filament of *Spirogyra* unisexual or bisexual? This has been a question for many years, but the reports of the great majority of modern workers would indicate that they regard the filament as wholly of one sex. The answer to the question hinges upon the presence of zygotes in both of the conjugating filaments. If they occur in only one of the two, the filaments may be said to be unisexual, since one functions as the male and the other functions as the female; if, on the other hand, they occur in both filaments and are formed by scalariform conjugation, the filaments may be said to be bisexual, since the empty cells in each have furnished the male gametes, while the ones in which the zygotes occur previously contained female gametes. The latter case is known as cross-conjugation.

The advocates of the theory of the unisexuality of the filament urge that as a rule all the male gametes arise in one filament and pass over into the other, so that zygotes appear in but one of the two conjugating filaments. They urge also that cases of cross-conjugation are so rare that they should be considered abnormalities and the result of forced conditions. Strangely enough they ignore lateral conjugation in so far as the sexuality of the filament is concerned. On the other hand, those who do not accept this theory have taken two different positions: one, that the gamete is absolutely sexless (HASSALL **17**, p. 130; also PRINGSHEIM, see BENNETT **2**); and the other, that the filament is bisexual, based

[1] This work was done in the Biological Laboratory of Trinity College, Durham, North Carolina, under the direction of Dr. J. J. WOLFE. The writer wishes at this point to thank Dr. WOLFE for his many kind and helpful suggestions. He further desires to acknowledge aid rendered on special questions arising in the course of the work by Professor G. S. WEST, of the University of Birmingham, England, and Professor J. M. COULTER, of the University of Chicago. Thanks are due also to Mr. J. P. BREEDLOVE, librarian of Trinity College, for his great assistance in securing literature on the subject.

principally upon the fact of lateral conjugation. Occasionally a case of cross-conjugation is presented as evidence, but these cases have been so isolated that they have been of little value. A clear case, then, of cross-conjugation occurring normally in a species would certainly strengthen the position of the advocates of the bisexuality of the filament.

VAUCHER (**28**), who was apparently the first to work upon *Spirogyra*, at least upon the problem of its reproduction, figures it in most cases in cross-conjugation. Figs. 1–4 of pl. XXIII are photographic copies from his plates. In his general description of the *Conjuguee* he says "ordinarily one of the filaments gives while the other receives throughout its entire length. Still it is not rare for the same filament to give in one part of its length and receive in another in such a manner that some of the cells of the tube are filled and some are emptied." Again, "while a tube usually gives or receives throughout its entire length, still it frequently happens, as I have said before, that some give and receive almost alternately." In regard to *Conjuguee condensee*, he says "the berries are indifferently lodged in either tube" (VAUCHER **28,** p. 69).[2] Such statements seem to point clearly to cross-conjugation, but when we remember that VAUCHER, probably because he was unacquainted with the phenomenon, did not figure or describe lateral conjugation, and that he was dealing in part with species which normally reproduce in this manner, we have reason to believe that he was really observing a combination of lateral and scalariform conjugation which might easily have been mistaken for cross-conjugation. Just such conditions have been observed by the writer. Pl. XXIII, fig. 9, shows diagrammatically such a case that came under his observation. At first glance the position of the zygotes in the 2 filaments gives the appearance of cross-conjugation. Upon closer investigation it is found that the contents of cell (*a*) probably passed into (*b*), (*c*) and (*d*) represent another lateral conjugation, (*e*) and (*f*) have conjugated in the regular scalariform manner with (*g*) and (*h*), (*i*) and (*j*) are lateral, and (*k*) entirely failed to conjugate.

[2] Free translations.

VAUCHER has figured 6 species of *Spirogyra*, 2 of which are shown in scalariform conjugation and 4 in cross-conjugation. I have arranged these species in table I so that the methods of reproduction assigned by the different writers may be compared.

TABLE I

SPECIES ACCORDING TO VAUCHER	METHOD OF REPRODUCTION		
	Vaucher	Wolle	DeToni
Allongee.....	Scalariform	Scalariform	Lateral
Adherente.....	Scalariform	Scalariform	Not stated
Majeure.....	Cross	Scalariform	Not stated
Portiquis.....	Cross	Scalariform and aplanospores	Not stated
Condensee.....	Cross	Scalariform	Often lateral
Renflee.....	Cross	Scalariform and lateral	Often lateral

At first glance there does not seem to be much uniformity, but if we assume that where DETONI does not state the particular method of conjugation that the scalariform method is meant, we find that WOLLE and DETONI agree that 5 of these species have scalariform conjugation. They also agree in assigning lateral conjugation to *renflee*. However, DETONI alone states that *condensee* conjugates laterally, while WOLLE alone states that *portiquis* forms aplanospores. In these 3 cases, therefore, it would be possible to have combinations with the appearance of cross-conjugation. *Majeure*, however, according to both WOLLE and DETONI, conjugates only in the usual scalariform manner. Figuring this species in cross-conjugation by VAUCHER is rather surprising, as will appear in the discussion which follows.

Since there have been but few cases of cross-conjugation reported, we have reason to doubt its occurrence in as many species as figured by VAUCHER. Furthermore, no other observer up to the present time has recorded its occurrence in any of these species. However, the species discussed in the present paper has been tentatively identified as *S. inflata* (Vauch.) Rahb., which according to RAHBENHORST would include *Conjuguee renflee* of VAUCHER.

Recognizing, then, the facts that (1) VAUCHER was unacquainted with lateral conjugation, (2) a combination of lateral

and scalariform conjugation resembles true cross-conjugation in appearance, (3) he has figured cross-conjugation in 3 species in which this appearance would naturally occur, due to this combination, (4) no other observer has reported any of these species in cross-conjugation, the writer feels that there is good ground to doubt the observation of true cross-conjugation by VAUCHER.

HASSALL (**17**) also has figured cross-conjugation, but does not describe it for any of the species thus figured, although he does mention it in the general description of the Zygnemaceae. Figs. 6 and 8 of pl. XXIII are reproductions from his plates showing this phenomenon. While HASSALL figures and describes cross-conjugation, he nowhere claims to have observed its occurrence. One of the contemporaries of HASSALL, in reviewing his book (HASSALL **18**), says "it is unfortunate that the author has not pointed out the cases in which the figures are not the result of his own observations but copied from published plates." Certain cases are cited in which HASSALL's plates were taken from published plates, and these tend to cast some doubt on the source of his plates on cross-conjugation, although they are not among those cited. The writer has been seeking the originals of these borrowed plates, but as yet has not been able to locate them, and is therefore uncertain as to whether or not they exist. BENNETT (**2,** p. 432), in reviewing the general field, says in regard to HASSALL "it is quite possible that the statement may be the result of an error of observation; I have often been deceived in this way." Further, HASSALL claims to have discovered lateral conjugation, and with this as his basis he lays great stress upon the act of conjugation as being without sex, explaining the movement of the gamete by the "law of universal gravitation" (HASSALL **17,** p. 132). He gives the following reasons for his belief: (1) both cells are alike; (2) reproductive bodies are surrounded by the heavy wall solely for protection; (3) spores arise in the same species, both with and without conjugation; and (4) there is conjugation but "no mixing of the endochrome." As a conclusion he says "thus, so far as can be presumed, the information already acquired would be opposed to the belief in the existence of sex as applied to the cells of *Conferva*."[3]

[3] This genus as used by HASSALL included *Spirogyra*.

With this as a basis one is not surprised to find him ascribing cross-conjugation as a character of the genus.

BENNETT (2) states that CLEVE (8) has figured 2 cases which may be called cross-conjugation. The writer, however, thus far has been unable to secure this monograph.

BESSEY (4) figures a case which he calls cross-conjugation. This figure is reproduced here (pl. XXIII, fig. 7). It is to be noted that in his description no mention is made of cross-conjugation, although it is made later (BESSEY 5). If the figure is complete, it is by no means a conclusive case, as it shows only one pair of conjugating cells. The presence of a zygote in filament *A* can be explained as the formation of an aplanospore after the cell had put forth the conjugating tube. WEST (29, fig. 64) shows a case of false cross-conjugation occurring in this manner. The idea is further supported by the observation of the writer, and diagrammed on pl. XXIII, fig. 9. Again, the cell in the filament *A*, if in cross with *B*, should bear an oval zygote, since the oval form seems to be dominant to the spherical. This fact was observed by BESSEY, as he states that the zygote of *S. protecta* is oval in shape, but those of *S. majuscula* are spherical, and that the hybrid between these two assumes the oval shape characteristic of *S. protecta*. He does not apply this, however, to the zygote formed in filament *A*, which is spherical. If the cell referred to is in cross with another filament, that filament should be shown. The figuring of the canal is not sufficient evidence, as has been shown. Furthermore, this hybrid is undoubtedly a forced condition. The strenuous efforts of *S. majuscula* to reproduce are shown in another case cited by BESSEY (6), in which this species tries to hybridize with *Mesocarpus*.

BENNETT and MURRAY (3, p. 266) say "as DEBARY (11) has pointed out, ... one of the two filaments is entirely emptied, while the other is completely filled with zygospores." To this they have added a footnote "HASSALL, however, figures and asserts to the contrary."

WEST (30, p. 125) refers to the footnote of BENNETT and MURRAY, but maintains that the phenomenon is rare. He states that he has seen but a single case, and that was in *S. gracilis* (WEST 29, p. 47). G. S. WEST, in a more recent personal com-

munication, says that he regards the phenomenon as rare, not having seen more than half a dozen cases of it. However, he sees no reason why it should not occur, as it represents much the same phenomenon from the sex standpoint as lateral conjugation. In both cases there must be a differentiation of sex in the one filament. A very distinct difference, however, will be pointed out later.

COULTER (10, p. 40) briefly describes cross-conjugation but does not assign it to any species.

On the other hand, the great majority of botanists doubt the occurrence of cross-conjugation, and with it the bisexuality of the *Spirogyra* filament. AGARDH (1), according to HASSALL (15), states that one filament is always giving and the other always receiving. WOOD (32) mentions scalariform and lateral conjugation, but not cross-conjugation. COOKE (9) has 11 plates of *Spirogyra*, but does not figure cross-conjugation. DEBARY (11) states that one filament gives and the other receives. WOLLE (31) figures and describes 39 species of *Spirogyra*, citing VAUCHER and HASSALL, but does not mention cross-conjugation. HABERLANDT (14), according to KLEBS, holds that the filaments are distinctly sexual. In order to verify this statement, KLEBS (22) grew *Spirogyra* on nutrient agar, but found that a filament would not conjugate with itself, and therefore concluded that it was all of one sex. This experiment might prove the case for one species, but it hardly appears just to use it as the basis for a sweeping statement that bisexuality does not occur in *Spirogyra*. MOTTIER (24) cites this experiment as a basis for belief in the unisexuality of the filament. DETONI (12) absolutely ignores cross-conjugation, although he cites the plates of both VAUCHER and HASSALL in his descriptions of the species figured in this condition by them. LOTSY (23) states that there is a distinct difference between the male and the female filaments. OLTMANNS (25, p. 64) states specifically that we have to do with male and female filaments. HERTWIG (19) makes a similar statement. ENGLER and PRANTL (13) speak of the visible difference between the male and female filaments. ROBERTSON (26), who grew *Spirogyra* extensively under abnormal conditions, did not find a case either of cross or lateral conjugation. Since cross-conjugation did not occur in his

own experiments and is so exceedingly rare in the work of others, he thinks it must be considered a very unusual abnormality. YORK (33), who worked several years on the sexuality of *Spirogyra*, seeking methods for determining the sex before conjugation, says "zygotes were never found in both filaments, but only in the one containing the greater amount of food. The male and female are morphologically and physiologically different."

Summarizing, it appears that the evidence for bisexuality is based (1) upon work done over 100 years ago, when the importance of cross-conjugation was not realized, and has not been verified since; (2) upon lateral conjugation, a strong basis ignored by the unisexualists; (3) upon the chance observations of CLEVE, BESSEY, and WEST. At this point it is interesting to note that HASSALL figures no species in cross-conjugation that was thus figured by VAUCHER; that BESSEY'S species is not that of either HASSALL or VAUCHER; and that the one cited by WEST is still different. Thus these would all appear to be abnormalities.

On the other hand, the advocates of unisexuality urge (1) that KLEBS found that a filament would not conjugate with itself, hence it is of one sex; (2) that the work of VAUCHER needs verification; (3) that the figures of HASSALL may have been taken from older works; (4) that, since the species figured by VAUCHER and HASSALL are common, the phenomenon should have been observed by modern investigators; (5) that specialists have seen but few cases, not more than a dozen, and these have been called abnormalities because of their rareness; (6) that experimentalists who have spent years on the sexuality and abnormal conjugation of *Spirogyra* have not observed cross-conjugation. All these things point to the unisexuality of the filament.

If, however, as stated in the beginning of this paper, a true case of cross-conjugation of *Spirogyra* should occur normally to any extent, it would settle the question, for one species at least. A species in this condition was found by the author while making a collection of algae along a stream near Durham, North Carolina, on April 1, 1915. The water stood in pools on the low ground, and it was from one of these pools that the collection was made. There was comparatively little of this species mixed with a larger *Spiro-*

gyra and some germinating *Vaucheria*. The phenomenon of cross-conjugation was not observed until the material had been brought into the laboratory, and heavy freshets prevented further collection. However, from this interwoven mass, not larger than a pea, more than 70 slides have been prepared showing cross-conjugation. Some of the slides have several distinct pairs of filaments in this condition. Considerable effort was made to secure long filaments, but this was unsuccessful on account of the intricate tangling of the mass. This species was again collected early in April 1916, in approximately the same locality, showing essentially the same phenomena as the earlier collection, but, owing probably to deficient rainfall, was not abundant and hence it was not possible to add any important facts not shown by the material gathered the year before.

A careful investigation of the material shows that all the known forms of reproduction in *Spirogyra* are represented in this species. While aplanospores occur, they are not found frequently, and they are hard to identify. The regular zygotes are formed by 3 distinct methods. The most common is the well known scalariform method, in which the 2 conjugating filaments have the appearance of a ladder, and the gametes travel in only one direction, so that one filament contains all the zygotes. This has been followed for 20 to 25 pairs of conjugating cells. Zygotes are formed also by lateral conjugation, the contents of one cell passing into the adjoining cell of the same filament. This is accomplished in this species by the bulging of the cell wall away from the septum at one side until there is a small opening left between the 2 cells through which the contents pass. In general appearance the zygotes are like those formed by scalariform conjugation. Usually the cells follow the law laid down by HASSALL (**16,** p. 34) that 2 males alternate with 2 females. This applies only to those filaments in which lateral conjugation occurs alone. In this species it is frequently accompanied by genuflexions. This method (lateral conjugation) occurs in filaments that are also conjugating in the usual scalariform manner. Zygotes are further formed by true cross-conjugation in which "there is the formation of a perfectly normal zygospore in each of the conjugating filaments." Here,

again, the zygotes have the appearance of those formed by scalariform conjugation. This has been followed for 16 pairs of conjugating cells. In this case they are all in conjugation, and, strangely enough, there are 8 zygotes in each filament. These filaments are shown schematically in fig. 1 of pl. XXV, and in part in fig. 1 of pl. XXIV. This is the only pair of filaments with any considerable number of zygotes thus far found which shows the same number in each of the conjugating filaments. A glance at the plates will emphasize the fact that, in general, there is no such regular order. Whole filaments would probably shed further light upon the occurrence. Moreover, filaments are found frequently in which both true cross-conjugation and lateral conjugation occur.

Much care is needed in the study of cross-conjugation, as there are many chances for error. As previously stated, there are combinations of scalariform and lateral conjugation that at first appear to be cases of true cross-conjugation. The writer has used the utmost care and has been compelled to discard a number of slides that at first were thought to show cross-conjugation. Only such cases as have complied with the following rules have been regarded as in cross-conjugation: (1) zygotes must occur in both filaments, the swelling of the egg cell is insufficient evidence; (2) the connecting tube must be visible; (3) the male cell must be empty; (4) end cells must be discarded unless the preceding conditions are met, since lateral conjugation may have occurred.

Slides have been permanently mounted in glycerine and from these the microphotographs of pl. XXIV were taken. They are made at 225 diameters. The plate shows the original photographs, and no "retouching" has been done either on the plates or the prints. Fig. 1 was made from a plain glycerine mount; while the others were from slides stained either with iodine or Magdala red in order that the cell walls might be made a little clearer. Figs. 2 and 6 show several filaments conjugating with each other. In the other figures only 2 filaments are involved. These are, however, very clear cases.

Schematic drawings have also been made (pl. XXV). In these the writer has laid the filaments parallel on the paper, regardless

of their twisting and turning on the slide. In each case, however, he has retained all the cells, whether conjugating or not, from the first to the last pair of conjugating cells in the filaments. When 3 or more filaments were found conjugating, they have been laid parallel also, and as nearly as possible in their relative position. The writer has adopted the schematic method in this paper in order that the reader may grasp more easily the relative position of the empty cells, unchanged cells, and the zygotes. Diagrammatic drawings can be followed more readily than the windings of the camera drawing, as may be seen by a comparison of fig. 1, pl. XXIV, with fig. 1, pl. XXV. The former is a microphotograph of a portion of the pair of conjugating filaments schematically represented in the latter. Furthermore, the diagrammatic method consumes considerably less space.

Two of these slides were exhibited at the meeting of the North Carolina Academy of Science in May 1915, where they were observed by a number of botanists. Other slides were sent to Professor G. S. WEST, who has referred it to *S. inflata*.

The species of *Spirogyra* under discussion is single banded, with 2 or 3 turns of the band, and has the cell membrane replicate at the ends. The length of the vegetative cell is about 80 μ, and the width about 15 μ. The zygote cell is swollen on both sides. The zygote length is about 43 μ and the width about 28 μ; zygote oval, considerably pointed, brown at maturity. In these characters it follows closely the descriptions for *Spirogyra inflata*. On the other hand, it is to be noted that the connecting tubes are always put out by the male cell and fuse directly with the cell wall of the female cell. This is true regardless of which filament furnishes the male gamete, and would indicate that the sex character was present even before the conjugation tube was put forth.

This species differs also from *S. inflata* in the phenomenon of cross-conjugation, which has not been ascribed to it by any modern work available to the writer. LOTSY (**23**, p. 198) states that there is a difference between the male and female filaments of *S. inflata*, which can signify scalariform conjugation only. WOLLE (**31**) asserts that conjugation may be either lateral or scalariform. DETONI (**12**, p. 766) gives *Conjuguee renflee* of VAUCHER (citing VAUCHER,

pl. V, fig. 3) as synonymous with *S. inflata*, and, although VAUCHER figures it in cross-conjugation (pl. I, fig. 4), DETONI merely states that conjugation is often lateral. Since *S. inflata* has been under observation for so long, and these conditions have not been recognized as characters, it would seem that we must either form a new species for this plant or include these conditions in the description of *S. inflata*. The writer is opposed to the multiplication of species, but these are such distinct characteristics that plants showing them should, it would seem, be classed separately. Final decision in this matter, however, must be reserved until the writer or someone else has had opportunity for further investigation.

The occurrence of this species presents some new problems in the general theory of sex as it applies to the filament of *Spirogyra*. The work on the cytology of this genus has not been entirely satisfactory, owing to the difficulty of staining and counting the chromosomes. CHMIELEWSKI (7), in 1890, saw evidences of reduction but was unable to count the chromosomes (JOHNSON 20). In 1899 KLEBAHN (21) followed the reduction in the desmids, and 12 years later TRÖNDLE (27) succeeded in counting the chromosomes in *Spirogyra* and found that a reduction takes place in the germination of the zygotes. He further found that 4 nuclei were formed, 3 of which degenerate, while "the fourth remains as the nucleus of the single embryonic plant." Evidently the sex factors are separated, and one or the other of them is thrown out in this reduction, since a filament wholly of one sex results.

In the case of lateral conjugation, however, it would seem that reduction cannot take place in the zygote, as both sexes are present in the filament. Moreover, it would seem that reduction takes place in the divisions just preceding reproduction. This may have occurred in the last division before conjugation. Let [♂ ♀] represent a cell which upon division separates the male and female factors [♂|♀]. These conjugating would give alternate zygotes and empty cells. In this case conjugation is assumed to take place between gametes derived from the same mother cell. If, however, they should so divide throughout the filament that the male of one mother cell should adjoin the female from another, we should have the alternation of male and female, so that con-

jugation might take place between daughter cells of different mother cells | ♂ | ♀ | ♂ | ♀ |. But if they should so divide that the female of one mother cell adjoins the female of another, we should have this occurrence | ♂ | ♀ | ♀ | ♂ |, which is very characteristic of lateral conjugation. HASSALL (**16**), observing such a phenomenon, stated that in lateral conjugation there were always 2 empty cells separated by 2 zygotes. WEST (**29,** pl. V, figs. 72, 73) also figures this condition. WOLLE (**31**) makes a similar statement which, however, is not borne out by his plates, since in several cases he has described as lateral conjugation conditions which according to his own figures are manifestly aplanospore formations (WOLLE **31,** pl. 132, figs. 5, 8; pl. 133, fig. 1; pl. 134, fig. 1).

This characteristic appearance may be brought about, however, by another method of division. If the cells adjoining should so divide that the male and female elements alternate | ♂ | ♀ | ♂ | ♀ |, and if this were followed by the subsequent division of all the cells, a filament would be produced which upon conjugation would contain 2 zygotes alternating with 2 empty cells | ♂ | ♂ | ♀ | ♀ | ♂ | ♂ | ♀ | ♀ |. If, however, the division should so occur that male adjoined male and female adjoined female | ♂ | ♀ | ♀ | ♂ |, then a further division would produce a filament containing 4 consecutive males and 4 consecutive females | ♂ | ♂ | ♀ | ♀ | ♀ | ♀ | ♂ | ♂ |.

Assuming that reduction has been retarded until just previous to reproduction in lateral conjugation, it would be possible for us to have an alternation of empty cell and zygote or an alternation of 2 empty cells and 2 zygotes. If these filaments should cross-conjugate (and there is no reason why they should not), they might produce results such as diagrammed in pl. XXV, figs. 9, 13; or pl. XXIV, fig. 3. The greatest number of consecutive zygotes would be 2. Assuming further that the second division has taken place, producing 4 nuclei, as usually occurs in the formation of sex cells, the greatest number of consecutive zygotes would be 4.

These numbers cannot explain the conditions figured on pl. XXV, figs. 1, 2, 3, or those of pl. XXIV, figs. 1 and 4, and many others

which the writer has observed but not diagrammed, since cases in which there are more than 4 consecutive zygotes or empty cells show that division has occurred once or more after reduction. Here, then, is a distinct and characteristic difference between lateral and cross-conjugation. In lateral conjugation there can be no further cell division after reduction is complete, but in cross-conjugating filaments there may be. A study of the plates shows that in the case of fig. 2, pl. XXV, division must have occurred 3 times subsequent to reduction in part of the cells at least, in order to produce the 11 consecutive males there shown.

From the foregoing it would seem that the phenomenon of cross-conjugation lies between lateral and scalariform and partakes of some of the characters of each. Like the former, the reduction does not occur in the zygote, but is retarded, and none of the potential gametes is lost. Like the latter, division continues after reduction has taken place. For these reasons it would seem that the filament of *Spirogyra*, in this species and in those with lateral conjugation at least, must be homologized with the sporophyte of higher plants. With these facts as a basis, the following conclusions seem to be justified:

1. Bisexuality of the filament does occur in certain species of *Spirogyra*, but not necessarily in all species.

2. Reduction may occur in the zygote, in which case a filament wholly of one sex arises, or reduction may occur just previous to reproduction, in which case none of the nuclei degenerates, and filaments of a bisexual nature are produced, which would conjugate either laterally or by cross-conjugation.

3. Cell division may take place subsequent to reduction, some cases showing 3 divisions, and this is an essential difference between lateral and cross-conjugation, since the latter may continue cell division after reduction is complete but the former apparently does not.

4. The filament of *Spirogyra*, in this species and those with lateral conjugation, is homologous with the sporophyte of higher plants.

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LITERATURE CITED

1. AGARDH, J. G., Species genere et ordines Algarum. London. 1848.
2. BENNETT, A. W., Reproduction of the Zygnemaceae. Jour. Linn. Soc. Bot. **20**:430–439. 1883.
3. BENNETT, A. W., and MURRAY, G., Cryptogamic botany. London. 1889.
4. BESSEY, C. E., Hybridization in *Spirogyra*. Amer. Nat. **18**:67–68. 1884.
5. ———, Note on cross-conjugation. Jour. Bot. **29**:173. 1891.
6. ———, Attempted hybridization between pond scums of different genera. Amer. Nat. **19**:800–801. 1885.
7. CHMIELEWSKI, V., Bot. Zeit. **48**:773. 1890.
8. CLEVE, P. T., Monografi ofer de Svenska arterna Försök till en af algens-familjen Zygnemaceae. *pls. 10*.
9. COOKE, M. C., British fresh water algae. London. *pls. 180*. 1882.
10. COULTER, J. M., BARNES, C., and COWLES, H. C., Textbook of botany. Vol. I. New York. 1910.
11. DEBARY, A., Untersuchungen über die Familie der Conjugation. Leipzig. 1858.
12. DETONI, J. B., Sylloge Algarum. I. Chlorophyceae. 725–777. 1889.
13. ENGLER, A., and PRANTL, K., Natürliche Pflanzenfamilien. Leipzig. 1895.
14. HABERLANDT, G., Quoted by KLEBS.
15. HASSALL, A. H., Ann. Nat. Hist. **10**:336.
16. ———, Ann. Nat. Hist. **9**:34.
17. ———, British fresh water algae. London. 1845.
18. ———, British fresh water algae; rev. in Ann. Nat. Hist. **16**:416–419.
19. HERTWIG, O., The cell; outlines of general anatomy and physiology. London and New York. 1895.
20. JOHNSON, D. S., The history of the discovery of sexuality of plants. Science N. S. **39**:299. 1914.
21. KLEBAHN, H., Bot. Jahrb. **22**:415. 1891.
22. KLEBS, G., Die Bedingungen der Fortpflantzung bei einigen Algen und Pilzen. 1896.
23. LOTSY, J. P., Vorträge über botanische Stammensgeschichte. I. Algen und Pilzen. 1907.
24. MOTTIER, D. M., Fecundation of plants. Carnegie Publ. no. 15. 1904.
25. OLTMANNS, F., Morphologie und Biologie der Algen. Jena. 1904.
26. ROBERTSON, R. A., On abnormal conjugation in *Spirogyra*. Trans. and Proc. Bot. Soc. Edinburgh **21**:185–191. 1899.
27. TRÖNDLE, A., Zeitsch. Bot. **3**:593. 1911.
28. VAUCHER, J. P. E., Histoire d'Conferveé d'eau douce. Geneva. 1803.
29. WEST, G. S., and WEST, W., Observations on the Conjugateae. Ann. Botany **12**:29–57. *pls. 2*. 1898.
30. WEST, G. S., The British fresh water algae. Cambridge. 1904.
31. WOLLE, F., Fresh water algae of the United States. 2 Vols. Bethlehem, Penn. 1887.

32. WOOD, H. C., Contributions to the history of the fresh water algae of North America. Smithson. Contrib. **19**:53–262. *pls. 21*. 1874.
33. YORK, H. H., Sexuality of *Spirogyra*. Science N.S. **38**:368–369. 1913.

EXPLANATION OF PLATES XXIII–XXV

PLATE XXIII

FIG. 1.—*Conjuguee majeure* (princeps), in cross-conjugation; copy VAUCHER, pl. IV, fig. 3; *Spirogyra nitida*.

FIG. 2.—*Conjuguee portiquis* VAUCHER, in cross-conjugation; copy VAUCHER, pl. V, fig. 1; *Spirogyra porticalis* (Meull.) Cleve.

FIG. 3.—*Conjuguee condensee* VAUCHER, in cross-conjugation; copy VAUCHER, pl. V, fig. 2; *Spirogyra condensata* (Vaucher) Kütz.

FIG. 4.—*Conjuguee renflee* VAUCHER, in cross-conjugation; copy VAUCHER, pl. V, fig. 3; *Spirogyra inflata* (Vaucher) Rabenh.

FIG. 5.—*Spirogyra gracilis* in cross-conjugation; copy West (**29**) pl. V, fig. 81.

FIG. 6.—*Zygnema intermedium* HASSALL, in cross-conjugation; copy HASSALL (**17**) pl. 38, fig. 8; *Spirogyra Weberi* Kütz.

FIG. 7.—*Spirogyra protecta* and *S. majuscula* hybridizing; claimed to be in cross-conjugation; copy of BESSEY (**4**).

FIG. 8.—*Zygnema orbiculare* HASSALL, figured in cross-conjugation; copy HASSALL (**17**) pl. 19, figs. 1, 2; *Spirogyra maxima* (Hass.) Wittr.

FIG. 9.—Schematic drawing of "false cross-conjugation" as observed by the writer.

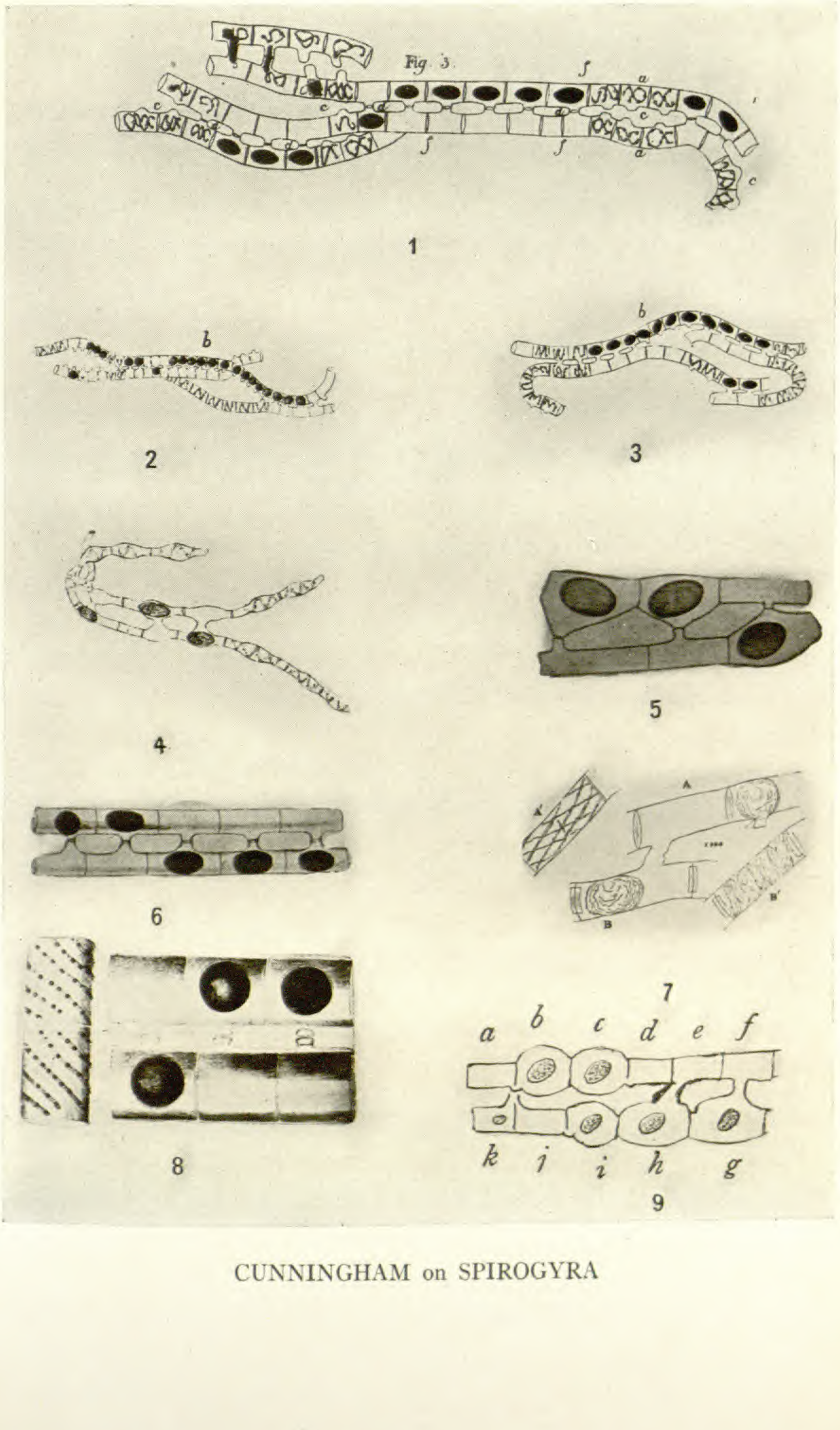
PLATE XXIV

FIG. 1.—Microphotograph of material mounted in glycerine and unstained; ×225.

FIGS. 2–7.—Microphotographs of material mounted in glycerine, and stained with iodine and Magdala red; ×225.

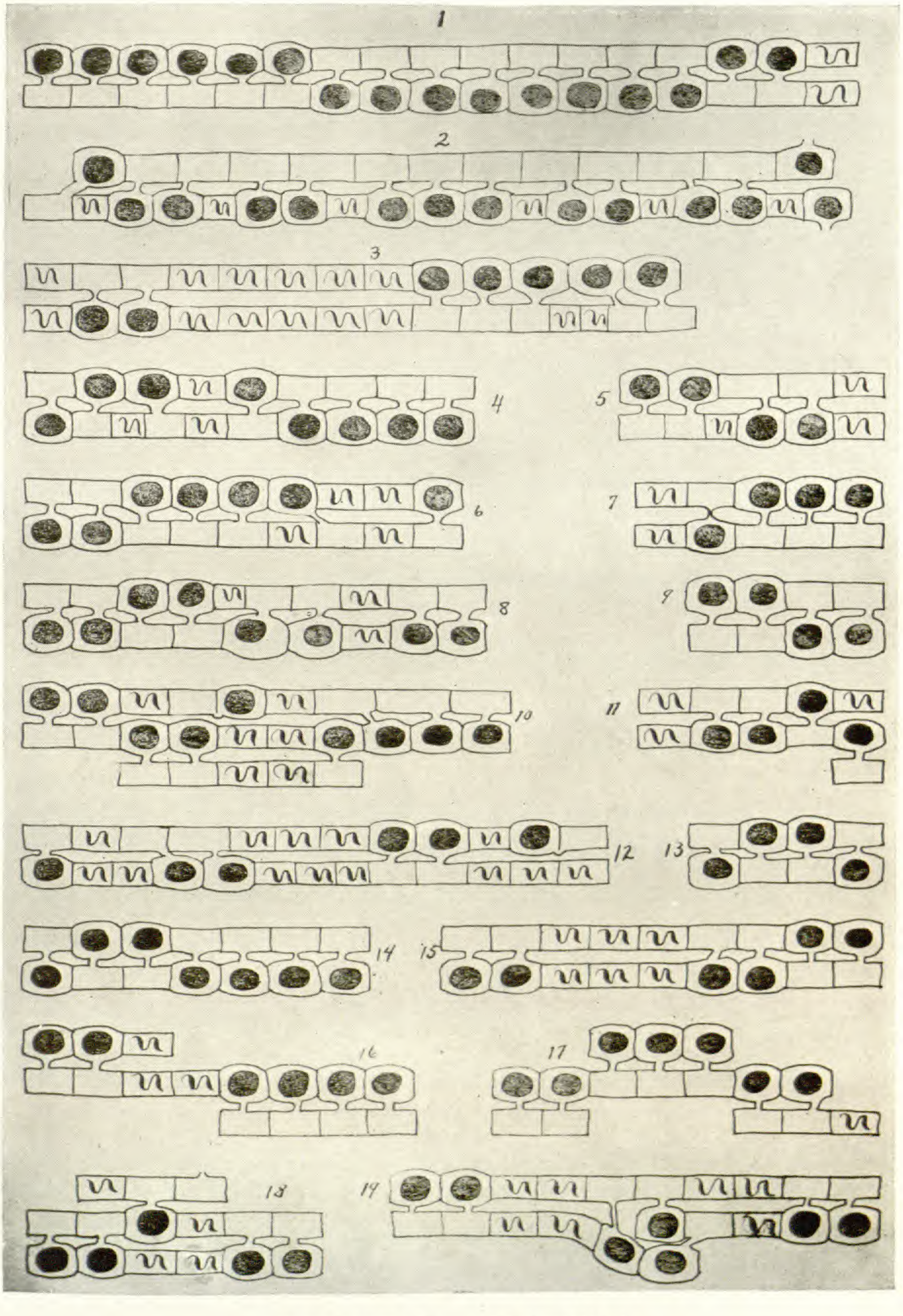
PLATE XXV

FIGS. 1–19.—Schematic drawings of cases of cross-conjugation observed by the writer.



CUNNINGHAM on SPIROGYRA

CUNNINGHAM on SPIROGYRA



CUNNINGHAM on SPIROGYRA

ORANGE RUSTS OF RUBUS

J. C. ARTHUR

(WITH ONE FIGURE)

Much interest has been taken recently in the short cycle rusts, especially since the startling discovery by KUNKEL,[1] 4 years ago, that a rust, indistinguishable from the aecia of *Gymnoconia interstitialis*, would produce promycelia. The year following, FROMME[2] and the writer demonstrated the telial nature of *Aecidium tuberculatum* Ellis and Kellerm., previously treated as a probable heteroecious rust. KUNKEL'S discovery stimulated researches by OLIVE and WHETZEL[3] upon the short cycle rusts of Porto Rico, leading to the detection of 5 aecidioid forms previously placed under the form genus *Aecidium*, and of one uredinoid form quite unlike anything heretofore known.

KUNKEL followed up his discovery with supplementary studies, which he has embodied in a paper[4] dealing with the facts, so far as known, pertaining to the blackberry orange rust and of their probable bearing upon questions of relationship and evolution. He has concluded that there are in the United States two independent but in part similar rusts on *Rubus*, one a long cycle form, which he identifies with the *Gymnoconia interstitialis* of Europe, and the other a short cycle form, for which he uses the name *Caeoma nitens*, first given by SCHWEINITZ to a collection made in North Carolina.

No clear morphological characters were found by which to distinguish the short cycle form from the aecia of the long cycle form, although in germination the two behave quite unlike. It is assumed

[1] KUNKEL, L. O., The production of a promycelium by the aecidiospores of *Caeoma nitens*. Bull. Torr. Bot. Club **40**:361. 1913; see also Amer. Jour. Bot. **1**:37. 1914.

[2] FROMME, F. D. and ARTHUR, J. C., A new North American *Endophyllum*. Bull. Torr. Bot. Club **42**:55. 1915.

[3] OLIVE, E. W. and WHETZEL, H. H., *Endophyllum*-like rusts of Porto Rico. Amer. Jour. Bot. **4**:44. 1917.

[4] KUNKEL, L. O., Further studies of the orange rusts of *Rubus* in the United States. Bull. Torr. Bot. Club **43**:559. 1916.

by KUNKEL that whatever the origin or degree of relationship of these two forms, they are to be considered and treated as distinct, a view in which the writer fully concurs.

A considerable difference in the distribution of the two forms is evident from KUNKEL'S studies, the long cycle form being more common northward and the short cycle form more common southward. So far the latter is not known in the Old World. Moreover, it is the short cycle form that appears to possess the chief economic importance in America by attacking cultivated varieties of *Rubus*, especially blackberries.

From these considerations it is evident that the two forms as a matter of convenience should be designated by different names, whatever views may be held as to their relationship. Since the appearance of KUNKEL'S first paper, appeals have been made to the writer a number of times for the correct application of the several names which have been in use. To meet an evident need, therefore, I have concluded to present the following arrangement of nomenclature and distribution, based upon such facts and material as are at hand.

The generic name *Gymnoconia* was founded by LAGERHEIM[5] in 1894 to more clearly recognize taxonomically the culture work of TRANZSCHEL[6] at Petrograd, Russia, performed during the summer of 1892, by which he genetically connected the caeomoid aecia of *Rubus saxatilis* with the puccinioid telia (*Puccinia Peckiana* Howe) on the same host. The same connection was made two years later by CLINTON[7] at Urbana, Illinois, using "*Rubus villosus*," the native blackberry of the region, being without doubt what is now called *R. nigrobaccus*. The genus *Gymnoconia*, therefore, represents the long cycle form, for which *interstitialis* is the oldest specific name. This was given to the aecia on *Rubus arcticus* from Kamchatka in 1820 by SCHLECHTENDAHL.[8] There can be no doubt,

[5] LAGERHEIM, G., Tromsö Mus. Aarsh. **16**:140. 1894.

[6] TRANZSCHEL, W., Culturversuche mit *Caeoma interstitiale* Schlechtd. (*C. nitens* Schw.). Hedwigia **3**:257. 1893.

[7] CLINTON, G. P., Relationship of *Caeoma nitens* and *Puccinia Peckiana*. BOT. GAZ. **20**:116. 1895.

[8] SCHLECHTENDAHL, D. F. L., Horae Phys. Berol. 96. 1820.

therefore, regarding the correct name for the long cycle form, unless exception be taken to the use of a specific name founded on the aecia, in which case the designation would become *G. Peckiana* (Howe) Trotter.

The short cycle form was first named by SCHWEINITZ[9] in 1822, as *Aecidium nitens*, in a paper on the fungi of North Carolina, the host being reported as *Rubus strigosus*, but a recent study of the type collection shows it to be in all probability *R. Enslenii* Tratt. This region is far south of the known range of the long cycle form, which may be considered sufficient evidence that SCHWEINITZ had only the short cycle form in hand. The specific name has been variously combined, usually in an unfortunate way, but in no case with a distinctive generic name.

Many short cycle genera have now been established. Those which come nearest to the desired characters for the *Rubus* rust are the aecidioid genus *Endophyllum*, and the uredinoid genus *Botryorhiza*. What is now needed is a caeomoid genus, for which no name exists. If any one feels reluctance in placing the telia of a short cycle form in a different genus from the aecia of a long cycle form from which they cannot be distinguished morphologically, let him reflect that he does not hesitate to call the common salt-grass rust *Uromyces Peckianus* Farl., when it has numerous meso-spores, and *Puccinia subnitens* Diet., when the mesospores are few, a closer relationship than in the blackberry rusts. Other such accepted anomalies, in the application of the genera *Uromyces* and *Puccinia*, could be cited. After all, we are not near enough to a nomenclature of the rusts along well considered genetic lines to debar us from using such names as enable one to form the clearest concepts and to convey thoughts in the least ambiguous manner. To meet these requirements short cycle forms require separate generic designation from long cycle forms.

In proposing a generic name for the short cycle orange rust of blackberries and raspberries, I take the opportunity to recognize the distinguished service which Dr. LOUIS OTTO KUNKEL has rendered to uredinology, not alone by the discovery of the true nature of this rust through the use of free surface germination of the

[9] SCHWEINITZ, L. D., Schrift. Nat. Gesell. Leipzig 1:69. 1822.

spores, but also by his subsequent studies and their clear and inspiring presentation.

Kunkelia, gen. nov.

Cycle of development includes subcuticular pycnia and subepidermal telia.

Pycnia conical or columnar, the hymenium applanate; ostiolar filaments wanting.

Telia caeomoid, erumpent, applanate, more or less indefinite in outline, without peridium or paraphyses. Teliospores catenulate, globoid or some elongated, 1-celled; wall colorless or pale, verrucose.

Kunkelia nitens (Schwein.), comb. nov.—*Aecidium nitens* Schwein. Schrift. Nat. Gesell. Leipzig **1**:69. 1822 (type on *Rubus* "*strigosus*," error for *R. Enslenii*, Salem, N.C.); *Caeoma luminatum* Link in Willd. Sp. Pl. **6**²:61. 1825 (founded on *A. nitens* Schwein.); *Caeoma* (*Aecidium*) *luminatum* Schwein. Trans. Amer. Phil. Soc. n. ser. **4**:293. 1832 (founded on *A. nitens* Schwein.).

VIII. IDAEI[10] (raspberry)

21. R. OCCIDENTALIS L. (*R. idaeus americanus* Torr.), black raspberry.—NEBRASKA: Peru, May 24, 1900, *John L. Sheldon;* INDIANA: Bourbon (cult.), May 22, 1889, *J. H. Parks;* NORTH CAROLINA: Leicester, June 12, 1909, *B. B. Higgins* (Barth. Fungi Columb. 2937); NEW YORK: Hempstead, Long Island, May 13, 1916, *Percy Wilson* 237; Sparrow Bush, Orange County, May 29, 1916, *Percy Wilson* 255; SOUTH DAKOTA: Lake Oakwood, June 1890, *Miss Stelter.*

XV. URSINI (western dewberry)

71. R. VITIFOLIUS Cham. and Schlecht.—CALIFORNIA: Chico, April 11, 1903, *E. B. Copeland* 3936 (SYDOW, Ured. 1785); Glendora, Los Angeles County, April 10, 1909, *C. F. Baker* 5273; OREGON: La Grand, July 20, 1914, *C. C. Cate* (cult., loganberry).

74. R. MACROPETALUS Dougl.—BRITISH COLUMBIA: Agassiz, June 1913, *James R. Weir* 84.

[10] The numbered divisions of the hosts and the numbers before the species are those employed by RYDBERG in his monograph of *Rubus* in N. Am. Flora **22**:428. 1913.

XVII. Discolores (sand blackberry)

77. R. cuneifolius Pursh.—North Carolina: Raleigh, without date, *F. L. Stevens* 136; Alabama: Auburn, April 26, 1914, *Fred. A. Wolf;* Florida: Lake City, April 13, 1900, *H. H. Hume* 16; Lake City, April 29, 1896, *P. H. Rolfs* 25, same without date 38.

XVIII. Arguti (high blackberry)

78. R. sativus (Bailey) Brainerd.—Indiana: Daleville (cult.), June 8, 1914, *Leslie V. Shoemaker.*

79. R. nigrobaccus Bailey (*R. villosus* Bigel. not Thunb., Ait., or Bailey, collections often labelled *R. allegheniensis*).—Maryland: Rosecroft, Prince George County, May 24, 1910, *E. Bartholomew* (Barth. Fungi Columb. 3238); New York: Arkville, Delaware County, May 30, 1915, *Percy Wilson* 69; Orient, Long Island, June 2, 1915, *Roy Latham* 625; Walden, Orange County, June 20, 1908, *M. E. Cummings;* Ohio: College Hill near Cincinnati, May 15, 1899, *W. H. Aiken* (Sydow, Ured. 1389); Kentucky: Dayton, June 21, 1910, *E. Bartholomew* (Barth. Fungi Columb. 3327); Indiana: Madison, May 6, 1910, *A. G. Johnson;* Wirt, May 7, 1910, *A. G. Johnson;* Illinois: Pine Hills, Union County, April 24, 1882, *A. B. Seymour* (Rab.-Wint. Fungi Eur. 3220*a*); Anna (cult.), June 13, 1888, *F. S. Earle* (Seym. and Earle, Econ. Fungi 27); Missouri: Columbia, May 1886, *Tracy* and *Galloway*, May 25, 1911, *G. M. Reed* 795; Cedar Gap, Ozark Mountains, alt. 1675 ft., May 22–June 3, 1911, *O. E. Lansing, Jr.* 2968; Kansas: Manhattan, May 23, 1889, *Miss May Varney* (Kellerm. and Sw. Kans. Fungi 31); Louisville, June 1912, *E. Bartholomew* (Barth. N. Am. Ured. 605), May 1912, *E. Bartholomew* (Barth. Fungi Columb. 4233); Minnesota: Minneapolis, June 17, 1914, *Bartholomew* and *Holway* (Barth. N. Am. Ured. 1113, Barth. Fungi Columb. 4629); Iowa: Charles City, May 30 and June 20, 1882, *J. C. Arthur;* Decorah, June 11, 1883, *E. W. D. Holway;* Decorah, June 2, 1886, *E. W. D. Holway* (Barth. N. Am. Ured. 211); Decorah, June 3, 1886, *E. W. D. Holway;* Fayette, May 3, 1908, *Guy West Wilson;* Oregon: Freewater (cult., northeastern section of state), June 27, 1913, *F. D. Bailey.*

81. R. ARGUTUS Link (*R. Andrewsianus* Blanch.).—NEW YORK: Hunter Island, New York City, June 14, 1912, *F. D. Fromme* 12, June 18, 1916, *Percy Wilson* 294; White Plains, June 7, 1914, *Percy Wilson;* Bedford Park, New York City, June 9, 1915, *Percy Wilson* 77; Cold Spring Harbor, Long Island, June 13, 1915, *Percy Wilson* 79.

84. R. FRONDOSUS Bigel.—NEW YORK: Sparrow Bush, Orange County, May 31, 1916, *Percy Wilson* 262.

89. R. CANADENSIS L. not A. Gray.—NEW YORK: Arkville, Delaware County, July 6, 1915, *Percy Wilson* 95.

XIX. PROCUMBENTES (dewberry)

99. R. ABORIGINUM Rydb.—TEXAS: Houston, March 6, 1914, *Arthur* and *Fromme* 6108.

102. R. PROCUMBENS Muhl. (*R. canadensis* A. Gray, not L., *R. subuniflorus* Rydb., *R. villosus* Ait.).—NEW YORK: Van Cortlandt Park, New York City, April 25, 1912, *F. D. Fromme* 29; White Plains, June 7, 1914, *Percy Wilson* 2; Williamsbridge, New York City, June 10, 1914, *Percy Wilson* 4; Pleasantville, Westchester County, May 14, 1915, *Percy Wilson* 63; Hunter Island, New York City, May 23, 1915, *Percy Wilson;* Mamaroneck, Westchester County, June 6, 1915, *Percy Wilson* 76; Yonkers, May 27, 1916, *Percy Wilson* 250; Sparrow Bush, Orange County, May 29, 1916, *Percy Wilson* 257; Ithaca, May 30, 1906, *Reddick* and *Frazer;* NEW JERSEY: West Englewood, Bergen County, June 19, 1915, *Percy Wilson* 83; PENNSYLVANIA: Lancaster, May 31, 1910, *E. Bartholomew* (Barth. Fungi Columb. 3239); MARYLAND: High Island, near Washington, May 1894, *P. A. Rydberg;* Cabin John Bridge, June 15, 1910, *Bartholomew* and *Swingle* (Barth. Fungi Columb. 3524); DISTRICT OF COLUMBIA: Takoma Park, May 1898, *C. L. Shear* 1568; MINNESOTA: Nichols, Aitkin County, June 1892, *E. P. Sheldon;* NEW HAMPSHIRE: Temple, June 20, 1888, *A. B.* and *A. C. Seymour* (Seym. and Earle Econ. Fungi 28); CONNECTICUT: Central Village, June 20, 1903, *John L. Sheldon;* NEW JERSEY: Newfield, June 1874, *J. B. Ellis* (Thüm. Myc. Univ. 446); Newfield, June 1893, *J. B. Ellis* (Ellis and Ev. Fungi Columb. 57); DELA-

WARE: Newark, May 15, 1907, *H. S. Jackson* 1620; Newark, June 6, 1907, *Mel. T. Cook* 1661; INDIANA: Greencastle, May 1893, *L. M. Underwood* (Und. Ind. Flora 19); Lafayette, May 21, 1899, *Wm. Stuart;* Brookville, May 8, 1915, *C. A. Ludwig* (Barth. N. Am. Ured. 1411; Barth. Fungi Columb. 4926).

103. R. ENSLENII Tratt.—SOUTH CAROLINA: without locality or date (*Ravenel*, Fungi Car. 1:91); GEORGIA: Darien, without date (*Ravenel*, Fungi Am. 276); NORTH CAROLINA: Salem, without date, *L. D. Schweinitz*.

(?) RUBUS sp. (mostly cultivated blackberry).—MARYLAND: Beltville, May 24, 1916, *H. S. Coe;* MISSOURI: Columbia, May 7, 1905, *H. S. Reed;* OKLAHOMA: Stillwater (cult.), May 14, 1915, *C. D. Learn*.

XX. HISPIDI (running swamp dewberry)

108. R. HISPIDUS L.—NEW YORK: Hempstead, Long Island, May 13, 1916, *Percy Wilson* 234.

XXI. TRIVIALES (southern dewberry)

109. R. LUCIDUS Rydb. (reported in N. Am. Flora 7:181 under *R. trivialis*).—SOUTH CAROLINA: Aiken, March 15, 1909, *Arthur* and *Kern;* FLORIDA: Lake City, March 30, 1895, and February 17, 1906, *P. H. Rolfs;* St. Augustine, March 27, 1903, *E. W. D. Holway* (Barth. N. Am. Ured. 507).

110. R. TRIVIALIS Michx.—FLORIDA: Lake City, February 1896, *P. H. Rolfs* 23; LOUISIANA: New Orleans, February 24, 1913, *E. Bethel.*

111. R. CARPINIFOLIUS Rydb. (reported in N. Am. Flora 7:181 under *R. trivialis*); TEXAS: Austin, February 27, 1901, *W. H. Long* (Barth. Fungi Columb. 1622), March 14, 1901, *W. H. Long* (Barth. N. Am. Ured. 1504), March 16, 1901, *W. H. Long;* Huntsville, without date, *Carl Hartmann*, communicated F. D. HEALD.

DISTRIBUTION: Central Florida to southern Texas northward to Baltimore, Maryland, and central Illinois, still farther north through the prairie region west of the Mississippi River nearly to the Canadian boundary, and along the Atlantic coast within about

100 miles of the sea as far as New York, then nearer to the sea as far as the coast of New Hampshire, also along the Pacific coast within 100 miles of the sea from southern California to southeastern extension of Alaska, the distance from the sea narrowing northward.

Kunkelia Rosae-gymnocarpae (Dietel), comb. nov.—*Caeoma Rosae-gymnocarpae* Dietel, Hedwigia **44**:334. 1905; *Gymnoconia Rosae-gymnocarpae* Arth. N. Am. Flora **7**:181. 1912.

ROSA GYMNOCARPA Nutt.—CALIFORNIA: Santa Cruz, without date, *C. L. Anderson*, communicated W. G. FARLOW; Modoc and Lassen Counties, "killing wild rosebushes," without date, communicated W. G. FARLOW; Jackson, Amador County, without date, *Geo. Hansen* 1012; Ione, Amador County, March 25, 1896, *Geo. Hansen* 2087.

DISTRIBUTION: From central California to northeastern California.

GYMNOCONIA INTERSTITIALIS (Schlecht.) Lagerh. Tromsö Mus. Aarsh. **16**:140. 1894.—*Caeoma* (*Uredo*) *interstitiale* Schlecht. Horae Phys. Berol. 96. 1820 (type on *Rubus arcticus* L., Kamchatka); *Uredo interstitialis* Schlecht. Horae Phys. Berol. 96. 1820 (variant of the preceding name); *Puccinia Peckiana* Howe, Peck, Ann. Rep. N.Y. State Mus. **23**:57. 1872 (type on *Rubus occidentalis* L., New Baltimore, New York); *Puccinia tripustulata* Peck, Ann. Rep. N.Y. State Mus. **24**:91. 1872 (type on *Rubus "villosus," Greig*, New York); *Uredo luminatum* Thüm. Bull. Soc. Imp. Nat. Moscou **55**:85. 1880 (type on *Rubus saxatilis* L., Minussinsk, Siberia); *Caeoma nitens* Burrill, Bull. Ill. Lab. Nat. Hist. **2**:220. 1885 (type on *Rubus occidentalis* L., et al., McLean County, Illinois); *Uredo* (*Caeoma*) *nitens* DeToni in Sacc. Syll. Fung. **7**:866. 1888 (type on *Rubus saxatilis* L., et al., Asiatic Siberia); *Puccinia interstitialis* Tranz. Hedwigia **32**:259. 1893 (founded on *Caeoma interstitiale* Schlecht. and *Puccinia Peckiana* Howe, supported by cultures on *Rubus saxatilis*, Petrograd, Russia); *Dicaeoma tripustulata* Kuntze, Rev. Gen. 3^3:467. 1898 (founded on *Puccinia tripustulata* Peck); *Gymnoconia Peckiana* Trotter, Fl. Ital. Crypt. 1^{12}:338. 1910

(founded on *Puccinia Peckiana* Howe and *Caeoma interstitialis* Schlecht., with *Rubus saxatilis* cited) *Gymnoconia Peckiana* Kleb. Krypt. Fl. Brand. **5***a*:665. 1913.

A. Hosts for aecia

IV. Arctici (northern dwarf raspberry)

4. R. stellatus Smith.—Alaska: Unalaschka (Bernhardi herbarium at Mo. Bot. Garden).

5. R. acaulis Michx. (distributed as *R. arcticus*).—Yukon: White Horse Rapids, June 16, 1899, *J. B. Tarleton*.

VIII. Idaei (raspberry)

21. R. occidentalis L. (*R. idaeus americanus* Torr.), black raspberry.—Vermont: Charlotte, June 12, 1880, *C. G. Pringle* 1128; Burlington, June 11, 1891, *Collins* F1363; Ontario: London, May 20, 1911, *J. Dearness* 1838 *c;* Glenora, June 7, 1912, *J. Dearness* (Barth. N. Am. Ured. 1208); Michigan: Ann Arbor, June 6, 1916, *C. A. Ludwig* 131; Ohio: Olena, Huron County, June 2, 1902, *O. E. Jennings* (Kellerm. Ohio Fungi 67); Massachusetts: Granville, June 1883, *A. B. Seymour;* Connecticut: Central Village, June 28, 1903, *John L. Sheldon;* New York: Onondaga Valley, June 1885, *L. M. Underwood;* Ithaca, June 27, 1907, *Whetzel* and *Barrus;* West Virginia: Seneca, May 30, 1904, *John L. Sheldon* 25; Morgantown (cult.), June 8, 1904, *John L. Sheldon* 502.

32. R. strigosus Michx. (*R. idaeus aculeatissimus* Rob. and Fern.), red raspberry.—Vermont: Burlington, June 10, 1891, *Collins* F1362; Burlington, June 14, 1893, *L. R. Jones;* Burlington, June 10, 1898, *W. A. Orton* F1807; New Brunswick: Salisbury, July 2, 1905, *C. L. Moore* 9; New York: Lyndonville, June 1, 1886, *C. E. Fairman;* Massachusetts: Newton, 1880, *W. G. Farlow* (Ellis, N. Am. Fungi 277, 278, Roumeguère, Fungi Gall. 874).

XVIII. Arguti (high blackberry)

79. R. nigrobaccus Bailey.—Vermont: Without locality, 1893, *A. J. Grout;* New York: Alcove, May and June 1892, *C. L.*

Shear (Shear, N.Y. Fungi 133); Taberg, Oneida County, June 1887, *L. M. Underwood;* Trumansburg (cult.), June 4, 1904, *H. H. Whetzel;* MAINE: Isle au Haut, May 31, 1912, *Arthur* and *Orton* 123; OHIO: Amanda, without date, *W. A. Kellerman* (Rab.-Wint. Fungi Eur. 3225 *b*); Columbus, May 5, 1901, *W. A. Kellerman* 3853 (Kellerm. Ohio Fungi 20); Columbus, June 2, 1901, *W. A. Kellerman* 3854 (Kellerm. Ohio Fungi 19); Johnston, June 18, 1910, *E. Bartholomew* (Barth. Fungi Columb. 3630); MICHIGAN: Portage Lake, Dexter, June 22, 1913, *E. B. Mains* 38.16; WISCONSIN: without locality, 1883, *L. H. Pammel;* Racine, June 26, 1887, *J. J. Davis;* Madison, May 24, 1911, *E. T. Bartholomew* (Barth. N. Am. Ured. 1007, Barth. Fungi Columb. 3911); ILLINOIS: Oregon, June 16, 1885, *M. B. Waite;* Peoria, June 15, 1894, *F. E. McDonald;* INDIANA: Greencastle, May 1878, *Mel. T. Cook;* Lafayette, June 7, 1894, *Miss K. E. Golden;* Greencastle, July 5, 1895, *Guy West Wilson;* Lafayette, May 18, 1896, *Miss Lillian Snyder;* Greencastle, May 27, 1897, *Mel. T. Cook;* Lafayette, April 28, 1898, May 19, 1899, *J. C. Arthur;* Lafayette, May 30, 1909, *Miss Evelyn Allison;* South Bend, June, 1909, *Miss Clara Cunningham;* Lafayette, June 6, 1911, *E. Trager;* Lafayette, June 21, 1912, *C. A. Ludwig;* Indianapolis, June 4, 1912, *N. K. Thompson;* VIRGINIA: Rosalyn, May 28, 1910, *C. L. Shear* (Barth. N. Am. Ured. 106).

81. R. ARGUTUS Link (*R. Andrewsianus* Blanch.).—MASSACHUSETTS: Barre, June 3, 1899, *Harold B. Smith.*

91. R. RANDII (Bailey) Rydb.—NOVA SCOTIA: Pictou, August 1, 1908, *W. P. Fraser* (accompanied with telia).

(?) RUBUS sp. (mostly cultivated blackberry).—ONTARIO: Muskoka, June 24, 1890, *Macoun;* Prince Edward Island: May 7, 1883, *Macoun;* VERMONT: Colchester, June 28, 1894, *L. R. Jones;* MAINE: Orono, June 1898, *P. L. Ricker;* NEW YORK: Onondaga Valley, June 1889 (Und. and Cook, Cent. Ill. Fungi 51); PENNSYLVANIA: Charter Oak, May 1, 1916; *J. C. Arthur;* WEST VIRGINIA: Morgantown, May 7, 1904, *John L. Sheldon* 24; INDIANA: Lafayette (cult.), May, 1901, *H. B. Dorner;* Broad Ripple (cult.), May 25, 1901, *Mrs. L. D. Dickey.*

B. Hosts for telia

V. Saxatiles (dwarf raspberry)

9. R. pubescens Raf. (*R. triflorus* Rich., *R. canadensis* Torr. not L.).—New Hampshire: Albany, August 1908, *W. G. Farlow;* Wisconsin: Oconto County, July 21, 1909, *J. J. Davis.*

VIII. Idaei (raspberry)

21. R. occidentalis L. (*R. idaeus americanus* Torr.), black raspberry.—Vermont: Burlington, August 20, 1890, *L. R. Jones* F1299; New York: Ithaca, September 30, 1912, *B. B. Higgins* (Barth. Fungi Columb. 4020); Ithaca, August 28, 1902, *H. H. Whetzel;* Poughkeepsie, August 1871, *W. R. Gerard* 853; Enfield near Ithaca, September 15, 1902, *J. M. Van Hook;* Glen near Ithaca, August 12, 1904, *H. S. Jackson;* Junius, September 13, 1904, *H. S. Jackson;* Massachusetts: Mt. Tom, August 20, 1883, *A. B. Seymour;* Newton, *W. G. Farlow* (Ellis, N. Am. Fungi 261); Illinois: Urbana, September 7, 1886, *M. B. Waite* 47.

22. R. strigosus Michx., red raspberry.—New York: Junius, September 16, 1904, *H. S. Jackson.*

XVIII. Arguti (high blackberry)

79. R. nigrobaccus Bailey (*R. villosus* Bigel. not Thunb., Ait., or Bailey, collections often labelled *R. allegheniensis*).—Vermont: Jamaica, September 12, 1890, *A. J. Grout* 426; New York: Alcove, August 1892, *C. L. Shear* (Shear, N.Y. Fungi 67); Alcove, August 1893, *C. L. Shear* (Ellis and Ev. Fungi Columb. 346); Ithaca, September 13, 1911, *B. B. Higgins* (Barth. Fungi Columb. 3631); Forest Home near Ithaca (cult.), July 22, 1906, *H. H. Whetzel;* Michigan: Leland, August 23, 1913, *J. C. Arthur;* Illinois: Urbana, July 29, 1884, *T. J. Burrill* (Seym. and Earle, Econ. Fungi 26); without locality, 1887, *T. J. Burrill* (Ellis and Ev. Fungi Columb. 653); Middlegrove, September 17, 1907, *E. Bartholomew* (Barth. Fungi Columb. 2568); Indiana: Lafayette, October 19, 1895, *Wm. Stuart;* Brookville, September 9, 1916, *C. A. Ludwig* 180.

89. R. CANADENSIS L. not A. Gray (*R. Millspaughi* Britton).—VERMONT: Stratton, August 7, 1894, *A. J. Grout* F30 (reported in N. Am. Flora **7**:181 under *R. vermontanus*); NEW YORK: sphagnum swamps at Junius, September 16, 1904, *Jackson* and *Whetzel;* Old Forge, August 25, 1913, *L. O. Kunkel;* Freeville, September 23, 1902, *C. H. Kauffman;* Malloryville, August 19, 1904, *H. S. Jackson;* Seventh Lake, Adirondack Mountains, August 18, 1909, *B. M. Duggar;* MAINE: Isle au Haut, September 10, 1899, *J. C. Arthur;* MICHIGAN: Neebish Isle, August 25, 1899, *E. T.* and *S. A. Harper;* WISCONSIN: Price County, September 17 and 22, 1911, *J. J. Davis;* WEST VIRGINIA: Cheat Bridge, August 18, 1906, *John L. Sheldon* 2433.

91. R. RANDII (Bailey) Rydb.—NOVA SCOTIA: Pictou, August 1, 1908, *W. P. Fraser* (accompanied with aecia).

DISTRIBUTION: From a southern boundary beginning in the vicinity of Boston, Massachusetts, diverging gradually to about 100 miles from the coast as far as central Maryland, then westward to central Illinois on the Mississippi River, and northward into Ontario and Quebec, especially along the Atlantic coast as far as Prince Edward Island, also on the Pacific coast from the vicinity of Mount St. Elias along the Aleutian Islands and the coast of Behring Sea into Asia. In the eastern hemisphere it occurs in northern Siberia and in northern Europe and the high mountains of central Europe.

In the foregoing lists of hosts and localities all the specimens now in the Arthur Herbarium have been entered. This has been done for three principal reasons. It will enable any person having one of these collections to know what disposition has been made of it in the present study, both as to fungus and host; it will serve to show in detail on what data the conclusions of this paper are founded; and, moreover, it is hoped that the long list of localities and hosts will lead to many germination tests. Such tests are very simple. The spores are dusted on the surface of water, and at intervals of 12–48 hours examined under the microscope to see if the germ tube be long and hyphoid, or short and septate with formation of basidiospores. The work will be enhanced in value

if drawings are made or the germinating spores preserved dry between thin sheets of mica, and if a large specimen of the host be pressed for the purpose of specific determination.

The *Rubus* hosts of the lists were carefully examined by P. A. RYDBERG of the New York Botanical Garden, on January 30, 1917, and the names used are in accordance with his judgment. Of course, no person can name species of *Rubus* with much confidence from leaves alone, as in many collections of the rusts, but the list presents the nearest approach to accuracy at present possible. The species have been listed under the divisions of the genus, and with the serial numbers of the species as given by RYDBERG in his monograph of the genus *Rubus* in the *North American Flora*, better to show the relation of the hosts and their possible susceptibility to the rusts. Whenever the specimen is said to come from a cultivated plant, it has been so indicated.

In only one instance have aecia and telia been found on the same plants. Only from Van Cortlandt Park and a few other places in the vicinity of New York City, and from Glen, New Hampshire, have the germination of the spores been satisfactorily observed. In all other cases the aecia of the *Gymnoconia* and the caeomoid telia of the *Kunkelia* have been separated largely upon arbitrary grounds. The geographical factor in connection with known localities for puccinioid telia has been given much weight, while various other collateral items of information have been utilized. In arriving at a conclusion I have had the valuable assistance of Dr. KUNKEL, who kindly went over all the material with me. One reason for making this assortment in detail is the hope of enlisting the interest of any botanist who may have the opportunity of testing the spore germination in moist air from the localities and hosts named, thus aiding in gradually verifying and rectifying the list.

All present evidence goes to show that the long cycle form on *Rubus* is essentially a northern species, while the short cycle form is essentially southern. Fig. 1 shows the present view regarding geographical distribution. The chart is based entirely upon the data given in the preceding lists of hosts, the southern limit of the *Gymnoconia* being in large part that indicated by the collections of

telia. It will be seen that the two forms overlap along an uncertain line running from eastern Massachusetts not far from the Atlantic coast to northern Delaware, then through northern Virginia and West Virginia, south-central Ohio and Indiana to central Illinois, thence northward along the Mississippi River. On the Pacific coast the region within 100 miles or less from the sea is occupied apparently by the short cycle form from Mount St. Elias

FIG. 1.—Distribution in North America of *Gymnoconia interstitialis* (vertical lines) and *Kunkelia nitens* (oblique lines).

to southern California, and by the long cycle form from Mount St. Elias northward and westward into Asia. No collections are known from the arid region of the plains and Rocky Mountains. To what extent the two forms overlap must be left to the future for decision. There seems to be no doubt, however, that the northernmost area of the continent is occupied by the long cycle form, and the southernmost area by the short cycle form, which appears to be

in accord with tendencies recently pointed out by the writer[11] in studying the rusts of tropical America.

Not much can be said regarding susceptibility of hosts. So far as now known, geographical range is more important in determining the susceptibility of the host than the species of *Rubus*. There appears to be some warrant, however, in thinking that the short cycle form is the one "so destructive to our cultivated blackberries and raspberries in this country," as suggested by KUNKEL.[12]

Probably no other species known are so well adapted for the study of the connection between closely related long and short cycle forms, and their possible evolutionary status.

The rose *Caeoma* of northern California is transferred to the genus *Kunkelia* with some confidence in advance of knowledge regarding the spore germination, partly because no puccinioid form has been found associated with it, and partly because of its general similarity to *Kunkelia nitens*.

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[11] ARTHUR, J. C., Rusts of the West Indies. Torreya **17**:26. 1917.

[12] KUNKEL, L. O., Bull. Torr. Bot. Club **43**:569. 1916.

ARBORES FRUTICESQUE CHINENSES NOVI. II

CAMILLO SCHNEIDER

CLEMATIS CHRYSOCOMA Fr., var. **sericea,** n. comb.—*C. montana* var. *sericea* Fr. apud Finet and Gagnepain in Bull. Soc. Bot. France **50**:525. 1903; Contr. Fl. As. Or. **1**:10. 1905.—*C. Spoonerii* R. and W. in Sarg., Pl. Wils. **1**:334. 1913.

Yunnan boreali-occidentalis: in sepibus ad viam principalem inter Yung-peh-ting et Tai-nao-ko, alt. circ. 2600 m., 3 Julii 1914, *C. Schneider* (no. 3929; flores magni albi, frutex scandens).

According to the material before me it seems impossible to separate *C. Spoonerii*, which is said by the authors to be identical with *C. montana* v. *sericea* Fr., as a distinct species from *C. chrysocoma*. I also collected the type on the eastern slopes of the Lichiang range at about 3600 m., August 1914 (no. 3396). This specimen agrees well with FRANCHET'S description in Bull. Soc. Bot. France **33**:362. 1886. With regard to *C. Spoonerii* the authors (l.c.) say: "It appears to us more closely allied to *C. chrysocoma* Franchet, in which, however, the flowers are pink and produced on the shoots of the current season." According to FRANCHET, "dans le *C. chrysocoma* les tiges adultes demeurent extrêmement raccourcies et produisent, seulement sous leurs sommet, le bourgeon floral." In my specimen (no. 3396) the flowers appear from buds on the older wood as they do in *C. montana* or in typical *C. Spoonerii*, while in a specimen of a cultivated plant (Hort. Chenault, 1911) they are produced on this year's shoots. In a specimen collected by G. FORREST that otherwise agrees well with var. *sericea* we can observe both sorts of flowers. After all, I believe that *C. chrysocoma* sensu stricto represents only a "forma depauperata xerophytica," and that var. *sericea* may be regarded as the phylogenetic type of the species. Of *C. chrysocoma* the flowers are rose pink, while the color is white in those of var. *sericea*, but I also collected a form with pinkish flowers which in its manner of growth is more like var. *sericea* than typical *chrysocoma*. To this pinkish flowered form belong the two following specimens: Szechuan australis, inter

Hoh-si et Te-li-pu, alt. circ. 2000 m., 7 Maji 1914 (no. 1128); and Yunnan boreali-occidentalis, ad latera orientalia montium niveorum prope Lichiang, alt. circ. 2800 m., 4 Julii 1914 (no. 1769).

The only real difference between *C. montana* sensu lato and *C. chrysocoma* sensu lato is furnished by the densely pilose achenes which are glabrous in *C. montana*. The yellowish silky pubescence of *C. chrysocoma* is not a reliable character, because I collected a form with almost entirely glabrous leaves but with distinctly pilose young fruits (in dumetis ad latera orientalia montium niveorum prope Lichiang fu, alt. circ. 3300 m., 19 Julii 1914, no. 1928). Unfortunately, I do not know the color of the flowers, having seen only young fruits. The leaves are very much like those of *C. montana* var. *rubens* Wils., and, I presume, this number represents a new variety of *C. chrysocoma*.

4313 CLEMATIS DELAVAYI Fr. var. **calvescens,** n. var.—A typo praecipue recedit foliolis subtus tantum laxe strigoso-sericeis viridescentibus non argenteo-micantibus margine ex parte distincte lobulato-dentatis, floribus ut videtur paullo minoribus.

Yunnan boreali-occidentalis: in declivibus montium in valle fluminis Yang-tze inter Lichiang et Chung-tien, Augusto 1914, *C. Schneider* (no. 2162; typus in Herb. Arb. Arn. et Hb. Schneider; flores albi).

The leaves of typical *C. Delavayi* are distinctly silvery white beneath, while in var. *calvescens* the under surfaces of the leaves are grayish green.

4445 CLEMATIS UROPHYLLA Fr. var. **obtusiuscula,** n. var.—*C. urophylla* R. and W. in Sargent, Pl. Wils. 1:323. 1913, non Franchet.—A typo praecipue recedit foliolis glabrioribus integerrimis vel parce serrato-dentatis sepalis obtusioribus ovato-oblongis stamina paullo non duplo superantibus.

Szechuan orientalis: in monte Omei, alt. 2000 m., 16 Octobris 1903, *E. H. Wilson* (no. 3121; typus in Herb. Arb. Arn.).—Yunnan australis: prope Mengtsze, in silvis versus orientem, alt. 2300 m., *A. Henry* (no. 11, 347; flores albi).

I have seen only one specimen collected by WILSON, November 1900, in western Hupeh, that agrees with FRANCHET'S description of *C. urophylla* in Bull. Mens. Soc. Linn. Paris 1:433. 1884. The two specimens which have been referred by REHDER and WILSON to this species look rather different. The leaves of no. 11347 are entire, while those of no. 3121 are mostly serrate-dentate at the margins between the base and the apex. The sepals of the type are narrow lanceolate, acuminate, and almost twice as long as the stamens.

The flowers of var. *obtusiuscula* are not yet open, but the sepals are distinctly obtuse and not much longer than the stamens and carpels, which are identical with those of the type. Both numbers look very much alike. The inflorescences are the same as in typical *C. urophylla*.

4447 **Clematis** (Sect. VIORNA Prtl., ser. CONNATAE Koeh.) **Kockiana,** n. sp.—Frutex scandens habitu *C. lasiandrae;* ramuli floriferi striato-sulcati, laxe villosuli. Folia ternata, longiter petiolata; foliola terminalia ovata, basi rotundata vel subcordata, apice subito acuminata, 5–9.5 cm. longa, 2.5–5 cm. lata petiolulis ad 2 cm. longis, superne satis viridia in costa nervisque lateralibus plus-minusve strigulosa, facie subglabra, subtus paullo discoloria, in costa nervisque lateralibus laxe sericeo-pilosa, venis elevatis conspicuis, margine inaequaliter subcrenulato-dentato-serrata, lateralia similia, paullo inaequilateralia, minora, brevius petiolulata. Inflorescentia axillaris, pedunculo quam petiolus breviore ad 3 cm. longo sustenta, paniculata, satis compacta, 3–13-flora et pedunculo incluso ad 10 cm. longa, sericeo-villosula, bracteis variabilibus partim foliaceis partim parvis lanceolatis instructa; pedicelli graciles, ut pedunculus sed densius pilosi, floribus vix vel paullo longiores; flores nutantes; sepala conniventia, apice revoluta, ovato-oblonga, 12–14 mm. longa, 5–6 mm. lata, in vivo flavescentia, sed extus versus basim purpureo-violacea, extus sericeo tomentella, margine tomentosa, intus glabriuscula; stamina exteriora sepalis vix breviora, ad 14 mm. longa, filamentis planis linearibus margine (et etiam in dorso partim) ima basi excepta dense longeque sericeo-pilosis quam antherae glabrae circ. $3\frac{1}{2}$-plo longioribus, interiora breviora, filamentis parte inferiore nudo dilatatis antheris vix ultra duplo longioribus, connectivo in dorso interdum pilis paucis praedito; carpella sericea, in stylum argenteo-plumosum staminibus interioribus subaequilongum desinentia. Achaenia ignota.

Yunnan boreali-occidentalis: in dumetis ad latera orientalia montium niveorum prope Lichiang-fu, alt. circ. 3200 m., 6 Septembris 1914, *C. Schneider* (no. 3898; typus in Herb. Schneider).

This species has the leaves of *C. urophylla* Fr. and the inflorescences and flowers of *C. lasiandra* Maxim., the sepals of which are much more glabrous. The plant is named in compliment to Rev. A. KOCK of the Pentecostal Mission at Lichiang-fu, in appreciation of valued service rendered to the author during the summer of 1914.

Mahonia Alexandri, n. sp.—Arbuscula ad 3 m. alta; ramuli hornotini flavo-viridi, dense foliati. Folia jugo infimo incluso 12-15-juga, ad 32 cm. longa, ad 28 cm. lata, rhachi lateraliter sulcata, jugis inter se 1.5–2 cm. distantibus; foliola lateralia sessilia, anguste lanceolata, versus apicem et basim folii paullo minora, ceterum subaequalia, crasse coriacea, laevia, superne dilute viridia, fere enervia, subtus pallidiora, in sicco flavescentia, 3—nervia, nervis secundariis fere invisibilibus, basi leviter inaequilaterali rotundato-subcordata, apice acuminata, spinosa, utrinque satis crasse sinuato-spinoso-dentata, dentibus distantibus 3–5 divaricatis 1.5–3 mm. longis, basalibus exceptis 4–7 cm. longa, versus basim 1. 2–1. 7 cm., lata, terminalia simillima, breviter petiolulata; stipulae in basi dilatata petioli lineari-lanceolatae. Inflorescentiae densiflorae, fructiferae ad 13 cm. longae; flores ut videtur lutei, ? 8 mm. diametientes; pedicelli 2–4 (–5) mm. longi, bracteis oblongis satis obtusis aequilongis vel paullo brevioribus suffulti; sepala 3 externa minima, late triangularia, 3 media late ovato-oblonga longiora, 3 interna maxima circ. 8 mm. longa, late ovato-elliptica, obtusa; petala late obovata, circ. 6 mm. longa, apice incisa, basi contracta, glandulis 2 subparvis instructa; stamina normalia, connectivo satis obtuso, filamentis edentatis quam antherae fere duplo longioribus; ovarium ovatum, in stylum attenuatum, ovulis 4–5 sessilibus instructum. Fructus globosi, valde pruinosi, seminibus maturis 2–3, stylo brevi instructi.

Szechuan australis: inter oppida Yen-yuan Hsien et Yung-ning inter viculos Wo-lo-ho et Cho-so, ad latera montium, alt. circ. 2600 m., 15 Junii 1914, *C. Schneider* (no. 1588; typus in Herb. Arb. Arn. et Hb. Schneider; arbuscula ad 3-metralis, fruticeta parva formans); Yunnan australis: prope Mengtsze, Lao Kwei-chou (?), 1 Novembris, *A. Henry* (no. 10309; frutex ad 1.8 m. altus; in Herb. Hort. Bot. New York).

My specimens with ripe fruits are identical with the flowering ones of HENRY. It is a distinct species, probably most closely related to *M. caesia* Schn. I take much pleasure in naming this species for my friend Mr. ALEXANDER SCHÖNBAUER of Vienna, in grateful recognition of the important services he has rendered to the dendrological society of Austria and Hungary during my absence from Europe.

Mahonia caesia, n. sp.—Frutex 1–3 m. altus; ramuli (indistincte reticulata costa subtus elevata) juveniles brunnescentes, leviter

pruinosi. Folia jugo infimo incluso 6–8-juga, jugis 1.5–2.5 cm. inter se distantibus, ad. 25 cm. longa (vel in surculis interdum fere duplo majora jugis distantioribus), rhachi tereto plusminusve glaucescenti; foliola lateralia sessilia, anguste lanceolata, versus apicem et basim folii paullo minora, ceterum subaequalia, crasse coriacea, laevia, utrinque in sicco concoloria, flavo-viridia, sed omnino glaucescentia (plusminusve caesia), basi subtruncato-obtusa vel latere inferiore rotundata, paullo inaequilateralia, apice breviter acuminata, spinosa, margine utrinque plusminusve undulato-sinuato-spinoso-dentata, dentibus 5–9 satis crassis subdivaricatis 1.5–2 mm. longis, basalibus valde minoribus ovatis vel rectangularibus exceptis 5–9 cm. longa, 1.2–2 cm. lata, terminalia similia; stipulae jugo infimo approximatae, lineares. Inflorescentiae ignotae.

Yunnan boreali-occidentalis: ad latera montium inter Lichiang-fu et fluminem Yang-tze ad viam principalem versus Yung-peh-ting, 3 Julii 1914, *C. Schneider* (no. 1723; typus in Herb. Arb. Arn. et Hb. Schneider; frutex 1–3 m. altus).

The leaves of this *Mahonia* are so well distinguished from those of any other species that I do not hesitate to describe it as a new species without having seen flowers or fruits. At first sight the leaves resemble those of *M. Alexandri*, but they may easily be distinguished from that species by their different color and nervation, and by the different number of leaflets as well as by their terete rhachis in which the leaflets are inserted in a different manner.

Mahonia philippinensis, n. sp.—Frutex ad 4-metralis. Folia 7-juga, ad 26 cm. longa et 10 cm. lata, jugo infimo multo minore basi petioli valde approximato, rhachi satis tenui lateraliter sulcato, internodiis 2–4 cm. longis; foliola coriacea textura subcrassa, superne ut videtur satis dilute viridia, paullo nitentia, subtus in sicco subflavescentia, nervis primariis utrinque distinctis leviter prominulis, lateralia inferiora superioraque quam media plusminusve minora, basalibus minimis ovatis exceptis lanceolata, basi inaequilaterali cuneata vel obtusata, latere inferiore rotundata (subcordata), apice acutissima, margine dentibus 3–4 mm. longis utrinque 4–6 sinuato-dentato-spinosa, minora 3:1 cm. magna, majora ad 7–7.5 cm. longa et 2–2.5 cm. lata, terminalia lanceolata, basi ovata, ceteris similia, sessilia? (in specimine unico viso foliolis 3 terminalibus basi plusminusve confluentibus), ad 6:1 cm. magna.

Inflorescentiae ad 29 cm. longae, laxiflorae; earum bracteae late triangulares, acuminatae, circ. 1.5 cm. longae; flores lutei?, extus rubescentes?, circ. 10 mm. diametientes; pedicelli graciles, 10–12 mm. longi, bracteis ovato-lanceolatis ad 4 mm. longis subacuminatis suffulti; sepala 3 externa minima, ovata, 3 media majora, late ovata, 3 interna maxima, ad 7 mm. longa, late ovata, apice subrotundata; petala circ. 6 mm. longa, obovata, apice subincisa, basi leviter contracta, glandulis 2 normalibus instructa; stamina petalis breviora, apice obtusa, filamentis edentulatis; ovarium ut videtur in stylum distinctum productum, ovulis 4 sessilibus. Fructus ignoti.

Insulae Philippinenses: Luzon borealis, Benguet, Baguio, 13 Novembris 1914, *R. S. Williams* (no. 1460; typus in Herb. Gray; frutex ad 4-metralis, flores lutei, fructus glauci).

This is a very distinct species, with its loose inflorescences and its long pedicels. I have not seen the fruits mentioned in the note of the collector. The texture of the leaves somewhat resembles that of *M. napaulensis* DC., to which it seems to be most nearly related.

Mahonia nivea, n. sp.—Frutex fide cl. Henry 0.9 m. altus. Folia 5–6-juga, ad 42 cm. longa et 15 cm. lata, rhachi lateraliter subsulcato, internodiis 4–5 cm. longis; foliola lateralia sessilia, late ovata, inferiora superioraque quam media subminora, ceterum inter se subaequalia, basi paullo inaequilaterali truncato-rotundata, apice acuta vel breviter acuminata, tenuiter spinosa, minimis basalibus exceptis 5–8 cm. longa et 2.5–5 cm. lata, utrinque distanter breviter indistincte spinoso-serrata, dentibus 4–7 graciliimis 2 mm. longis porrectis, tenuiter coriacea, superne viridia, laxe tenuiter reticulata, subtus albida, pruinosa, laxius elavato-reticulata, terminalia distincte petiolulata, late ovato-subcordata, ad 8:5 cm. magna, infima ovato-orbicularia distanter spinoso-dentata; stipulae non visae. Flores fructusque ignoti.

Yunnan australis: prope Mengtsze, Pi-che-shen, 21 Novembris, *A. Henry* (no. 9863; typus in Herb. Hort. Bot. New York).

According to the note on the label, HENRY also collected "fl. buds" which, however, are wanting in the specimen before me. Nevertheless, the leaves of this *Mahonia* are so distinct that it undoubtedly represents a good new species. It may easily be distinguished from all the species of the old world by the snowy white under surface of its finely serrate sessile leaflets.

SCHISANDRA GRANDIFLORA Hk. f. and Thoms., Fl. Brit. Ind. 1:44. 1872.—King in Ann. R. Bot. Gard. Calcutta 3:219, *pl. 69, fig. A*. 1892; *Kadsura grandiflora* Wall., Tent. Fl. Nepal. 10, *pl. 14*. 1824.—The typical *S. grandiflora* has large white or pinkish white flowers about 1 in. or more in diameter, and the male flowers have 7–9 sepals. The anthers are elliptic or ovoid-elliptic, with lateral or subextrorse cells, the filaments of the lower ones being of about the same length as the anthers. By FINET and GAGNEPAIN and also by REHDER and WILSON, some forms from western and southwestern China have been united with *S. grandiflora* which, in my opinion, form a distinct variety that may be described as follows.

5-5617 SCHISANDRA GRANDIFLORA, var. **cathayensis,** n. var.—*S. grandiflora* Finet and Gagnep. in Bull. Soc. Bot. France **52:** Mém. IV. 48. 1905, pro parte; non Hk. f. and Th.; Contr. Fl. As. Or. **2:**48. 1907, pro parte; REHDER and WILSON in Sargent, Pl. Wils. **1:**412. 1913; *S. chinensis* Diels in Not. R. Bot. Gard. Edinbgh. **7:**398. 1913, non Baillon.—A typo praecipue recedit floribus minoribus vix ultra 2 cm. diametientibus roseis vel sanguineis antheris partim distinctius extrorsis ovato-ellipticis vel fere ovato-subglobosis apice paullo apiculatis vel interdum leviter emarginatis loculis subrectis vel partim satis curvatis.

Yunnan boreali-occidentalis: in vallibus ad latera orientalia montium Tsang prope Tali-fu, alt. 2800–3200 m., Junio–Augusto 1906, *G. Forrest* (no. 4797); in dumetis montium niveorum prope Lichiang-fu infra glaciem magnam, alt. circ. 3500 m., 14 Sept. 1914, *C. Schneider* (no. 2807; fructus maturi rubri); eodem loco, Octobri 1914, *Schneider* (no. 3303); in silvis umbrosis ad angustias montium inter Sung-queh et Teng-chuan, alt. circ. 3200 m., 29 Sept. 1914, *C. Schneider* (no. 2686); Szechuan australis: in regione Yen Yüan Hsien inter viculos Ka-la-pa et Liu-ku, in dumetis ad ripas, alt. circ. 3300 m., 17 Maji 1914, *C. Schneider* (no. 1276; frutex scandens, flores intense rubri); in silvis supra Hua-li ad flum. Yalung, ad angustias montium boream versus, alt. circ. 3800 m., 28 Maji 1914, *C. Schneider* (no. 3936; typus in Herb. Schneider; frutex scandens, flores rubri).—Vide etiam specimina a cl. Wilson in Hupeh occidentali collecta in Pl. Wils., l.c. indicata.

The color of the flowers of the plants collected by myself is almost blood red, while FORREST says "flowers crimson" and WILSON "flowers flesh pink" or "deep fleshy pink." They are smaller than those of typical *S. grandiflora*, and the shape of the anthers seems rather variable, the cells being often almost entirely extrorse and sometimes distinctly curved. In the male flowers I found mostly 6 sepals, but apparently there are about 9, the outermost being

rather small and very deciduous. Regarding the fruiting aments and the shape, texture, serration, and reticulation of the leaves, it is impossible to detect sufficient differences between this variety and the type. The under surface of the mature leaves is often very glaucescent and without a distinct reticulation, which seems to be much more prominent in the leaves of the type and also of var. *rubriflora*. The last one does not, in my opinion, represent a more distinct form than var. *cathayensis*. I think it best to make *S. rubriflora* another variety of *S. grandiflora*, the main characters of which apparently have been somewhat misunderstood by REHDER and WILSON, and I propose the following combination:

5-5619 SCHISANDRA GRANDIFLORA, var. **rubriflora,** n. comb.—*S. chinensis*, var. *rubriflora* Franchet in Nouv. Arch. Mus. Paris. **8:** 192 (Pl. David. II. 10). 1886; *S. grandiflora* Fin. and Gagnep. in Bull. Soc. Bot. France **52:** Mém. IV. 48. 1905, pro parte, non Hk. f. and Thom.; Contr. Fl. As. Or. **2:** 48. 1907, pro parte.—A typo differt floribus fusco-rubris (?atro-sanguineis) pedicellis fructuum longioribus 6–8 cm. longis antheris fere ut in var. *cathayensi* et etiam saepe satis distincte extrorsis.

Szechuan occidentalis: in dumetis montis Niu-tou, prope Kuan Hsien versus occidentem, alt. 2000–2600 m., 20 Junii 1908, *E. H. Wilson* (no. 921 b; typus in Herb. Arn. Arb.). Vide etiam specimina altera in Pl. Wils., l.c., enumerata.

The flowers of var. *rubriflora* are as large as those of typical *S. grandiflora*, but "very dark red" according to WILSON'S notes. The number of the sepals of the male flowers varies from 5 to 7, and I never saw more than 9 in any form of this species. The shape of the leaves is rather variable, as is also the shape of the anthers. The true *S. chinensis* Baillon is a northern plant, and is readily distinguished by its 5–6 stamens with very short filaments or almost sessile anthers. The male flowers of *S. grandiflora* always possess more than 10 stamens, the filaments of the lower ones becoming almost as long as the anthers.

ARNOLD ARBORETUM
JAMAICA PLAIN, MASS.

CURRENT LITERATURE

NOTES FOR STUDENTS

Temperature and respiration rate.—BLANC[1] has studied the effect of sudden changes in temperature upon the rate of respiration of plant parts. He reviews the work of ZIEGENBEIN[2] with germinated seeds of *Vicia Faba*, and that of PALLADIN[3] with etiolated seedlings of the same species. These two investigators, although working with very similar material, came to very different conclusions as to the influence of sudden changes of temperature upon the rate of respiration. PALLADIN'S conclusion that passing from a low temperature or from a high temperature to a medium temperature excites the respiration is considered doubtful on account of the fact that, previous to the change in temperature, the seedlings had been cultivated at different temperatures on sugar solutions.

BLANC worked with the embryos of *Phaseolus vulgaris* deprived of their cotyledons, with the ends of etiolated seedlings of *Vicia Faba*, and with young leaves of *Secale cereale*. The *Vicia* seedlings had previously been cultivated on 10 per cent saccharose or 5 per cent glucose solutions. The *Phaseolus* embryos and *Secale* leaves were used both with and without previous cultivation on 10 per cent saccharose solution. Raising the temperature at which the experiment was conducted invariably increased the rate of respiration, and lowering the temperature always decreased the rate. After having undergone one such change of temperature, samples of the material studied were returned to the original temperature for a short period (15–30 mins.). It was found that the rate of respiration during this second period at a given temperature was higher than that during the first period whenever the temperature had been raised during the intervening period, and lower whenever the temperature had been lowered during the intervening period.

In a third series of experiments, embryos of *Phaseolus vulgaris* and leaves of *Secale cereale* were changed from one temperature to a temperature about 20° C. warmer or 20° C. cooler, and the rate of respiration was determined for 3 successive 20-minute periods at the new temperature in comparison with the

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[1] BLANC, M. L., Recherches experimentales sur l'influence des variations de température sur la respiration des plantes. Rev. Gén. Bot. **28**:65–79. 1916.

[2] ZIEGENBEIN, E., Untersuchungen über den Stoffwechsel und die Athmung keimender Kartoffelknollen sowie anderer Pflanzen. Jahrb. Wiss. Bot. **25**:595–596. 1893.

[3] PALLADIN, W., Influence des changements de température sur la respiration des plantes. Rev. Gén. Bot. **11**:241–257. 1899.

rate at the original temperature. It was found that after a change in temperature, the corresponding change in respiratory activity took place only gradually, apparently not having reached an equilibrium even at the end of the third 20-minute period. Although the main point is proved, the value of this part of the work would have been increased by continuing the observations over a longer time.

Many students will regret that the author did not study oxygen consumption as well as the production of CO_2, to see whether the respiratory coefficient was altered by temperature changes. It should be remembered, too, that the conclusions reached may not hold good for other sorts of material, such as dormant seeds, the germination of which is greatly stimulated by alternations of temperature.—G. T. HARRINGTON.

Ecology of bryophytes and lichens.—Ecological studies of liverworts and mosses have not been numerous in the past, largely because bryologists have not been interested in ecology and ecologists have not been sufficiently acquainted with bryophytes. There are also some difficulties peculiar to the application of ecological principles to these plants. Some of these have been pointed out by WATSON[4] in attempting, among other things, to define a xerophytic bryophyte. This he decides must be a plant capable of withstanding long periods of dryness and of having at the end of such periods sufficient living cells to enable it to resume its growth quickly when water becomes available. He proceeds to consider the "xerophytic adaptations" under the two principal heads of structures causing (1) reduction of water output and those resulting in (2) water storage. The former is accomplished by such means as cushion forms, investments of dead cells, thick cell walls, leaf arrangement, and capillary structures; the latter by water sacs, water-storing cells, mucilaginous cells, and succulent tissue. The writer, however, warns us that many bryophytes exhibiting "xerophytic adaptations" are not xerophytes.

A second paper by the same author[5] gives in detail the zonation of bryophytes in a wet heath. The shallow water zone is dominated by *Aneura pinguis*, *Pellia epiphylla*, *Hypnum scorpioides*, and *Sphagnum cymbifolium;* the second zone, just above water level, is dominated by *Aneura multifida;* a third zone consists of *Sphagnum subnitens*, *Hypnum intermedium*, and associated forms, passing imperceptibly into a fourth zone, characterized by *Hypnum cuspidatum*, and closely followed by a fifth zone dominated by *Brachythecium pyrum* and *Cephalozia connivens*. This is frequently the end of the series, although occasionally the drier tussocks show a sixth zone of *Hypnum cupressiforme* var. *ericetorum*. Drainage and the accumulation of humus are the chief

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[4] WATSON, W., Xerophytic adaptations of bryophytes in relation to habitat. New Phytol. **13**:149–169, 181–190. 1914.

[5] ———, A Somerset heath and its bryophytic zonation. New Phytol. **14**:80–93. 1915.

factors in determining the succession. The probable history of the heath is well discussed and the diagrams are decidedly good and appropriate.

A remarkable instance of the vitality of moss protonema is recorded by BRISTOL,[6] who found resting protonemal cells, rich in oil, in dry soil stored in air-tight bottles for 46–49 years. In cultures these grew and produced protonema of the ordinary type.

In a series of notes WEST[7] has recorded the bryophytes and lichens found upon trees in parts of Scotland, Wales, and Ireland, and has arranged them according to abundance. He has found the percentage ratio of some of the principal forms to be: *Stereodon cupressiformis* 16, *Parmelia saxatilis* 6, *Isothecium myosuroides* 2, *Frullania dilatata* 2, *Parmelia fuliginosa* 2, *Lecanora tartarea* 2, and *Platysma glaucum* 1.—GEO. D. FULLER.

Variations in wood structure.—Several recent articles have called in question some of the "laws of Sanio" for variation in the size of tracheids in conifers, more particularly that law which states that tracheids increase in size from the pith radially outward until they reach a definite size, which remains constant for the following annual rings. SHEPARD and BAILEY[8] found the gradual increase in size up to 30–60 years, but in succeeding years no constant length was attained. Later the same authors maintained their points in this journal.[9]

Their results were for the greater part confirmed by a detailed study of *Pinus palustris* and *Pseudotsuga* by Miss GERRY,[10] who also finds the longest tracheids in the early spring wood and the shortest in the late wood. LEE and SMITH[11] now supplement this with an extended study of *Pseudotsuga* from British Columbia. Their results, in general, agree with those already cited except that after a gradual and fairly rapid increase up to the age of 50 years the tracheid length varies comparatively little, but tends to increase slightly. They also find an increase in tracheid length up to 42 ft. above the ground, and then a gradual decrease up to 154 ft., where the measurement ceased. It is interesting also to note that trees from the coast region appear to have slightly longer tracheids than those from the mountains.

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[6] BRISTOL, B. MURIEL, On the remarkable retention of vitality of moss protonema. New Phytol. **15**:137–143. 1916.

[7] WEST, W., Ecological notes; chiefly cryptogamic. Jour. Linn. Soc. **43**:57–85. 1915.

[8] SHEPARD, H. B., and BAILEY, I. W., Some observations on the variation in length of conifer fibers. Proc. Soc. Amer. Forest. **9**: 1914.

[9] BOT. GAZ. **60**:66–71. 1915.

[10] GERRY, ELOISE, A comparison of tracheid dimensions in longleaf pine and Douglas fir. Science **43**:360. 1916.

[11] LEE, H. N., and SMITH, E. M., Douglas fir fiber, with special reference to length. Forest Quart. **14**:671–695. 1916.

Extending their work to angiosperms, TUPPER and BAILEY[12] found the average length of their wood elements to be twice that of the corresponding structures in gymnosperms except in the vesselless angiosperms, *Tetracentron*, *Trochodendron*, and *Drimys*, which seem to have the typical gymnospermous length of wood elements. More recently, PRITCHARD and BAILEY[13] examined *Carya ovata* and reached the general conclusion that both in conifers and in woody dicotyledons there is a period in the early stages of the life history during which the woody elements increase in size comparatively rapidly, the length of the period varying in different groups. Furthermore, different types of xylem elements, such as tracheids, wood fibers, and vessel segments, behave very differently, but their size generally fluctuates more or less during the later stages of the development of the stem.—GEO. D. FULLER.

Taxonomic notes.—COOK[14] has made a comparison of the peculiar branching and flowering habits of Cacao (*Theobroma cacao*) and Patachte, formerly referred to *Theobroma*, but recently made the basis of a new genus (*Tribroma*) by COOK.[15] The comparison deals with morphological and ecological features of the two genera, as exhibited under cultivation in eastern Guatemala.

GREENMAN[16] has described a new species of *Senecio* (*S. Hollickii*), collected by BRITTON and HOLLICK in Jamaica in 1908.

GROVE[17] has described, along with other new fungi, a new genus (*Diploöspora*) of Ascomycetes.

ORTON[18] has monographed the North American species of *Allodus*, a genus of Uredinales whose most conspicuous feature is the frequent close association of aecia and telia on the same plant parts, and the absence of distinct uredinia. The most interesting fact in connection with its host relationships is that no host occurs among the Rosales. There are 47 species recognized, including 4 new species and 20 new combinations.

SPRAGUE and HUTCHINSON,[19] in connection with a report upon a collection of African Anonaceae, call attention to the great increase in our knowledge of

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[12] TUPPER, W. W., and BAILEY, I. W., The secondary xylems of gymnosperms and angiosperms. Science **43**:323. 1916.

[13] PRITCHARD, R. P., and BAILEY, I. W., The significance of certain variations in the anatomical structure of wood. Forest Quart. **14**:662–670. 1916.

[14] COOK, O. F., Branching and flowering habits of Cacao and Patachte. Contr. U.S. Nat. Herb. **17**:609–625. *pls. 44–54.* 1916.

[15] Jour. Wash. Acad. Sci. **5**:288. *pls. 46–50, 52, 54.* 1915.

[16] GREENMAN, J. M., A new *Senecio* from Jamaica. Ann. Mo. Bot. Gard. **3**:201, 202. 1916.

[17] GROVE, W. B., New or noteworthy fungi. V. Jour. Botany **54**:217–223. 1916.

[18] ORTON, C. R., North American species of *Allodus*. Mem. N.Y. Bot. Gard. **6**:173–208. 1916.

[19] SPRAGUE, T. A., and HUTCHINSON, J., African Anonaceae. Kew Bull. no. 6. pp. 145–161. *figs. 3.* 1916.

the tropical African flora, as illustrated by this family. In 1868, the date of publication of the first volume of the *Flora of tropical Africa*, only 13 genera and 59 species of Anonaceae were known; while in 1901 there were 23 genera and 170 species recorded, and at present 27 genera are known. In the present paper the limits of certain genera are revised and several species are transferred. New species are also described in *Artabotrys*, *Isolona*, *Oxymitra* (3), *Uvaria*, and *Xylopia*.

WERNHAM[20] has published a new genus (*Pseudomussaenda*) of Rubiaceae from the "Nile-land districts" of tropical Africa. It includes 3 species formerly referred to *Mussaenda*, to which a new species is added.—J. M. C.

Extraction of sap.—GORTNER, LAWRENCE, and HARRIS[21] have repeated and extended the work of DIXON and ATKINS on the extraction of sap from plant tissues. Their primary purpose was to determine something concerning the nature, amount, and regularity of the change in the concentration of the sap extracted from a mass of tissue under continuous pressure. The results secured fully substantiate the conclusions of DIXON and ATKINS that samples of sap pressed from unfrozen tissues cannot be taken as typical of the original concentration of the juices in the tissues. In general, successive samples extracted by continuous pressing become more concentrated. The authors have shown that such, however, is not always the case. In some instances the fluid may become less and less concentrated, for example, extractions from cabbage leaves. In other instances all fractions may be about the same in concentration. The development of the freezing method to render tissues permeable and thereby obtain typical samples of sap has marked a great advance in the study of the properties of vegetable saps.—CHAS. O. APPLEMAN.

A new soil constituent.—An unusual organic soil constituent has been isolated and identified as α-crotonic acid by WALTERS and WISE.[22] This unsaturated acid was found associated with infertility in a Texas soil where drainage is poor, basic compounds deficient, and oxidizing power low. The physical and chemical properties of the purified soil acid agree with the properties of the synthetic acid. The occurrence of this acid in nature had not been certainly established previously. The authors suggest that it may be formed from aliphatic β-hydroxy acids which are produced during the destruction of cellulose, or by hydrolysis of allyl cyanid which occurs in the essential oils of some plants.—CHARLES A. SHULL.

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[20] WERNHAM, H. F., *Pseudomussaenda*, a new genus of Rubiaceae. Jour. Botany **54**:297–301. 1916.

[21] GORTNER, ROSS AIKEN, LAWRENCE, JOHN V., and HARRIS, J. ARTHUR, The extraction of sap from plant tissues by pressure. Biochem. Bull. **5**:130–141. 1916.

[22] WALTERS, E. H., and WISE, LOUIS E., α-Crotonic acid, a soil constituent. Jour. Agric. Research **6**:1043–1045. 1916.

GENERAL INDEX

Classified entries will be found under Contributors and Reviewers. **New names** and names of new genera, species, and varieties are printed in **bold face** type; synonyms in *italic*.

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