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ERRATA

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P. 57, line 10, for higher read lower
P. 181, footnote line 4, for FINDFUSZ read RINDFUSZ
P. 300, line 7, for even read do not even
P. 316, fig. 1, lowest filled circle of 1921 curve should be open circle of 1919 curve

VOLUME LXXIV NUMBER 1

THE BOTANICAL GAZETTE

September 1922

DEVELOPMENT OF PLANT COMMUNITIES OF A SAND RIDGE REGION IN MICHIGAN

W. G. WATERMAN

(WITH TWELVE FIGURES)

Plant synecology is the study of the relations of plant communities to their environment. The subject may be divided into three major sections.

1. *Morphological and physiological synecology.*—The physiognomy, ecological structure, and floristic composition of plant communities and their relationship to the factors of the environment. This includes not merely an enumeration of the species and ecological forms present, but also the sociological value of the different members of the community, as suggested in the following topics: abundance, dominance, affiliation, genetic importance, constancy, etc.

2. *Geographic synecology.*—The distribution of plant communities, with special reference to the influence of the factors of environment.

3. *Genetic or dynamic synecology.*—The study of the development of plant communities on unit areas as the result of the action of biotic factors, modified by physiographic influences and by changes of climate.

In the early days of ecology, the distributional phase of synecology was more evident and was followed with almost no suggestion of the others. In recent years the other phases have been increasingly studied, but all have generally been combined in a more or less haphazard fashion. In a geographically extended treatment of the subject, the distributional (geographic) division may either precede or follow the developmental (genetic) division. If the communities are considered merely from the standpoint of their floristic content, their distribution may be studied first, and this has been the historical order. Plant geography has been studied with increasing interest since the days of VON HUMBOLDT, and it is still an important branch of synecology. It is evident, however, that the distribution of communities identified and named in accordance with their position in a developmental series (associations, formations, etc.) cannot be carried out adequately until a genetical study of those communities has been completed. In a limited area the study of the distribution of communities is correspondingly limited, and is of value only as it helps to determine the developmental relationships of these communities.

As genetic synecology is the most recent branch of the subject, and its content is not yet fully organized, a brief historical statement will be in order at this point. COWLES (3) was the first to form a comprehensive system based on the dynamic element in plant communities, as a result of his difficulty in classifying the communities of the Chicago region in accordance with WARMING's principles. He was so strongly impressed with the influence of the physiographic factors that he outlined his system on that basis. Later (4) he recognized climatic, physiographic, and biotic factors as the three great causes of plant succession.

CLEMENTS (1) pointed out that climatic and physiographic causes produce succession but not true development, that is, the building up of a quasi-organism; and that this was possible only by the action of biotic factors, and especially by the influence of the plants which compose the different communities. CLEMENTS bases his main divisions on his climaxes or "formations," his next division is into primary and secondary successions, and his third into hydrosere and xerosere, based on the water content of the initial area. For these divisions he also uses as adjective modifiers the term hydrarch and xerarch, suggested by COOPER (2). As these latter refer especially to the beginning of the succession, they are more suitable in this connection than such terms as hydrosere, etc., which apply to the moisture content of the whole succession, because in most cases the initial moisture condition does not persist, and the substratum generally approaches a mesophytic condition. While this classification may be logically defensible, CLEMENTS does not give sufficient consideration to the fact that the actual lines of development are determined by the nature of the substratum, that the floristic content of the pioneer stages is absolutely different in clay, sand, or rock, and that it is only as the seres approach the climax stage that they begin to converge and to resemble each other. Furthermore, standing water should be regarded as a type of substratum, because its pioneer stages are practically identical in ponds on rock, sand, or clay, and are quite different from the pioneer stages of wet sand or clay, to which stages the term hydrarch should be restricted. The subdivisions of the primary succession (prisere), therefore, should be sand succession (psammosere), clay succession (geosere), rock succession (lithosere), and aquatic succession (hydrosere). The first three successions have wet and dry initial stages (hydrarch, xerarch). It is evident that this classification does not distinguish the many types of substratum containing mixed sand, clay, and gravel. It does not seem, however, that these are sufficiently well marked or sufficiently different as to vegetation to warrant establishing one or more additional seres for them at present.

The terminology of the units of genetic synecology is being much discussed at present. It is generally agreed that the fundamental unit in the developmental classification of communities is the association. At first this was defined in terms of the habitat, but in 1921 NICHOLS (**7**), as a result of several questionnaires sent to eighty-five ecologists, reported at the recent meeting of the Ecological Society of America at Toronto that a large majority of the ecologists consulted favored the following statements: (1) That the term plant association be recognized as applicable both to the abstract vegetation concept and to the concrete individual pieces of vegetation upon which this concept is based; (2) that plant association in the abstract be defined somewhat as follows: an ecological vegetation-unit characterized by an essentially definite physiognomy and ecological structure, and by an essentially definite floristic composition as regards dominant species; (3) that plant association in the concrete be defined somewhat as follows: a plant community of essentially uniform (or homogeneous) physiognomy and ecological structure and of essentially uniform (or homogeneous) floristic composition as regards dominant species. This simply formulates the more or less unconscious practice of most ecologists, who, when speaking of "a *Scirpus-Typha* association" have a concrete community in mind, while "the *Scirpus-Typha* association" of a certain region is plainly an abstract concept.

The next higher unit is also generally recognized as the formation, but there is not yet the same agreement in regard to its content as there seems to be for the association. The following brief survey of the progress of opinion in regard to the formation is summarized from TANSLEY (**9**). According to the definition adopted by the Brussels Congress, the formation is composed of associations which differ in their floristic composition, but are in agreement (1) with the conditions of the habitat, and (2) as regards their growth forms. TANSLEY says, "Though this concept is apparently accepted by most European phytogeographers, it has little real hold on actual concrete research because it is abstract and one sided." In 1907 MOSS (**5**) proposed a unit, later embodied by TANSLEY (**8**), in which all associations developed on the same habitat or on one of essentially constant character were considered as belonging to one formation. Not all the stages of a succession were necessarily included in one formation. If the habitat obviously changed its character completely, it was recognized that a new formation had been initiated. This conception was widely criticized, and TANSLEY admitted the validity of criticisms of the habitat element in the definition. CLEMENTS (**1**) refused to recognize any formations except those determined by climate, regarding all communities in a region where forests are climatically possible only as stages in the development of forest formations. TANSLEY believes that this view has not been generally accepted in Europe or in America, and feels that the universal dominance of climatic factors as determinants of climax vegetation has not been proved.

NICHOLS (6) recognized this and returned to SCHIMPER'S distinction between climatic and edaphic formations. His unit next above the association was the edaphic, later called the physiographic formation, which he defined as the association-complex occupying a physiographic unit area, while the climatic formation was a complex of physiographic formations forming the vegetation, taken in its entirety, of any region in which the essential climatic relations are similar or uniform throughout. This TANSLEY criticized, because "nothing like a sharp line can be drawn between one climatic region and another, so that it becomes impossible to delimit climatic formations in NICHOLS' sense." TANSLEY accepts CLEMENTS' "associes" for all stages which have not reached a relatively stable (climax) condition, and defines the association as a mature quasi-organism which is relatively fixed and stable. He then defines the formation as including "all the vegetation which is naturally grouped around the association, determined by the particular collection of environmental factors which make up the ecological conception of the habitat." NICHOLS has not published as yet any further statement on the formation, but in his paper at the Toronto meeting he seems to adhere to his division of formations as physiographic and climatic.

As a result of a study of literature on formations, as well as actual conditions in the field, especially in connection with the preparation of the present paper, the writer has reached the following conclusions, on which the definitions of the terms involved will be based.

1. That there is a distinct advantage in omitting from the definition of the formation all reference to the habitat, as was done in the case of the association.

2. That it is inadvisable to connect the idea of the formation with a climax association, because the determination of climax is one of the purposes of a genetical study, and it is clearly undesirable to define a term which should be usable from the beginning of a study in such a way that it cannot properly be used until the study has been completed. In such a case it would be necessary to secure an additional term for the community in the process of development. This is cumbersome and unsatisfactory, as is illustrated in CLEMENTS' use of "associes" and "association," which does not

seem to meet with general approval in this country at least. In this case there is the additional objection that the more familiar and convenient term is restricted in use to a minority of cases.

3. That the double aspect, abstract and concrete, approved for the association be recognized also for the formation. The abstract concept of the formation, indicated by the use of the definite article, would thus correspond with the Brussels Congress description, and would constitute the formation abstract as a sort of ecological species. The formation concrete might then be regarded simply as any association complex, characterized by a dominant association but including all adjacent associations, whether mature or immature, and other more or less anomalous or unidentified communities connected with them. Thus the formation concrete in general would correspond to Nichols' physiographic formation, although the habitat is omitted from the definition, and contiguity made the basis on which the communities are united in the formation. Individual formations may be named either from the dominant association or from the physiographic nature of the area occupied.

4. If a unit above the formation is desired, it will be found convenient to associate the formations of a region in a larger group, which Nichols (**6**), following Schimper, has characterized as a climatic formation. Tansley has demonstrated the inadvisability of the term, but the fact remains that the concept is a convenient one, especially for field use, and the writer suggests that the term "formation complex," or simply "complex," be used for this concept. If it be objected that complex is equally applicable to lower grades of units, and is actually in use with them, a special term, such as "aggregate," might be employed. It does not seem advisable to use the term formation, even if qualified by a descriptive adjective, for two classes of units of different grade. Indeed this concept may be identified sufficiently by general expressions already in use, such as vegetation, formations, or even forest (as in "vegetation of Connecticut," "formations of the Great Lakes region," "temperate deciduous forest," etc.).

On the whole, it seems advisable to follow Tansley and Nichols in emphasizing the vegetational content of the community and regarding the habitat as the sum total of the environmental factors, and therefore not employing it to indicate any definite portion of the surface of the earth. There is an advantage in employing a special term for the ground occupied by each synecological unit, and the writer tentatively uses the word "locality" for the ground occupied by an individual association, "area"[1] for that occupied by a formation, and "region" for that occupied by a formation complex.

The present study is to be regarded as a preliminary reconnoissance rather than as a completed work. Its purpose is to indicate the lines along which such a study should proceed, and to suggest some tentative conclusions. It is the intention of the writer to make a thorough study of the morphology and physiology of these communities, and in the light of those results to review the tentative conclusions now reached. This preliminary survey will also serve to introduce the region to ecologists, and to show the unusual opportunity for the study of the very diverse communities of a region in relatively primitive condition. Incidentally the writer regards the region as one which should be included in a list of regions to be preserved in their natural condition.

Description of habitat

GEOGRAPHY.—The region is located in Benzie County, Michigan, and adjoins on the north the Crystal Lake Bar region already reported (10). It may be described as a right-angled triangle whose base is about eight miles long, extending south-southeast from a point on the shore of Lake Michigan about two miles northeast of Point Betsie, almost to the town of Honor on Platte River. The east side of the region is the perpendicular of the triangle, and extends north from Honor nearly to the town of Empire on Lake Michigan. The shore forms the hypothenuse, curving slightly to the south with a projection at the mouth of Platte River. It has a total area of about twenty-five square miles, of which perhaps one-fifth is occupied by lakes and ponds. The region is locally known as the Platte Plains, although it is composed of sand ridges and hills, and the general relief is distinctly rolling rather than flat (fig. 1).

The Lake Michigan shore is bordered by a strip of moving dunes ranging from 200 to 500 yards wide. As the prevailing winds are

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[1] This is an ecological use of the term, and differs materially from its floristic use.

from the southwest, this dune strip has been protected by the morainic ridge (fig. 2) north of Point Betsie, and the dunes are relatively low and do not have the scenic features to be found in similar areas elsewhere. South of the narrow belt of moving dunes are found the sand ridges, roughly parallel with the shore, with intervening depressions, some still containing small ponds. These ponds occur almost exclusively in the portion of the region west of Platte River, probably because of the protection of the morainic ridge which has prevented them from being filled by blown sand. Between the sand ridges lies a wide trough of relatively slight depth, which contains a series of six lakes, more or less completely connected and draining into the Platte River, and three small lakes draining to the north through Otter Creek.

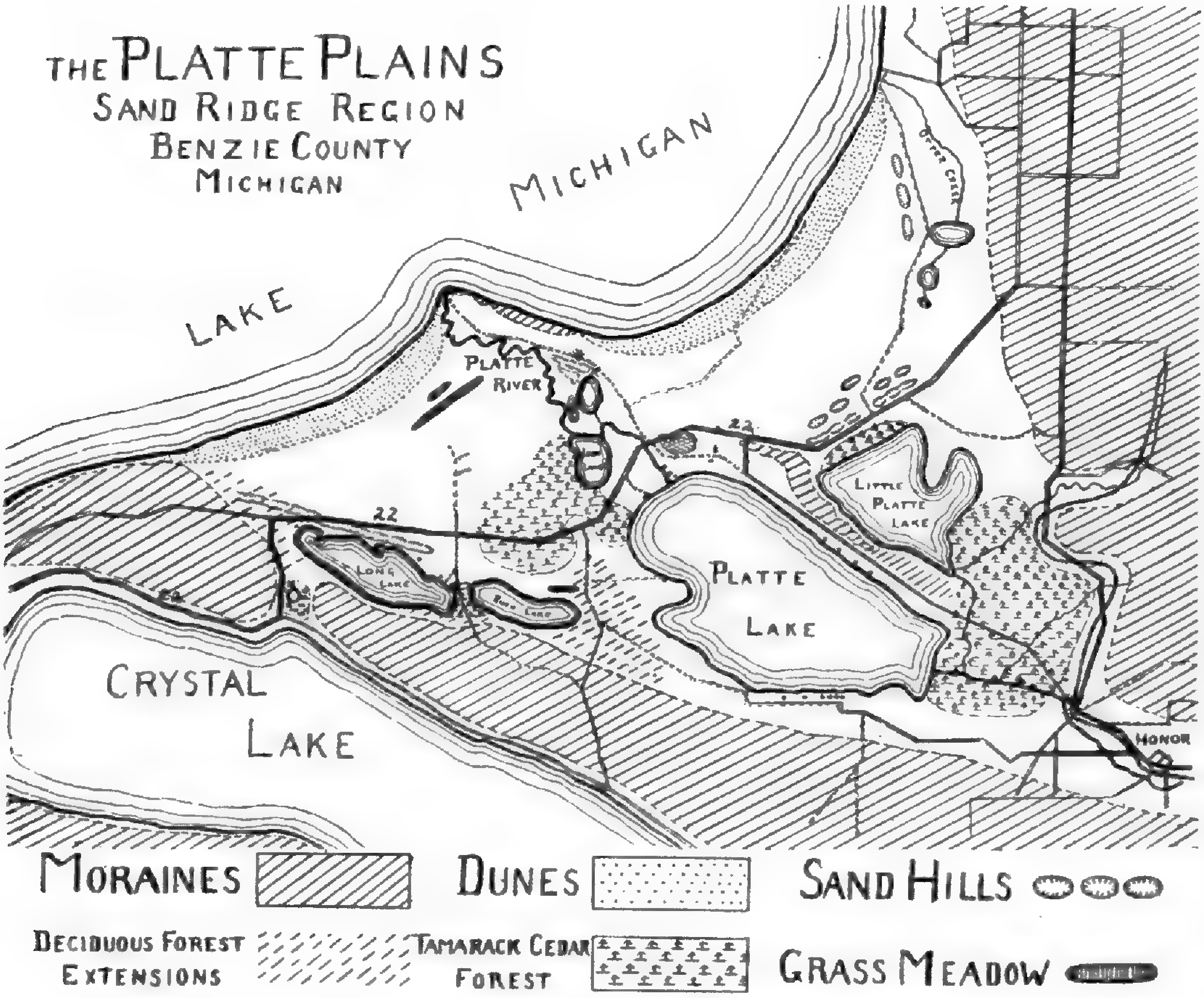

FIG. 1.—Map of Platte Plains sand ridge region

GEOLOGY.—This region is regarded as having been a shallow embayment of Lake Algonquin, whose shores were formed by morainic uplands. The shore line of the embayment is clearly traceable along the southern and eastern border of the Platte Plains as shown on the map. On the west the old shore line, protected by the morainic ridge, is marked by low rounded knolls, but on the south and east it still shows the characteristics of a wave-formed bluff (fig. 3). This feature has been continued by wave action in the lakes on the south, and it is especially marked on the east, where it borders the floodplain of Otter Creek (fig. 7). There it still remains as a steep bank rising 150 or 200 feet above the plains. The present sand ridges with intervening depressions were formed originally as sand bars by the receding waters of Lake Algonquin, assisted by the winds, which piled dunes of varying heights up to 100 feet above Lake Michigan on the successive beaches left bare by the receding lake. One morainic fragment is found on the present shore line half a mile east of the mouth of Platte River, in shape like a hogback, with a very steep forested slope on the south and an equally steep bluff of erosion facing the lake (fig. 10). The line of hills between Platte Lake and Little Platte is a morainic remnant exactly in line with the fragment on the shore. Morainic gravel has been found

FIG. 2.—View toward southwest over shore dunes to morainic ridge which formed the Algonquin shore on the south.

in at least one spot midway between the two; so it seems probable that these represent the remains of a moraine which originally bisected the triangle, and which later determined the location of Platte River.

Environmental conditions.—The factors of the environment are similar to those of the Crystal Lake Bar region (**10**), and will not be repeated here. A study of the soil acidity by Wherry's method is planned for the near future, and will probably yield interesting results. The variations in soil and moisture content are evidently of great importance. The substratum consists in the main of beach and dune sand, but there is a mixture of morainic material around the edges where moraine clay and gravel were washed down by the waves of Lake Algonquin and by atmospheric agencies since that time. The materials of the low morainic ridge which bisect the area are also of considerable importance, and their significance will be considered in connection with the migration of the deciduous forest elements into the sand ridge vegetation.

Fig. 3.—View toward the southwest over Platte Lakes, showing Algonquin shore line in distance.

While the slight moisture content of the superficial layer of sandy soil is generally recognized, it has been thought that the moister conditions of lower layers and the low wilting coefficient of sand would prevent a serious deficiency of moisture for plants adapted to that habitat. The very dry weather in the first part of the summer of 1921, however, apparently caused serious results, although the full effects of these conditions cannot yet be determined. The leaves of the blueberries and other small shrubs dried up early, and while the plants were not killed, their vegetative development for the following summer was greatly reduced. A number of oak and pine seedlings up to two feet in height were evidently killed.

Fig. 4.—Typical sand ridge vegetation, with *Pteris aquilina* in center

Similar "red summers" were reported by the farmers as having occurred at intervals of eight or ten years. It is evident that they would profoundly affect the development of a dense growth of trees on these ridges. Various fires, chiefly prehistoric, have also had an influence. The areas which were affected by these fires should be determined and mapped, and their influence on the present vegetation more definitely determined.

Morphology and distribution of communities

Moving dune formation.—The dune vegetation is practically the same as that of the Point Betsie dune complex already described

(**10, 11**), with the important exception that there are numerous groves of *Pinus Banksiana* occupying depressions just back of the foredune, and apparently originating in pannes.

SAND RIDGE FORMATION.—The sand ridge area was originally covered by a forest of pine and oak whose trees had reached considerable size. This forest had been burned before the white settlers came to the region. Many of the dead trees were cut while still standing, and many of their stumps still remain. A few patches were not burned, perhaps being protected by neighboring bodies of water, and these give some idea of what the original forest might have been (fig. 8). Reproduction has been good all over the region, and with fifty years' growth behind it, the forest might be regarded as half-way to maturity. While strictly this region should be regarded as a secondary succession, most of the area has practically been untouched by man, and the development seems to be well on its way toward a reproduction of its original condition, so that with the aid of apparently untouched portions it should be possible to work out the stages of the original succession.

The trees of this formation in the order of their importance are *Pinus Strobus*, *P. resinosa*, *P. Banksiana*, *Quercus ellipsoidalis*, *Q. alba*, *Q. rubra* (*Q. velutina* apparently should belong here, but has not been certainly identified), *Acer rubrum*, *Betula alba papyrifera*, *Populus tremuloides*, *P. grandidentata*, *Amelanchier canadensis*, and *Prunus pennsylvanica*. The shrubs found on the sand ridges are *Cornus stolonifera* near the dune belt and *Rhus typhina* farther south (fig. 4). In the undergrowth are found *Pteris aquilina*, *Gaultheria procumbens*, *Vaccinium vacillans*, *V. pennsylvanicum*, *Melampyrum americanum*, and *Ceanothus virginiana*, with *Cladonia rangiferina*, several cushion mosses, and occasionally *Selaginella rupestris* in the drier portions. In the more mesophytic spots occur also *Pedicularis canadensis*, *Galium* sp., *Maianthemum canadense*, *Diervilla Diervilla*, and *Aster cordifolius*. Near the dunes are found *Artemisia canadensis*, *Smilacina stellata*, *Arctostaphylos uva-ursi*, *Apocynum cannabinum*, *Rosa blanda*, *Juniperus communis*, and *J. horizontalis*.

ASSOCIATIONS OF SAND RIDGE DEPRESSIONS.—The depressions between sand ridges are small oval bowls or pockets averaging only a few hundred yards in greatest length. They show all types of aquatic communities, from those which are nearly all floating aquatics to the grass meadow or the swamp shrub thicket. There are few characterized by a true bog mat, but many contain characteristic bog plants and shrubs. The grass meadows vary in size from a few yards in diameter to one 100 yards wide, and one-half to three-quarters of a mile long; while two others of equal length are 200–300 yards wide (fig. 11). These are usually bordered by a narrow shrub zone between the sand ridge and the meadow, including *Alnus incana*, *Pyrus arbutifolia*, *Rosa carolina*, and *Cornus stolonifera*. With the grasses and sedges in the smaller meadows are found also *Hypericum virginicum* and *Spiraea salicifolia*, while occasional specimens of aquatics occur, as *Iris* and *Sagittaria*. In one case a remarkable growth of *Lobelia cardinalis* covered one acre of meadow with its scarlet flowers.

FIG. 5.—*Chamaedaphne* meadow with *Picea Mariana* and *Larix laricina*

The bogs are generally found at or near the border of a lake or river, and are of two general types, one an ericad heath, the other a tamarack thicket. The heath type has a more or less continuous cover of sphagnum with its usual accompaniments: *Sarracenia purpurea*, *Vaccinium macrocarpon*, *Drosera rotundifolia*, *Menyanthes* *trifoliata*, *Aspidium Thelypteris*, and in the wetter portions *Typha latifolia*, *Iris versicolor*, and *Phragmites communis* (fig. 5). The shrubs are *Chamaedaphne calyculata*, *Andromeda glaucophylla*, *Vaccinium corymbosum*, *Betula pumila*, and rarely *Ledum groenlandicum*. The trees if any are scattered, and include *Larix laricina*, *Picea mariana*, and where the substratum is very dry *Pinus resinosa* and *P. Strobus*.

The bogs of the thicket type are covered with a dense growth of *Larix* with some *Thuja occidentalis*, mingled with shrubs of *Alnus incana*, *Betula pumila*, *Rosa carolina*, *Chamaedaphne calyculata*, *Andromeda glaucophylla*, *Cornus stolonifera*, *Eupatorium perfoliatum*, *Myrica Gale*, and occasional specimens of *Cypripedium* sp. These thickets are usually surrounded by a zone of *Thuja*, sometimes narrow but occasionally of sufficient area and maturity of trees to be called a "cedar swamp." Here are found in addition *Fraxinus americana*, *Betula alba*, *Abies balsamea*, and *Acer rubrum*. Around the outer edges next the sand ridge, small specimens of *Tsuga canadensis* are locally found.

FIG. 6.—Long Lake, with pines on sandy point; Algonquin shore line in distance

In the cedar forests the shade is very heavy. The roots of the trees interlace above the water or semiliquid muck, while the undergrowth grows on the peaty soil which has collected in hummocks or on the layer of tree roots. Shade-tolerant mosses are abundant, and occasional restricted patches of sphagnum occur. Among flowering plants the most common are *Coptis trifolia, Cornus canadensis, Trientalis americana, Maianthemum canadense, Aralia nudicaulis, Gaultheria procumbens, Viola* spp., and occasionally *Clintonia borealis. Aspidium spinulosum* and *Osmunda regalis* are frequently found, and some *Taxus canadensis.*

FIG. 7.—Algonquin shore, kept steep by erosion of Otter Creek

VEGETATION OF RIVER AND LAKE BORDERS.—In the Platte Plains there are no true alluvial floodplains, and the shores of streams and bodies of water are either rather steep sandy slopes, or else shallow bays or lagoons inhabited by one of the types of communities already described (figs. 6, 12). The lakes are frequently shallow some distance from shore, and these shallows generally contain extensive colonies of *Scirpus.* There is usually a fringe of aquatic plants, including *Typha latifolia, Sparganium, Sagittaria, Scirpus,* etc., or of shrubs including *Myrica Gale* and *Decodon verticillatus.* On flowing streams the latter does not seem to advance from year to year, probably on account of being torn away by ice in the spring. In this region *Decodon* is chiefly confined to streams, as it has been observed on lakes or ponds in only one or two instances.

VEGETATION OF MORAINIC UPLANDS.—The morainic uplands were covered with a typical climax beech-maple-hemlock forest, which has been sufficiently described elsewhere (**10, 12**). In some places this is almost untouched, and in at least two places, near Lake Michigan on the west and south of Long and Rush Lakes, the tension zone between it and the sand ridge vegetation is in practically its original condition. In the first locality this zone is about a quarter of a mile wide, and its elements mingle with those of the sand ridge formation. On the south it descends the steep Algonquin terrace to the shores of Long Lake, and merges with the cedar forest between Long and Rush Lakes, and south and east of Platte and Little Platte Lakes (fig. 7). North of Little Platte it originally stopped on the crest of the steep bluff which borders the Otter Creek valley, and the bluff was occupied by a xerophytic conifer association, of which only a few patches now remain.

Development of communities

As already stated, the purpose of genetic synecology is to indicate the successional relationships of the communities of a region, and the place of each in a developmental series. In the present study there is no rock substratum present, and only a very restricted amount of clay or gravel, so that the communities found are largely confined to the psammosere and the hydrosere. Secondary successions are present, both in burned areas and to a limited degree in clearings, but in the present paper the chief concern will be to trace the stages of the original succession (prisere). With one or two minor exceptions the influence of climatic and physiographic factors is so slight as to be negligible, and the region is so young geologically that there seems to be no necessity for the consideration of paleoecological relationships.

SAND SUCCESSION (PSAMMOSERE)

PIONEER STAGES.—*Xerarch.*—The pioneer stages of this succession are confined to the strip of shifting sand along the lake. The initial vegetation includes *Ammophila arenaria* as the absolute pioneer, associated with *Artemisia caudata*, *Calamovilfa longifolia*, *Cirsium Pitcheri*, *Campanula rotundifolia*, *Cakile maritima*, *Lathyrus maritima*, *Prunus pumila*, *Salix longifolia*, *S. syrticola*, *Senecio vulgaris*, *Solidago* spp., *Aster* sp., *Zygadenus chlorantha*, *Hudsonia tomentosa*, *Arctostaphylos uva-ursi*, *Juniperus communis*, and *J. horizontalis*. All of these are able to germinate on dune sand, but only *Ammophila* is able to become established in pure sand. The others are stunted and soon die, unless their roots come in contact with buried plant material, from which apparently they are able to obtain the necessary mineral elements. *Ammophila* and perhaps *Calamovilfa* are the only plants which cover the ground thickly enough to act as stabilizers. The other herbaceous plants are also scattered, and never grow close enough to form a ground cover in the moving dune belt. *Arctostaphylos* and *Juniperus horizontalis* germinate occasionally on the open sand, but it is doubtful whether they can stabilize. Whenever they occur in sufficient quantity to cover the ground, it is usually by invasion from a patch already established.

Hydrarch.—*Juncus balticus* and willows are the usual pioneers in pannes. Occasionally a local patch of *Pinus Banksiana*, with more or less *Thuja occidentalis*, *Abies balsamea*, and *Betula alba*, forms a clump or grove, which may reach the size of several acres and spread over small valleys or local patches of level sand. There is no evidence of any extensive permanent stabilization by coniferous trees in the belt of moving sand next to the lake. They frequently occur as narrow strips or tongues between advancing lee slopes. The transition from this area to the pine-oak ridges is very marked as one crosses the irregular line of crescent-shaped lee slopes and comes to the lower rounded ridges, where the force of the wind is much less marked and the plants of the pine-oak stage have become established. From this it would appear that the coniferous tree stage originates in pannes, but does not really become widespread so as to form a forest into which the more mesophytic pines and oaks gradually migrate and become dominant, but that the coniferous patches are relatively scattered, and their only influence is as humus formers and as centers of distribution for certain elements of the pine-oak stage. Stabilization, therefore, is due to a diminution of the force of the wind, either on account of the increasing distance from the shore as the lake waters recede, or by the building up by the wind of high dunes which form a windbreak and protect the sand ridges.

PINE-OAK STAGE.—With the relative cessation of sand movement, coupled with increase of humus, the pine-oak stages begin. As might be expected, the content of vegetation on the ridges shows a certain amount of progressive change, both in floristic content and in the density of growth and mesophytism, as we traverse the region from the vicinity of the lake toward the south (that is, from younger to older ridges). On the first ridges there is a preponderance of conifers and many relics of the herbaceous pioneers, especially *Artemisia*, *Smilacina stellata*, *Arctostaphylos*, and *Juniperus* spp. The first tree of this stage to come into the coniferous association of the open dunes is *Pinus Strobus*, followed shortly by *P. resinosa*. *Quercus ellipsoidalis*, *Q. alba*, and possibly *Q. velutina* follow rather slowly, and now may be found fairly evenly distributed in the more advanced portions of the area. *Acer rubrum* is very frequently met near Platte River, or in other somewhat moist

FIG. 8.—Grove of old pine, oak, and hemlock, probably protected by proximity to Platte River.

localities. *Betula alba*, *Populus grandidentata*, and *P. tremuloides* are fairly common all through the region. As has been stated, the region has been burned probably more than once, although fortunately not in recent years. The extent of area covered by any one burning is uncertain. Certain local differences in distribution can best be explained on the assumption that the burnings have not been very complete; certainly some patches which bear old pines and hemlocks must have escaped (fig. 8), but patches of white birch and poplars indicate a secondary succession after fires.

FIG. 9.—Luxuriant growth of *Pteris aquilina* in mesophytic habitat, second growth birch and poplar in background.

The undergrowth varies from the modified pioneer type of the ridges nearest the lake to a mesophytic type containing many forms belonging to the climax beech-maple-hemlock forest. These mesophytic associations are not distributed in accordance with the geologic age of the ridges, but are determined rather by edaphic conditions. They will be considered in the section on the beech-maple-hemlock invasion. In addition to the typical mixed ground cover already described, there are two types of undergrowth societies unevenly distributed over the ridges, an almost pure *Pteris aquilina* society and a *Vaccinium* society including *V. penn-*

sylvanicum, *V. vacillans*, with considerable *Gaylussacia baccata*. On the whole, the *Pteris* communities are more characteristic of the portion of the ridge area west of the Platte River and the *Vaccinium* of that east of the river. No definite factors determining the distribution of these communities have yet been established. The *Pteris* seems to be more moisture-requiring than the *Vaccinium*, and it certainly grows more luxuriantly in the moister habitats (fig. 9). The *Vaccinium* species are usually regarded as more acid-tolerant, and investigations along these lines may yield definite results.

BEECH-MAPLE-HEMLOCK STAGE.—It is generally recognized throughout Michigan that the deciduous hardwood forest is confined to rich clay or loamy soil, white pine forests are found on sandy loam, and the pioneer conifers on poor sandy soil. The development of a climax deciduous forest on fixed dunes, as found in the Point Betsie region and at other points along the Michigan shore, is an interesting problem, the solution of which should materially be aided by the evidence to be obtained from a study of this relatively untouched region. As suggested by the preceding morphological study, the climax forest which developed on the morainic upland has begun to invade the sand ridge area along all lines of contact between the moraines and the sand, the undergrowth having gone farthest, and the tree species migrating more slowly. The first tree of the climax forest to appear in the sand ridges is *Tsuga canadensis*, scattered specimens of which are found up to a quarter or half a mile from the morainic border in the tension zone on the west, and for varying distances on the other borders of the sand ridge area. Many parts of the lowland border on the south have been cleared of trees, but in the small triangle between Long and Rush Lakes the deciduous forest is in contact with a cedar swamp, and we can find there a horizontal succession in practically untouched condition. The first hardwood pioneers in the swamp are *Fraxinus americana*, *F. nigra*, *Ulmus americana*, *Tilia americana*, and *Acer rubrum*. As *Thuja* disappears, the trees of the beech-maple-hemlock forest begin to come in on an alluvial substratum, forming a lake plain washed down from the Algonquin terrace on the south. The largest trees have been cut, but hemlock stumps up to four feet in diameter are still to be seen.

On the strip of land between Long and Platte Lakes a scattered and somewhat stunted growth of *Tsuga canadensis*, *Acer saccharum*, and *Fagus americana* extends north almost to Michigan Highway 22, over soil which at first is somewhat alluvial, but which gradually changes into the usual sand ridge type. Toward the north the hard maples disappear first and the hemlocks last, as the oaks and pines become more frequent. On the east very little uniform advancement of the deciduous trees was found, probably because of the steep xerophytic bluff occupied by conifers (fig. 7). The valley of Otter Creek has not been studied in detail as yet, but it seems to be largely occupied by conifers, although the soil conditions are more those of an alluvial floodplain than in any other part of the sand ridge region.

Apart from this rather uniform invasion along the borders, there are several isolated spots where hemlocks at least are found in some frequency and of considerable size. On the morainic ridge between Platte and Little Platte Lakes the deciduous forest was apparently well developed, with, however, a large proportion of pines and some oaks. Large hemlocks are found in several places along the east bank of Platte River north of Platte Lake (fig. 8), and in the valley on the south slope of the morainic fragment on the shore of Lake Michigan (fig. 10). In the last locality they are in poor condition, and some have recently died. Small specimens are to be found on the edges of many of the smaller swamp depressions, especially on the southwest of Loon Lake, that is, on the opposite side of Platte River from the morainic ridge extension just mentioned. *Fagus americana* has not been found away from the borders and the wedge-shaped invasion between Platte and Little Platte Lakes. A solitary specimen apparently about fifty years of age is growing by Michigan Highway 22, about a quarter of a mile west of Platte River, and therefore in the heart of the sand ridge area. Its shape shows that it grew in the open, but its age does not preclude the possibility of its having been planted by the first settlers. The mixed coniferous-deciduous lake bluff border association described in the Crystal Lake Bar region (10) does not appear in this region, probably because the corresponding habitat is not present. This association contained *Thuja occidentalis*, *Abies balsamea*, *Tilia americana*, and *Ostrya virginiana*, mixed with the trees of the climax deciduous forest on the crest of the bluffs facing Lake Michigan. It was apparently due to the exposure to sun and lake winds, combined with low soil moisture content. The only place where it might have been expected was on the high bank at the eastern edge of the sand ridge region, and there the only conifers seem to have been *Pinus Strobus* and *P. resinosa*.

FIG. 10.—Morainic remnant on shore of Lake Michigan; landward slope equally steep, with hemlocks growing in valley.

The preceding discussion indicates that the migration of the beech-maple-hemlock forest into the sand ridge formation has been of two sorts, one a general advance along all lines of contact between the two formations, the other in a long slender belt on the morainic ridge and its remains, to the shore of Lake Michigan (fig. 10). Unpublished investigations by the writer indicate that for the establishment of *Thuja occidentalis* and *Abies balsamea* on sandy soil it is necessary that there should be present in the sand enough moisture to carry the young tree root below the drought zone. This may be held by humus in the soil, or by a high moisture content in sand relatively free from humus, and it seeems possible that this may also be true for *Tsuga*, but not for *Fagus*. *Acer saccharum* seemed to hold an intermediate position between the two, and its apparent absence from any advanced positions on the sand ridge habitat was a distinct surprise. Morainic soil equally with humus seems to afford a suitable substratum for the establishment of all the species mentioned, either because of the supply of necessary mineral elements, or because these minerals make possible a better utilization of the water present. For these reasons the advancement of the deciduous formation seems to be the result of increased humus content and mesophytism, and also a pushing forward by sheer force of numbers. The parent seed trees being so near at hand and supplying so many seeds, it follows that in time a fair number of seedlings have been able to find conditions favorable to growth and so become established. In the other case, the morainic substratum affords a soil peculiarly favorable to the deciduous trees, and while a much smaller number of seeds have lodged on it, a relatively larger proportion have become established. In view of the fact that the shade and moisture conditions vary greatly on different parts of this ridge, it would seem to be the chemical constituents of the soil which give to it its favorable characteristics.

The question may be raised as to whether this condition may not be the result of the prehistoric fires, previous to which the beech-maple-hemlock elements may have been more widely distributed, and the present isolated groups may be relics preserved because of the protection of bodies of water. Against this view may be set the evidence of tradition and the entire absence of stumps in other moist habitats of the region which seem to have been untouched by fires. Unquestionably even hemlock stumps and logs do not last as long as pine, but it might be expected that some traces would remain if they had been at all widespread in comparatively recent times. This, however, would not account for the appearance of hemlocks on the edges of swamps at some distance from these relic patches. It is also reasonable to suppose that reforestation after a fire would proceed along general lines similar to those of the original advance, so that in either event the stages of the prisere would be approximately as outlined.

AQUATIC SUCCESSION (HYDROSERE)

The substratum in this succession is standing water, either in closed depressions such as ponds and small lakes, or in open depressions as bays or lagoons along the banks of lakes or of Platte River. These various bodies of water show practically the same vegetation for the first three stages, commonly designated as the *Potamogeton*, *Nymphaea*, and *Scirpus* stages. After that different lines of succession are found, depending on the condition of the habitat.

SAND RIDGE DEPRESSIONS CONTAINING STANDING WATER.—In these depressions the water is fairly shallow, but the depression is surrounded by sand ridges whose slopes rise directly from the water's edge. Here are to be found either swamp or bog stages according to the condition of the substratum. In the swamp type the fourth stage is a narrow sedge zone, the shrub growth is scanty, mostly willows, and the sand ridge vegetation descends the slopes almost to the water's edge. In the bog type the quaking mat is seldom found, but there is a dense growth of sedges and grasses on a fairly solid muck foundation. This contains such bog plants as *Menyanthes trifoliata*, *Potentilla palustris*, and occasionally *Sarracenia purpurea*, and *Sphagnum* sp. The shrub zone is a dense thicket of *Alnus incana*, *Pyrus arbutifolia*, with some *Hamamelis virginiana*, and sometimes with scattered specimens of *Thuja occidentalis* and even *Tsuga canadensis* mingling with poplars and the first trees of the pine-oak association. The water is frequently shallow on the north side of the pond, possibly from sand blown in by the winds from Lake Michigan, and of course is exposed to the heat of the sun, but protected from the colder winds. The south side has deeper water, and is more sheltered from the heat of the sun on account of the shade of the pines and oaks, but exposed to the cold winds. In depressions of this kind the swamp vegetation is found on the shallow, warmer, northern side, while the bog type occurs on the deeper, cooler, southern side. In depressions which are sheltered on all sides and in which the water is deep all over, the bog type generally prevails over the whole pond.

Grass meadow type.—Here the fourth stage is one dominated by grasses and sedges forming a relatively solid turf. In the smallest depressions the sedge society may be only a few yards square, and in it there are often found swamp plants such as *Hypericum virginicum*, *Spiraea salicifolia*, and *Rosa carolina*, as well as occasional relics of the aquatic stages. The later stages resemble those of the swamp type already described. The larger grass meadows are relatively limited in number, only four or five having been discovered so far, and they have certain peculiar features which seem to demand special consideration. The first is the mature condition of the extensive grass turf, and the other is the absence of any tendency of the shrubs and trees to invade the meadow. Where the grass meadow is surrounded by a shrub zone of the swamp type, this may be accounted for on the assumption that the depression was originally all very shallow, thus favoring a development of turf so rapid that the shrubs and trees had no chance to become established before the mat of grass roots had completely occupied the substratum. There are, however, some features which indicate that the grass mat was formed recently and very rapidly, indicating perhaps a physiographic change in comparatively recent times. These are best shown in a grass meadow visited only once, as it was discovered in a hurried reconnoissance trip, and so far there has been no opportunity for a second visit. This meadow is located just west of the lower reaches of the Platte River very near the strip of moving dunes on the shore. It extends from northeast to southwest practically in a straight line for rather more than half a mile, but is less than a hundred yards wide at the point crossed, although somewhat wider to the east and the west. At this point there were imbedded in the grass on both edges of the meadow trunks of dead trees extending out from both banks, and on each tree was growing a row of tamaracks apparently not over twenty-five years old. There were scattered tamarack trees on the lower edges of the sand ridges. While the localization of the tamaracks on the dead logs and not in the grass is not surprising, the preservation of the logs long enough for the grass turf to form and the apparent youth of the trees makes a very interesting problem. This meadow apparently has never been mowed or burned. The logs may have come from trees killed by prehistoric fires, as they had no bark on them, but they were not charred, and otherwise seemed well preserved, with many dead branches extending up among the young tamarack trees. In fact, the whole situation suggested the sudden freezing of the surface of a pond, solidifying into a green grass mat, instead of a covering of ice. In the case of the large meadow occupying a shallow swale between Long and Rush Lakes, which at one time might have been a water connection between the two lakes, the shore showed the regular horizontal stages of a bog-cedar forest succession, but the center of the swale is occupied by a meadow with solid turf (fig. 11). The meadow has been mowed for years, and was recently ditched for draining, but this treatment apparently has not changed the general relations. The shrub zone at its southern edge is the usual bog shrub stage, followed by a belt of tamaracks of considerable size. A mature cedar forest adjoins this on the south, with a fairly dry substratum and some of the undergrowth elements of the deciduous forest. Next come deciduous swamp trees, and finally the trees of the climax forest. Here we have a bog forest left high and relatively dry, with a grass meadow formed at its edge.

FIG. 11.—Grass meadow near Long Lake; bog shrubs on left, with tamaracks and cedar swamp behind them.

Each of these formations, although differently situated (one in the heart of the sand ridges, the other between two lakes), seems to indicate the same physiographic change, that is, a sudden lowering of the water table by several feet. This change might be referred to the activities of the first white settlers about fifty years ago. The Long Lake area is very close to Crystal Lake, and might have been partially drained by seepage when Crystal Lake level was lowered in 1871 (**10**). A low terrace on the south bank of Long Lake adds weight to this hypothesis. The other meadow must have come very close to Platte River at its eastern extremity, and may have been lowered in connection with the first lumbering operations at about the same time.

Another explanation of the lowering of the water is based on diastrophic changes. Observations on the shore of Lake Michigan, both on the Michigan side and on the Green Bay Peninsula opposite, indicate that fifty years ago the lake was several feet higher than the highest levels of recent years, and this fall of level might have affected the level of Platte River in its lower reaches (fig. 12). There are also extensive meadows bordering Platte River and the sluggish stream connecting Platte and Little Platte Lakes, whose origin may be connected with the lowering of water levels at about the same time. Further study, both of the floristic content and of the nature of the substratum, is necessary before definite conclusions can be reached. There is no indication of any migration of trees into a grass meadow with solid grass mat, whether large or small, but there seems to be some evidence that they can come in on a floating mat of the swamp type. Further investigation may show that the latter case is really a bog mat, in which event it would not be available as evidence, and the presence of trees would be quite in accordance with the rule for bog mats.

BOG TYPE.—Here the fourth stage develops as a bog mat composed of sphagnum and the usual accompanying bog plants. In several cases this has developed into an ericad heath composed largely of *Chamaedaphne*, *Andromeda*, occasionally *Ledum* and similar shrubs, including *Betula pumila*, and scattered trees of *Larix*, *Picea mariana*, and occasionally *Pinus Strobus* and *P. resinosa*. In other cases the tamaracks with some bog shrubs and

Thuja have come in very thickly, forming a bog thicket which in some cases apparently may develop into a cedar forest. The tamaracks in this region are all small, with the exception of those between the shrub belt and the cedar forest already noted between Long and Platte Lakes. The occurrence of bog vegetation in the depressions among sand ridges has already been noted. The variation in depth on opposite sides also applies to some extent to the larger ponds and lakes. In the latter cases the shallow portions are characterized by extensive *Scirpus* colonies, but the bog associations do not appear along the shores of the lakes. In open bays and lagoons there was no general uniformity, but swamp or bog types were found corresponding to the varying local conditions. From this it is concluded that the development of the later stages of the hydrosere into the swamp or the bog type is dependent chiefly on depth of water and temperature. Investigations as to acidity have not been made as yet, but it is assumed that here, as elsewhere, the swamp type will be associated with a neutral or alkaline condition and the bog type with high acidity. The tree stage in the hydrosere was found to follow only the quaking mat

FIG. 12.—Lower reaches of Platte River, bordered by grass meadows, with some second growth birches; sand ridges in distance.

stage, and not the grass meadow. If we accept the hypothesis for the very recent formation of the large grass meadows, the absence of trees might be attributed to shortness of time, but the same condition is found in the smallest meadows, which from their position and general appearance must be regarded as contemporaneous with the wet depressions and with the sand ridges themselves, and here there should have been ample time for invasion. It does not follow necessarily that the grass meadow is an edaphic climax, but it is evident that that association will remain stable for a very long time. As already noted, the bog mat passes relatively rapidly into a tamarack cedar forest, which quickly receives hydrophytic deciduous elements, and thus passes into the climax deciduous forest.

Summary

1. Genetic synecology is that part of ecology which deals with the developmental relations of plant communities. In a limited region the development of successions (seres) is definitely related to the character of the substratum. In this region two such seres are found, the sand succession (psammosere) and the aquatic succession (hydrosere). The clay-gravel succession (geosere) has reached its climax on the surrounding moraines, and is observed only as it invades the sand ridge region.

2. In this study the successional units, the concrete association and formation, are defined as follows. The association is a plant community of essentially uniform (or homogeneous) physiognomy and ecological structure, and of essentially uniform (or homogeneous) floristic composition as regards dominant species. The formation is an association-complex characterized by a dominant association, but including all adjacent associations, whether mature or immature, and other more or less anomalous or unidentified communities associated with them. The unit above the formation is a formation complex, or aggregate, and is composed of the formations of a definite region which may be limited by climatic or geographic boundaries. The ground occupied by an association is called a locality, that occupied by a formation an area, and that occupied by a formation complex a region.

3. The vegetation of the region studied is found to be a formation complex consisting of a sand dune formation, a sand ridge

formation, and swamp and bog formations. It occupies a region which consists of sand ridges with depressions containing bodies of water of all sizes, from a few yards to a mile or more in diameter. Geologically the region was a shallow bay of Lake Algonquin drained by the recession of the waters of the lake with the melting of the ice barrier in the Straits of Mackinaw.

4. The first stages of the sand succession (psammosere) are found in the moving dune belt along the shore, but they do not lead to a complete stabilization of the sand. The later stages appear when the sand stops moving as a result of a checking of the force of the wind, due to distance from the shore or the formation of high dunes which act as windbreaks. The pine-oak stage shows a progressive change from the less mature areas near the lake to the more mesophytic areas in the southern portion of the region. Soil moisture content and the amount of humus in the sand seem to be important factors in this change.

5. The clay-gravel succession (geosere) has reached the climax stage as a beech-maple-hemlock forest on the surrounding morainic upland, and is found invading the pine-oak formation along the borders of the region, and especially along a narrow morainic tongue which extends completely through the sand ridge substratum to Lake Michigan. The controlling factor in this invasion seems to be primarily the chemical character of the soil, glacial material ranking close to humus in importance, and secondarily the soil moisture content.

6. The various ponds and lakes all show the normal early stages of the aquatic succession (hydrosere), which lead either to swamp meadows or to bog forests, the line of development followed being determined chiefly by depth of water and exposure to the heat of the sun.

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LITERATURE CITED

1. Clements, F. E., Plant succession. Carnegie Publ. no. 242. 1916.
2. Cooper, W. S., The climax forest of Isle Royale, Lake Superior, and its development. Bot. Gaz. **55**:1–44. 1913.

3. COWLES, H. C., The physiographic ecology of Chicago and vicinity, a study of the origin, development, and classification of plant societies. BOT. GAZ. **31**:73–108; 145–182. 1901.
4. ———, The causes of vegetative cycles. BOT. GAZ. **51**:161–183. 1911.
5. MOSS, C. E., Geographical distribution of vegetation in Somerset. Royal Geog. Soc. 1907.
6. NICHOLS, G. E., The interpretation and application of certain terms and concepts in the ecological classification of plant communities. Plant World **20**:305–319; 341–353. 1917.
7. ———, Abstract in printed program of Ecological Society of America for Meeting at Toronto, December, 1921.
8. TANSLEY, A. G., Types of British vegetation. Cambridge. 1911.
9. ———, The classification of vegetation and the concept of development. Jour. Ecology **8**:118–149. 1920.
10. WATERMAN, W. G., Ecology of Crystal Lake Bar region. Ann. Report Mich. Acad. Sci. **19**:197–208. 1917.
11. ———, Development of root systems under dune conditions. BOT. GAZ. **68**:22–53. 1919.
12. WHITFORD, H. N., The genetic development of the forests of Northern Michigan. BOT. GAZ. **31**:289–325. 1901.

SULPHUR CONTENT OF SOILS AND ITS RELATION TO PLANT NUTRITION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **297**

SCOTT V. EATON

(ITH ONE FIGURE)

Introduction

Ever since the ten essential elements for plant nutrition were established by the work of SACHS, BOUSSINGAULT, NOBBE, and other investigators, sulphur has been recognized as one of them. The ash analysis method of determining sulphur in plants, however, which was in use during this early period, showed such a small amount present that the needs of the plant were thought to be amply taken care of by the supply in the soil. Contributions during the last twenty years by BERTHELOT, BARLOW, FRAPS, GOSS, BESTLE, SHERMAN, and others have shown that in ashing plant material much of the sulphur may be lost. The amount of sulphur in plants as determined by analyzing the ash may be only a fraction of the real amount. Thus the whole question of the relation of sulphur to plant nutrition has been reopened, for if plants use several times as much sulphur as had been supposed, then perhaps the supply in the soil is not sufficient for the needs of the plant. Recently there have been a number of contributions to the subject. The first questions to be considered have naturally been how much sulphur do crops use and what are the supplies to meet these needs. Thus the first problems to be investigated have been the sulphur content of crops, the sulphur content of soils, the amount of sulphur brought down by the rain, and the amount lost by drainage, etc. Next, sulphur was added to soils found to be low in it to see whether the yield of crops would be increased. In the present paper no attempt is made even to approximate a resumé of the sulphur literature, rather complete digests of which may be found in papers by CROCKER (4) and OLSON (19).

ROBINSON and co-workers (**22, 23**) have analyzed a number of soils from different parts of the United States. The sulphur content is not high, the average for thirty-five important agricultural soils being 0.052 per cent, with a range of 0.012–0.156 per cent. SHEDD (**24**) finds the soils of Kentucky much poorer in sulphur than in phosphorus, and is inclined to place sulphur in the same class with phosphorus, potassium, and nitrogen as one of the chief limiting factors in crop production. In pot experiments with some of these soils, tobacco, soy beans, turnips, radishes, mustard, and alfalfa were benefited by sulphur fertilization. AMES and BOLTZ (**1**) report analyses for certain Ohio soils. The unfertilized soils range in sulphur content from 0.020 to as high as 0.055 per cent. BROWN and KELLOGG (**2**) find nearly twice as much sulphur as phosphorus in some of the larger soil areas of Iowa. The Mississippi loess proves to be lowest, the soil samples in this area ranging in sulphur content from 441 to 847 pounds per two million pounds of soil. SWANSON and MILLER (**27**) have analyzed a number of the soils of Kansas and find that the cultivated soils analyzed have an average sulphur content of 0.027 per cent. Certain cultivated soils of Wisconsin, analyzed by HART and PETERSON (**8**), prove to be low in sulphur, the average being 0.020 per cent. They summarize the results of their analyses of a number of crops by stating that cereal crops remove from the soil about two-thirds as much sulphur trioxide as phosphorus pentoxide, the grasses of mixed hay as much sulphur as phosphorus, while the legume hays may take from the soil about as much sulphur as phosphorus, or, as in the case of alfalfa, more sulphur than phosphorus. Such crops as the cabbage and the turnip may remove two to three times as much sulphur trioxide as phosphorus pentoxide. REIMER and TARTAR (**21**) give analyses for a number of Oregon soils. The range in the sulphur content of the surface soils is 0.015–0.038 per cent. The phosphorus content is much greater. The sulphur fertilization of alfalfa grown on these soils produces greatly increased yields. Increased tonnage yields of 50–1000 per cent are secured, and the protein content is increased in some cases almost 2 per cent. In experiments in Washington by

Olson (**19**), sulphur fertilization of alfalfa caused increased yields of 200–300 per cent.

The purpose of the present investigation was (1) to increase our knowledge of the sulphur content of soils, and (2) to study the relation of sulphur to chlorophyll development in certain plants and its effect on the yield of these plants. The phosphorus content of all the soils was also determined. Phosphorus, together with nitrogen, is considered the most generally limiting element of crop production in the soils of the United States. It was thought that it would be interesting to compare the sulphur content of the soils with their content of such an element as phosphorus.

Investigation

Soil analysis

It is important for American agriculture to discover how many soils in the United States are suffering from lack of sulphur, as are the Oregon soils to which reference has already been made. The Oregon results might be duplicated, perhaps, in the case of many other soils; on the other hand, many soils are probably not lacking in sulphur. The samples were chosen with a view of giving some idea of what range in sulphur content might be expected in the soils of the eastern and central United States. Thus, samples from the Atlantic and Gulf coast regions, from one of the southern states, from certain of the north central states, and from Chicago were analyzed. Investigations on the Atlantic coast during the early history of the United States showed great benefits from the use of gypsum as a fertilizer. It was thought that the analysis of certain of the coast soils might give some interesting results. On the other hand, soil analyses and sulphur fertilization tests in the central states may be said to indicate, in general, a higher sulphur content in the soils of this section than in the coast soils. It was desired to analyze a number of soils of the central states to compare with the coast soils. It is well known that rain carries down much more sulphur from a smoky atmosphere than from one less contaminated with smoke. It was thought that the Chicago soils might prove to have a very high sulphur content, owing to this fact.

Methods

Three methods of total sulphur determination were tested, the sodium peroxide method as evolved by HART and PETERSON (**8**), the VAN BEMMELEN method as modified by the same investigators, and a sodium carbonate fusion method, which was really a combination of KOCH'S (**12**) sodium carbonate method for the determination of total sulphur in organic material and HILLEBRAND'S (**10**) sodium carbonate method for the determination of total sulphur in rocks. The sodium peroxide method gave as high results as the other methods and better duplicates, and also was easier to manipulate. It was therefore adopted, but modified somewhat, and so it is given in some detail.

Ten grams of soil was placed in a 100 cc. nickel crucible, made quite moist with water, and 10 gm. of sodium peroxide added, a little at a time, stirring thoroughly with a nickel rod as the sodium peroxide was being added. This was heated slowly with a microburner until dry, and 10 gm. more of sodium peroxide added, spreading it over the surface, and continuing the heating until the surface layer melted. With a blast burner the mass was then brought to red heat and kept in this condition for ten minutes, stirring thoroughly. This was allowed to stand over a moderate flame for one hour, cooled, and the fused mass removed with boiling water, transferring it to a 600 cc. beaker. This was neutralized with concentrated HCl and then 10 cc. excess added, and allowed to stand on the steam bath for five or six hours, or until there was no undecomposed material in the bottom. It was next transferred to a 500 cc. volumetric flask, cooled, filled to the mark, and allowed to stand for four or five hours, shaking at intervals. A 250 cc. aliquot was filtered off, transferred to a beaker, a quantity of filter paper pulp added, and while stirring the iron, aluminum, etc., was precipitated out with ammonium hydroxide. This was heated for an hour on the steam bath, filtered into an 800 cc. beaker, and the precipitate washed with hot water until 600 cc. was obtained. After this it was heated to boiling, 10 cc. of hot 10 per cent barium chloride added to the boiling solution, and allowed to stand on the steam bath over night and at room temperature for the remainder of the twenty-four hours. The volume must not be allowed to decrease while on the steam bath. The solution was then filtered off from the barium sulphate, washed until no test for chlorides was obtained, the precipitate dried in an oven, and ignited to constant weight in a muffle furnace.

Part of the sulphur determinations reported later in this paper were obtained by igniting over a microburner, taking care that the paper was consumed without flaming up, but it was found that more uniformly successful results were obtained by igniting in a muffle furnace.

The iron and aluminum were removed because it was found that, in the case of a number of the soils analyzed, the barium sulphate precipitate was quite seriously contaminated by the iron. In the case of some of the soils analyzed during the preliminary work, the iron collected in masses on the bottom of the beaker. In other cases there was no contamination, or so little that the error introduced was small. It was decided, however, to make it a general practice to remove the iron and aluminum before adding the barium chloride solution. The chief difficulty encountered in the process was in washing out the sulphate ion from the iron and aluminum hydroxides. By using hot water, however, and having the precipitate well separated with paper pulp, the sulphate ion was completely washed out. It was found that there was some sulphur in the reagents used. Blanks were run and correction made for this.

The phosphorus was determined by the magnesium nitrate method as given in the Methods of Analysis of the Association of Official Agricultural Chemists (17). No important modification was made in the method. The ignition value given is the loss in weight obtained by heating the soil in the muffle furnace at red heat for an hour and a half. Two or three grams were used, and it was found that after heating for this length of time there was no loss of weight on further heating.

Data

In the following results of the analyses, the samples that proved to have the lowest sulphur content will be given first. The chief lack in the data is information as to the previous history of the soils from which the samples were taken. In a number of instances I have data as to the productivity of the soils, and information as to the amount of manure and fertilizer that had been applied to the soils in recent years, but in some cases it was impossible to secure this information. Also, the data would have more general significance, perhaps, if in all cases the names of the soil types could be given, but this was only possible for the Maryland soils. In most instances, however, the samples were taken from important agricultural soils, and therefore the data should have significance in adding to the information as to the sulphur content of the agricultural soils of different sections of the United States. All the results are figured on the basis of the oven-dry weight. The data concerning the pounds of sulphur per acre were obtained from the percentage of sulphur determined by analysis, by assuming that an acre of soil 6.5–7 inches in depth weighs 2,000,000 pounds.

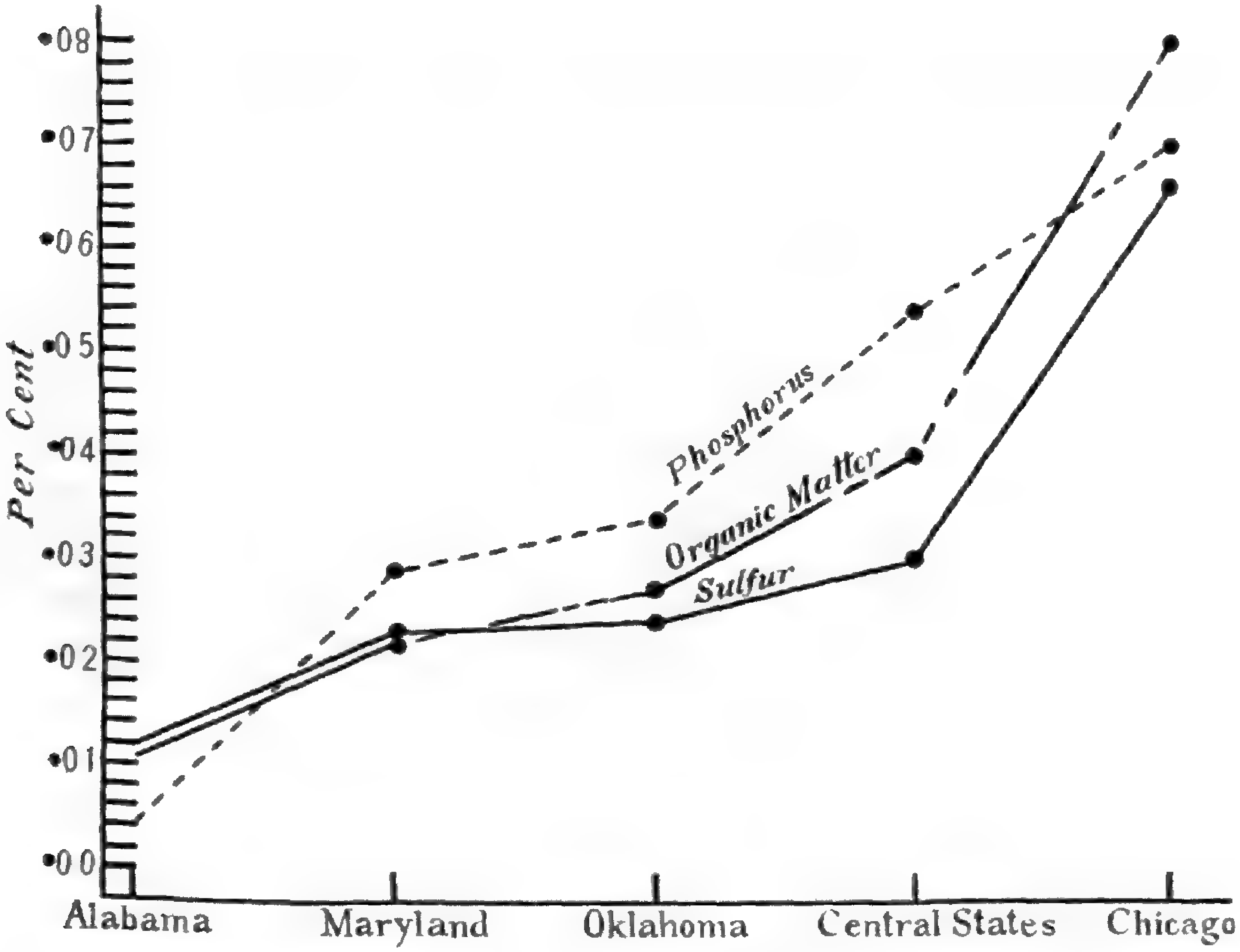

FIG. 1.—Curve comparing sulphur, phosphorus, and organic matter content of five groups of soils; organic matter divided by 200.

Tables I and II give the results of the analysis of some soil samples from the Gulf coast and the Atlantic coast. I am indebted to Dr. A. G. McCall, of the University of Maryland, for the Maryland samples, and also for the information in regard to the productivity of the soils. Little information was obtainable in regard to the samples of table I, except that they came from soils on which the attempt was being made to grow pecans. The soils are so low in sulphur, phosphorus, and organic matter that it would seem impossible to grow any crop successfully on them without considerable fertilizing.

TABLE I

Sulphur, phosphorus, and organic matter content of certain Alabama soils (near Mobile Bay), taken from surface

Sample no.	Percentage sulphur	Lb. per acre	Percentage phosphorus	Lb. per acre	Ignition value
15..........	0.0090	180	0.0028	56	2.189
16..........	0.0148	296	0.0044	88	2.127
17..........	0.0151	302	0.0067	134	2.256
18..........	0.0126	252	0.0044	88	2.204
Average...	0.0128	256	0.0045	90	2.194

TABLE II

Sulphur, phosphorus, and organic matter content of certain Maryland soils

Sample no.	Location (county)	Depth inches	Percentage sulphur	Lb. per acre	Percentage phosphorus	Lb. per acre	Ignition value
23.......	Worcester	0–7	0.028	560	0.015	300	2.48
24.......	Worcester	7–28	0.023	460	....	....	1.58
25.......	Talbot	0–7	0.023	460	0.026	520	4.39
26.......	Talbot	7–28	0.015	300	0.012	240	4.71
27.......	St. Mary's	0–7	0.018	360	0.026	520	4.66
28.......	St. Mary's	7–28	0.020	400	0.018	360	4.54
29.......	Howard	0–7	0.019	380	0.048	960	6.33
30.......	Howard	7–28	0.014	280	0.044	880	6.14
31.......	Prince George	0–7	0.030	600	0.030	600	4.81
32.......	Prince George	7–28	0.019	380	0.026	520	4.91
Average surface soils............			0.023	460	0.029	580	4.53
Average subsoils................			0.018	360	0.025	500	4.37

Samples 23 and 24 of table II belong to the soil type known as Norfolk fine sandy loam. The field from which the samples were taken is fairly productive, being capable of producing ten bushels of wheat or thirty bushels of corn per acre. The samples from Talbot County belong to the Elkton silt loam type of soil. It is not very productive, and has to be fertilized rather heavily to produce very good crops. The soil represented by samples 27 and 28 is known as the Leonardtown silt loam type of soil. It has rather low productivity, producing about 500 pounds of tobacco or seven bushels of wheat per acre. The samples from Howard County belong to the Chester loam soil type, which is one of the best soils in the state, producing sixty-five bushels of corn or twenty bushels of wheat per acre. Samples 31 and 32 belong to the sassafras silt loam type. This is a fairly good soil, producing ten bushels of wheat or thirty-five bushels of corn per acre. The samples from St. Mary's County and from Howard County are from soils that had not been fertilized in recent years. The other soils have probably received recently little if any fertilizers. The Maryland samples are rather few in number, but are well distributed over the state. They are probably typical for the cultivated soils of Maryland. The soils are low in sulphur, phosphorus, and organic matter; somewhat lower in sulphur on the average than in phosphorus. It would seem that they should be benefited by the use of both sulphur and phosphorus as fertilizers.

Table III is an attempt to make a further study of the Maryland soils, using as a basis the productivity data and the data for the surface soils of table II. The second column shows the relative order of the five soils in productivity, beginning with the most productive. There does not seem to be any relation between the sulphur content and the productivity. When we consider the phosphorus content, however, the two best soils as to productivity are also highest in phosphorus. This relation between phosphorus content and productivity does not hold in the case of the other three soils, but here the phosphorus content is so low that other factors may be limiting production. It would seem possible, therefore, especially in the case of the fields from which the Chester loam and sassafras silt loam samples came, that phosphorus rather than sulphur was limiting production. The Chester loam soil especially should be considered. It is one of the best soils of the state and in its phosphorus content also it is decidedly higher than any other of the soils analyzed. The order of the soils as to the organic matter content is about the same as their order considered on the basis of the phosphorus content. It might be that the relatively large amount of organic matter in the Chester loam and the sassafras silt loam soils is a factor in their productivity. Organic matter improves the tilth of soils, adds plant food, and has other important effects.

Their relative ability to produce cereal crops is used as a measure of the productivity of the soils. Cereal crops require more phosphorus than sulphur. It might well be that, if the production of a high sulphur containing crop (alfalfa, for instance) was taken as the criterion, the order of the soils in table III would be different. Even if phosphorus, rather than sulphur, is at present the limiting factor in these soils, sulphur would no doubt soon become the limiting factor if the level of supply of the phosphorus is raised by adding phosphorus fertilizers. The sulphur supply is so low that, with phosphorus removed as the limiting factor, it might become the limiting factor to production.

TABLE III

RELATIVE ORDER OF THE FIVE MARYLAND SOILS IN PRODUCTIVITY, SULPHUR, PHOSPHORUS, AND IGNITION VALUE

Soil type	Productivity	Sulphur	Phosphorus	Ignition value
Chester loam	1	4	1	1
Sassafras silt loam	2	1	2	2
Norfolk fine sandy loam	3	2	5	5
Leonardtown silt loam	4	5	3	3
Elkton silt loam	5	3	3	4

Of course it is realized that too great reliance should not be placed in a soil analysis, especially such a soil analysis as this, where only two of the several elements needed by plants are determined. At the most, a soil analysis only shows the total amount of plant food present and does not tell anything as to the availability of the elements. Also, other factors than plant food may be limiting production, but a soil analysis should develop some leads, which can be followed up by other methods of attack.

Since the data show a rather low sulphur content in the few Atlantic coast and Gulf coast soils analyzed, it might be of interest to see what results have been obtained from using sulphur as a fertilizer in these regions. Very little work of this kind has been done. Several stations report a favorable effect from using phosphorus or potassium as a fertilizer for alfalfa, when the carrier of the phosphorus or potassium also contained sulphur. The Delaware station (**7**), for example, reports greatly increased yields of alfalfa due to acid phosphate. Experiments in Oregon (**21**) have shown a decided increase from applying acid phosphate to soils, but no increase due to phosphorus in any other form. Here it has been definitely proved that the increased yield caused by acid phosphate was due to the sulphur of the acid phosphate, and not to the phosphorus. It would seem worth while to test this in the case of the Delaware soils. The Virginia station (**3**) secures increased yields of alfalfa due to phosphorus in the form of acid phosphate and basic slag, but not in the case of other forms of phosphorus, such as rock phosphate. Here again we have the possibility that sulphur is responsible for the increased yields. The Massachusetts station (**16**) finds sulphate of potash a better fertilizer for alfalfa than muriate of potash. The alfalfa of the sulphate of potash plats was also a darker green. Clearly these results are due to the sulphur present in the sulphate of potash, and not to any differences in the potassium.

The best experiments on the Atlantic coast to show the effect of sulphur fertilization on crops are those of the investigators of the colonial period, whose work is summarized by CROCKER (**4**). PETERS and BINNS were the most prominent of these investigators. They performed numerous experiments showing the effect of gypsum on crop yield. Leguminous crops especially were benefited, red clover giving increased yields of two to threefold. BINNS reported like increased yields for corn and wheat. Although the reports of the experiments do not make this clear, it seems likely that the beneficial effects of gypsum on the non-leguminous crops was due to the increased nitrogen supply brought about by the greater growth of the legumes of the rotation. RUFFIN was also greatly impressed by the results obtained from the use of gypsum as a fertilizer. In his *Essay on calcareous manures* he speaks of the magic effects obtained from applying gypsum as a fertilizer to clover. Considering the results of the writer's analyses, together with the other experimental work to which reference has been made, it would seem worth while to test sulphur as a fertilizer throughout the Atlantic coast region.

The samples of table IV were taken in cultivated fields near Miami, Oklahoma, two of the samples from one field and two from another. Nothing is known as to the previous treatment of the soils from which the samples came, or it might be possible to answer some questions which arise from a study of the data, such as the reason for the much greater phosphorus content of samples 21 and 22 than of samples 19 and 20.

TABLE IV

SULPHUR, PHOSPHORUS, AND ORGANIC MATTER CONTENT OF CERTAIN SOILS NEAR MIAMI, OKLAHOMA

Sample	Depth	Percentage sulphur	Lb. per acre	Percentage phosphorus	Lb. per acre	Ignition value
19........	Surface	0.0202	404	0.0107	214	4.346
20*.......	Subsoil	0.0287	574	0.0087	174	7.764
21........	Surface	0.0278	556	0.0587	1174	6.710
22*.......	Subsoil	0.0136	272	0.0543	1086	5.290
Average surface soil...		0.0240	480	0.0347	694	5.528
Average subsoils......		0.0211	422	0.0315	630	6.527

* It is not certain that subsoils 20 and 22 go with soils 19 and 21 respectively; they were arranged with these soils on the basis of color.

Table V contains the results of the analyses of certain soils of the central states. Samples 3 and 4 were taken in an alfalfa field; samples 5 and 6 in an oat field. The alfalfa field had been manured with one and one-half tons of cow manure per acre in 1918. The oat field had received in the same year an application of two tons per acre of cow manure. Both fields had been fertilized with gypsum in 1920. Samples 7, 8, 9, and 10 were all taken in one field of seven acres, which had been in grass for many years. This field was put in corn in 1920, producing only a fair crop. In the fall of 1920 it was put in alfalfa. Sample 33 is a composite sample taken in a clover field near Paris, Illinois. Trouble was being experienced in growing clover on part of the field. It was thought that this might be due to the low sulphur content of this part of the field, but soil analysis indicated that such was not the case. In fact, one of the samples from soil supporting a good stand of clover contained decidedly less sulphur than did any of the samples from the part of the field where there was no clover. It would seem that some other factor than the sulphur content was preventing the growth of clover. Since the results of analysis revealed no reason for the failure to secure a good stand of clover on part of the field, the data for all the samples were averaged in order to secure an average value for the entire field.

TABLE V

SULPHUR, PHOSPHORUS, AND ORGANIC MATTER CONTENT OF CERTAIN SOILS OF CENTRAL STATES

Sample no.	Location	Depth	Percentage sulphur	Lb. per acre	Percentage phosphorus	Lb. per acre	Ignition value
1....	Fremont, Ohio	0–7	0.029	580	0.056	1120	6.87
2....	Fremont, Ohio	7–20	0.015	300	0.048	960	6.72
3....	Plattesville, Wis.	0–7	0.028	560	0.034	680	4.47
4....	Plattesville, Wis.	7–20	0.038	760	0.040	800	5.62
5....	Plattesville, Wis.	0–7	0.034	680	0.040	800	5.26
6....	Plattesville, Wis.	7–20	0.019	380	0.036	720	6.74
7....	Naperville, Ill.	0–7	0.021	420	0.060	1200	10.17
8....	Naperville, Ill.	7–20	0.030	600	0.040	820	8.40
9....	Naperville, Ill.	0–7	0.040	800	0.051	1002	9.32
10....	Naperville, Ill.	7–20	0.020	400	0.052	1004	9.71
11....	Gilman, Ill.	0–7	0.058	1160	0.086	1720	13.53
12....	Gilman, Ill.	7–20	0.035	700	0.120	2400	10.76
13....	Gilman, Ill.	0–7	0.029	580	0.045	900	6.71
14....	Gilman, Ill.	7–20	0.036	720	0.057	1140	8.43
33....	Paris, Ill.	0–7	0.030	600	0.056	1120	7.40
Average surface soils............			0.030	600	0.054	1080	8.00
Average subsoils................			0.027	540	0.056	1120	8.05

Particular attention is called to the samples from Gilman, Illinois. These were received from Mr. F. I. MANN, who also supplied the information in regard to the previous treatment of the land from which the samples came. They were all taken in the same field. Samples 11 and 12 came from a part of the field that during sixteen years had received applications of rock phosphate and ground limestone. No other fertilizer had been applied to the land for at least twenty years, and not much before that. Samples 13 and 14 came from the check part of the field, which had received no fertilizer of any kind. Clover had been grown on the field once in four years, about half of the crop being plowed under. Clover was also grown on the check part of the field. Very little grew here, however, and so there was not much to plow under. Mr. MANN stated that the amount of phosphorus applied to the land where samples 11 and 12 were taken would just about equal that naturally present in the soil, so that these samples would be expected to contain about twice the phosphorus of the samples from the check portion. Table V shows this to be the case, but the sulphur content of the surface soil of the fertilized land is also double that of the check portion. This is rather to be expected, when we compare the two in their organic matter content. Sample 11 is about double that of sample 13, and a high organic matter content usually means a high sulphur content. The question, however, is as to the source of supply of the sulphur. No sulphur fertilizers have been applied to the land. There is the possibility that, since the clover plant makes considerable growth during the time of the heavy rains of the spring and again in the fall after the rains start (times when the sulphur content of rainwater is rather high), some of the sulphur might come from this source. It is not believed, however, that the sulphur brought to the land by the rain results in a net increase in the sulphur content of the soil, on account of the large amount of sulphur lost in drainage, although the amount lost in drainage is greatly decreased when the land is covered by a crop. There is the additional possibility that the clover roots bring up sulphur from the subsoil, depositing it in the surface layers. As shown by the data, the subsoil of the fertilized part of the field has about the same sulphur content as the soil and subsoil of the check portion. Some of the other soil analyses have shown that the sulphur content of various parts of the same field may vary widely, when all parts of the field have been treated alike so far as sulphur fertilization is concerned. If this is true in the present case, then the difference in the sulphur content of the two parts of the field would not be significant, but the high organic matter content of the fertilized part of the field would seem to indicate that these samples are representative, and that there really is here a high sulphur content.

Considering all the samples of table V, it may be said in summary that the sulphur content on the average is not high in amount, although somewhat greater than the Maryland and Oklahoma soils, and decidedly greater than the Alabama soils. The phosphorus content is also rather low, although much higher than the sulphur content. There is a fair amount of organic matter present on the average in the soils. Reference was made previously to certain soil analyses in Kentucky, Iowa, Kansas, Wisconsin, and Ohio. Judging from my analyses and those referred to in the introduction, many soils in the middle states need sulphur. Some of them are well supplied, however, and on the average they seem to have a higher sulphur content than the soils of either the Atlantic or Pacific coasts, although not enough analyses or fertility experiments have been made to make a positive statement as to this. On the other hand, some of the soils are as low in their sulphur content as any of the coast soils, so that it would not be surprising if sulphur should prove beneficial on these soils. Demonstration experiments on as many of the central states soils as possible are needed to determine how generally sulphur is deficient.

Not many experiments of this kind have been performed. Certain investigators in Kentucky (**25**), Wisconsin (**9, 28**), and other states, in pot experiments, have secured increased yields from sulphur fertilization in the case of alfalfa, clover, radishes, rape, turnips, mustard, tobacco, and soy beans. In field experiments, JARDINE and CALL (**11**) attribute the increased yields in Kansas secured by fertilizing alfalfa with acid phosphate to the phosphorus of the acid phosphate, but here again there is the possibility that the sulphur contained in the acid phosphate is at least partly responsible for the increased yields. During the last few years the Gypsum Industries Association has conducted a number of experiments, seeking to determine the value of gypsum as a fertilizer for crops. Beneficial effects have already been secured in a number of cases. Such work should be extended.

Table VI records the results of the analysis of a few samples taken within the environs of Chicago. Each sample includes a number of borings and is therefore composite. The sample from the South Chicago region was taken from what seemed to be a natural prairie. This soil had probably never been fertilized. The Midway, where samples 39 and 40 were taken, is quite often manured. It was learned after analyses were made that samples 41 and 42 were taken from a part of the botany gardens that had been filled in. The subsoil especially of this sample is not typical, its higher sulphur content than the subsoils of the other soils probably being accounted for by the filling in. The few Chicago soils analyzed are all much better supplied with sulphur, phosphorus, and organic matter than any of the other soils analyzed. It may be that soils of as high a sulphur content as these Chicago soils might not need any sulphur fertilization, although in the case of certain high sulphur-using crops the available sulphur might not be sufficient. All the samples were taken from soils overlaid with sod, and have a high organic matter content. There are probably many soils in Chicago of much lower sulphur and organic matter content, which might need sulphur fertilizers.

TABLE VI

SULPHUR, PHOSPHORUS, AND ORGANIC MATTER CONTENT OF CERTAIN CHICAGO SOILS

Sample no.	Location	Depth	Percentage sulphur	Lb. per acre	Percentage phosphorus	Lb. per acre	Ignition value
39....	Midway	0–8	0.060	1200	0.100	2000	15.25
40....	Midway	8–26	0.021	420	0.055	1110	10.24
41....	Botany Gardens	0–8	0.055	1110	0.073	1460	15.79
42....	Botany Gardens	8–26	0.045	900	0.068	1360	13.08
43....	South Chicago	0–8	0.069	1380	0.038	760	17.11
44....	South Chicago	8–26	0.023	460	....	....	15.12
Average surface soils............			0.061	1220	0.070	1400	16.05
Average subsoils................			0.029	580	0.060	1200	12.81

It was thought interesting to determine how much of the total sulphur of the Chicago soils might be accounted for by the sulphate sulphur content. As is well known, where much soft coal is burned, much sulphur is given off. It would be expected, therefore, that rain would carry to the soil much more sulphur from a smoky atmosphere than from one free from smoke. WARRINGTON (quoted by HART and PETERSON (**8**)) gives the amount of sulphur carried to an acre surface of soil at Rothamsted as about seven pounds per year. Judging from their limited data, HART and PETERSON (**8**) estimate about the same figures are correct for University Hill Farm, Madison, Wisconsin. Data indicate a much higher sulphur content of the rainwater of cities. Some of my determinations show several times as much sulphur in Chicago rainwater as in rainwater collected in the country some distance from Chicago. Most of the sulphur in rainwater is in the sulphate form. It might be expected, therefore, that the sulphate sulphur present in the Chicago soils might account for much of the total sulphur. Roughly quantitative determinations showed an average sulphate sulphur content of the three surface soils of table VI of 158 pounds per two million pounds of soil. This is high, compared with the Iowa soils as analyzed by BROWN and KELLOGG (**2**). They found an average sulphate sulphur content of 59 pounds per two million pounds of soil, but the sulphate sulphur present in the Chicago soils accounts for comparatively little of the total sulphur. Most of this is in the organic form, and the high sulphur content of the soils is due mainly to the high organic matter content. That the sulphate sulphur content is not higher may be accounted for probably by the ease with which sulphur in a soluble form is leached from the soil.

LYON and BIZZELL (**14**), MACINTIRE and co-workers (**15**), and other investigators have performed lysimeter experiments. LYON and BIZZELL show that 3–6 times as much sulphur is lost in drainage as is used by the crop, and when put in a soluble sulphate added to the tanks, over one-half of the amount added in any one year was removed in drainage the same year.

Table VII summarizes the data of tables I, II, IV, V, and VI. Fig. 1 compares in a graphical way the sulphur, phosphorus, and organic matter content of the five groups of soil. The Alabama, Maryland, and Oklahoma soils are all low in sulphur, phosphorus, and organic matter, the Alabama soils being especially deficient in all three substances. The phosphorus, on the average, is not much greater in amount than the sulphur. Although the central states soils are better supplied with sulphur and phosphorus than these three groups of soils, they would not be considered high in either. The range in the amount of sulphur and phosphorus present in the various soils is rather great. Certain of the soils would be considered fairly well supplied with both sulphur and phosphorus. The organic matter content of the soils is on the average fairly good, although here also the range is very great, and certain of the soils are deficient in this respect.

The sulphur and phosphorus content of the Chicago soils is fairly good, while the organic matter content is high. As is brought out in connection with table VI, the samples are not typical for the cultivated soils of Chicago and its environs. They were taken in places where the organic matter had had a chance to accumulate. Their high sulphur content is to be accounted for mainly by their high organic matter content, the sulphur brought down by the rain accounting for little of the total sulphur. Although the Chicago samples should not be considered typical for cultivated soils, they are perhaps typical of soils of any section of the United States which have been in grass or any form of plant life for a number of years, or have been heavily manured. Such soils would be expected to be well supplied with organic matter, and to have a correspondingly high sulphur content. If conditions are right for active sulphofication, there should be an abundance of available sulphur.

TABLE VII

SUMMARY OF TABLES I, II, IV, V, AND VI, GIVING AVERAGE OF SURFACE SOILS; SULPHUR AND PHOSPHORUS IN POUNDS PER ACRE, IGNITION VALUE IN PERCENTAGE

	Alabama	Maryland	Oklahoma	Central states	Chicago
Sulphur	256	460	480	600	1220
Phosphorus	90	580	694	1080	1400
Ignition value	2.194	4.53	5.528	8.00	16.05
Ratio ignition value to sulphur	182.5	196.9	230.0	263.3	263.1

A further study has been made of this relation between the organic matter and total sulphur of the different groups of soils by determining the ratio of the organic matter to the total sulphur. As shown by table VII, this ratio is far from a constant. A 100 per cent increase in the organic matter content does not mean a corresponding 100 per cent increase in the sulphur content. A comparison of the ratios for the different groups of soils, and of the figures for the sulphur content and the organic matter content seems to justify the statement, however, that there is a general correlation between the two, that a soil with a large amount of organic matter also contains a large amount of sulphur. That the correlation is not closer may be accounted for, at least in part, by the fact that plants differ greatly in their sulphur content. The source of the organic matter present in the soil has a great deal to do with the amount of sulphur the soil contains. This fact may account, at least partly, for the cases (shown by the tables giving the detailed data of the soil analyses) in which there does not seem to be any correlation at all between the organic matter and sulphur content. A high organic matter content may be correlated with a low sulphur content, but these cases should be considered exceptions to the general rule that a soil containing a large amount of organic matter also contains a large amount of sulphur, a rule which is seen more clearly when the sulphur and organic matter content of a number of soils are averaged. In general, the sulphur content of soils is greater than that of the corresponding subsoils.

Table VIII gives the number of crops that could be grown from the amount of sulphur present in the various groups of soils as summarized in table VII. BROWN and KELLOGG'S (2) figures for the amount of sulphur removed by maximum yields of these crops have been used. They assume that the entire crop is removed from the soil. In the Maryland and central states soils, which include the most important agricultural soils, the number of crops supply of sulphur in the poorest soil and in the best soil is given in the column "Range." Table VIII shows that there is enough sulphur present in most of the soils for comparatively few maximum crops of such high sulphur-containing plants as alfalfa and potatoes. The other crops contain less sulphur, and therefore a greater number of maximum crops of these could be grown.

Most of the sulphur of the different soils is in the organic form and unavailable for the plant, and it is not known how rapidly sulphofication is making it available. When the sulphur content of a soil is as low as it is in the Alabama, Maryland, Oklahoma, and several of the central states soils, however, sulphofication may not produce enough available sulphur to secure maximum yields of most crops. Considered from this standpoint, table VIII may not be very significant, except as another way of comparing the sulphur content of the different groups of soils.

The sulphur content of maximum yields of the six crops given in table VIII, according to the figure of BROWN and KELLOGG, totals 134.3 pounds; the total phosphorus content 128 pounds. These are five of the most common crops, especially in the central states. Judging from the soil analyses that have been made by various investigators, the agricultural soils of the United States are even more deficient in sulphur than in phosphorus. Although considerable sulphur is added to the soil of rainwater, a larger amount seems to be lost in drainage, some investigators stating that three times as much sulphur is lost from the soil in drainage as is added to the soil by the rain. It would seem possible, therefore, that further investigation would prove that sulphur is as generally needed as a fertilizer as is phosphorus.

TABLE VIII

NUMBER OF MAXIMUM CROPS THAT MAY BE GROWN FROM AMOUNT OF SULPHUR PRESENT IN THE FIVE SOIL GROUPS AS GIVEN IN TABLE VII

	Alabama	Maryland	Range	Oklahoma	Central states	Range	Chicago
Corn	16	28	22–37	30	37	26–72	76
Wheat	25	45	35–58	47	58	41–113	119
Oats	15	27	21–36	29	36	25–70	73
Potatoes	7	14	11–17	14	18	12–35	37
Clover	19	35	27–45	36	45	32–88	93
Alfalfa	5	10	7–13	10	13	9–25	26

EFFECT OF SULPHUR ON CHLOROPHYLL DEVELOPMENT, AND GROWTH OF RED CLOVER AND SWEET CORN

Several investigators have reported a better color in plants due to sulphur fertilization. REIMER and TARTAR (**21**), as already stated, secured greatly increased yields of alfalfa from sulphur fertilizers. They emphasize the poor color of the alfalfa on the plats not fertilized with sulphur. OLSON (**19**) speaks of the same thing in connection with experiments in Washington. The Massachusetts station (**16**) reports like results, but the beneficial effect on the color does not seem to be confined to the legumes. DULEY (**6**) reports the same thing in the case of sweet corn, and DEMOLON

(5) observed a darker green in the foliage of rutabagas, parsnips, and beets fertilized with sulphur than in the check experiments. An experiment was planned to try to determine the relation between the sulphur and the chlorophyll content of the plants. STOWELL'S evergreen sweet corn and mammoth red clover were grown in ordinary 12-inch flower pots. Thirty-six pounds of sand were added to each pot. For series 1, 2, and 3 pure quartz sand was used; for series 4, 5, 6, 7, and 8 a fine grade of torpedo sand sifted free from stones and coarse material was used. Sulphur was added to the sand, as shown in table IX. The figures in the table mean that sodium sulphate and flowers of sulphur were added in such amounts as to give the same amount of sulphur as contained in 100, 300, and 500 pounds of gypsum per acre, or two million pounds of sand. Each series was run in triplicate.

TABLE IX

AMOUNT OF SULPHUR ADDED TO SAND CULTURES OF CLOVER AND SWEET CORN

Series	Flowers of sulphur (lbs.)	Sodium sulphate (lbs.)	Gypsum (lbs.)
1	....	....	....
2	....	100	....
3	100	....	....
4	....	300	....
5	300	....	....
6	....	500	....
7	500	....	....
8	....	....	500

The gypsum and flowers of sulphur were thoroughly mixed with the sand at the time the pots were filled. The sodium sulphate was added in solution in three applications. The corn was harvested sooner than had been planned, and received only two applications of sodium sulphate. The sodium sulphate series in the case of the corn, therefore, received two-thirds of the amount given in the table. In addition lime was added at the rate of 1000 pounds per two million pounds of sand. One week after the cultures were started 1 gm. of ferric chloride was added to the sand of each pot. The corn was planted February 7. It had previously been placed between moist filter paper, and at the time of planting all the seeds were fully imbibed and most of them had sprouted. The clover was sown January 22, and on February 6, when the plants were 4 inches high, they were transplanted to the sand. A pure culture inoculum obtained from the Department of Bacteriology of the University of Wisconsin was added to the sand containing the clover on February 13.

The nutrient solution used was the same as that used by Kraus and Kraybill (**13**), except that magnesium chloride was substituted for magnesium sulphate. Perhaps a solution better suited to corn and clover might have been found, but it gave good growth in both cases. It was made up as follows:

Solution *A*	Per cent	Solution *B*	Per cent
Magnesium chloride	2	Calcium nitrate	8
Dibasic potassium chloride	2		
Potassium nitrate	2		

Equal parts of *A* and *B* were diluted 1 to 70 with water and then mixed. The solution was applied in this strength to the corn. The solution applied to the clover was just half this strength. Five hundred cc. of these solutions were added on an average of once a week to the corn and the clover. While the plants were small and the light poor, not so much was applied, but later the supply was increased. Both the corn and clover grew well, but no marked differences in color or size of plants developed in either In fact, in the case of the corn, that in the control series was as green as the corn of any of the other series. It was not deemed the right kind of material for studying the effect of sulphur on chlorophyll development, and no chemical analyses were made.

Since no marked differences in color or growth due to sulphur deficiency had developed in the clover of the different series, it was decided to modify the experiment somewhat. It was thought the nitrate supply might be too high. On April 27 each series was divided into two parts. The nitrates were kept up in one-half of the pots and discontinued entirely in the other half, but no marked differences in color had developed at the time the experiment was stopped, on May 27. At this time the clover of the control series was somewhat paler than the clover of the high sulphur pots, but it was not very marked. Also, no definite gradation in color from the control series to the high sulphur series was discernible. The microchemical analyses were made on the clover of the control series and the clover of the high sulphur series, in the latter case using mainly plants from series 8, the gypsum series.

The most noticeable point in table X is that the nitrates, protein, and sulphates are greater in amount in the plants of the high gypsum series. There was not much difference in the carbohydrate situation in the two series. Leaves from plants of the two series had about the same amount of reducing sugars and starch. The petioles of the control plants contained more reducing sugar than in the case of plants from the high gypsum series, but the starch content of the petioles was about the same in both. In the roots, the reducing sugar was about the same in amount in both series, while the starch was greater in amount in the gypsum series. These differences, while clearly evident, were not great enough to permit any definite conclusions as to the relation of sulphur to chlorophyll development in the clover plant.

TABLE X

MICHROCHEMICAL ANALYSES OF CLOVER PLANTS OF CONTROL SERIES AND OF HIGH SULPHUR SERIES; NITRATES DISCONTINUED MAY 27

	$CaSO_4$ HIGH			$CaSO_4$ NONE		
	Leaves	Petioles	Roots	Leaves	Petioles	Roots
Nitrates.....	+	++	++	+	+	+
Protein......	+	++	++	+	+	+
Sulphates....	++	++	++	+	+	+
Sugar.......	+	+	++	+	++	++
Starch......	+	++	++	+	++	+

Reimer and **Tartar** (**21**), **Miller** (**18**), **Duley** (**6**), and **Hart** and **Tottingham** (**9**) have shown that root development and nodule formation are increased in clover and alfalfa by the use of sulphur fertilizers. **Pitz** (**20**) has shown that sulphur causes an increase in the nodule-forming bacteria of 2–3 fold. **Reimer** and **Tartar** have also demonstrated that sulphur increases the nitrogen content of alfalfa 2–3 per cent. **Schertz** (**26**) has shown a close connection between the nitrogen and chlorophyll content of *Coleus* leaves, so that it is possible that sulphur has at least part of its effect through increasing the nitrogen content of the plant. My work seems to indicate this, but more work is desirable before coming to any definite conclusions. In the case of the non-legumes, the activity of ammonifying and nitrifying bacteria of the soil might be increased. The evidence is conflicting as to the effect of sulphur on these organisms, some claiming a favorable effect and some little effect. It is hoped to repeat this experiment, both in the case of corn and clover, omitting the nitrates entirely or keeping them very low. This should be done from the start; then, if the sulphur does have its effect indirectly by increasing the nitrogen supply through an increase in the number and activity of these organisms, this effect should be apparent. Special precautions should be taken to exclude sulphur. Sulphur-free salts, of course, should be used. The sand should be thoroughly washed with distilled water, perhaps even boiled in acid and then washed with distilled water, to eliminate any sulphur that it may contain. If decided differences in color develop, as a result of sulphur deficiency, the microchemical analyses should be followed by quantitative chemical analyses.

Table XI gives data concerning the effect of sulphur in different forms and different amounts on the growth of sweet corn. As before stated, the corn was grown with the idea of obtaining material to study the effect of sulphur on chlorophyll development in non-legumes. Since no difference in color in the different series developed, the corn was harvested and the dry weight determined, to see the effect of sulphur on the growth. It had been growing about two and one-half months and was in tassel. The details of the plan of the experiment have already been given. The numbers in the column "Treatment" indicate that flowers of sulphur and sodium sulphate were added in such amounts as to contain the same amount of sulphur as present in 100, 300, or 500 pounds of gypsum per acre, or two million pounds of sand. The percentage increase or decrease is based upon the dry weight. The minus sign indicates a decrease.

Series 4 should not be considered, for from the first the corn in two of the pots of this series did not grow well. At the time of harvest most of this corn was short and spindling. This is believed to be due not to the sulphur treatment but to poor seed. Leaving this series out of consideration, we see that flowers of sulphur and sodium sulphate containing the same amount of sulphur as 100 pounds of gypsum, and gypsum at the rate of 500 pounds per acre gave marked increased dry weights over that of the control. The flowers of sulphur caused the greatest increase, 66.16 per cent, and gypsum was next with 41.6 per cent, sodium sulphate causing 35.3 per cent increased dry weight. It is hard to say why flowers of sulphur and sodium sulphate in the larger amounts did not bring about increased growth. The dry weight of these series is about the same as the check.

TABLE XI

EFFECT OF DIFFERENT SULPHUR TREATMENTS ON GROWTH OF STOWELL'S EVERGREEN SWEET CORN IN GREENHOUSE

Series	Treatment	Moisture	Green weight (gm.)	Dry weight (gm.)	Percentage increase or decrease
1........	Control	87.62	266.7	33.1	....
2........	Na_2SO_4 at rate of 100 lb. gypsum per acre	86.60	334.0	44.8	35.34
3........	Flowers of sulphur at same rate as 2	85.12	369.6	55.0	66.16
4........	Na_2SO_4 at rate of 300 lb. gypsum per acre	85.00	164.3	24.7	−25.37
5........	Flowers of sulphur at same rate as 4	84.95	217.8	32.8	− 0.90
6........	Na_2SO_4 at rate of 500 lb. gypsum per acre	85.44	222.5	32.4	− 2.11
7........	Flowers of sulphur at same rate as 6	85.93	237.2	33.4	0.90
8........	Gypsum at rate of 500 lb. per acre..............	82.76	271.7	46.9	41.69

There has been work indicating injury to plants by the acid resulting from the oxidation of flowers of sulphur. Also some have claimed injury from the alkalinity developed in the soil by sodium salts. While this is a possibility, it is not emphasized. The acidity should have been taken care of by the calcium carbonate added to the sand. The literature shows that sulphur fertilization of cereals has not given consistent results, and as a rule not a very marked increased growth has been caused, so perhaps no particular significance should be attached to the fact that series 5, 6, and 7 show about the same dry weights as the check. The corn of all the series where sulphur fertilizers were used had a lower moisture content than the corn of the control series, the corn fertilized with gypsum having about 5 per cent less moisture than the control.

Summary

1. The Alabama, Maryland, and Oklahoma soils analyzed are low in sulphur, phosphorus, and organic matter; the phosphorus being not much greater in amount than the sulphur. The central states soils are better supplied, on the average, in all three respects, and decidedly better supplied with phosphorus than with sulphur. Some of these soils might be considered to have a fair amount of sulphur, phosphorus, and organic matter, while others are deficient in these respects. The Chicago soils have a fairly good content of phosphorus, and a rather high content of sulphur and organic matter. Although the sulphate sulphur content of the Chicago soils is high, this accounts for little of the total sulphur, most of it being due to the large amount of organic matter present.

2. Most of the sulphur of soils is in organic form. There is a general correlation between the sulphur and organic matter content, soils of a high organic matter content having in general a high sulphur content. The surface soils are in general higher in sulphur than the subsoils.

3. Judging from the results obtained and the work of other investigators, sulphur fertilization should prove quite generally beneficial on the Atlantic coast and the Gulf coast. The same thing may be true of the Pacific coast. Sulphur fertilizers are probably not as generally needed in the central states, many soils no doubt needing them, and many others not. Soil of a high organic matter content, such as the Chicago soils, may not need sulphur fertilizers except for high sulphur-using crops. In case the sulphate sulphur is as great in amount as it is in the Chicago soils, sulphur fertilizers might not be needed, even if the organic matter content is low. Attempts are made, in the case of several soils, to correlate the sulphur, phosphorus, and organic matter content with the production of the soils, previous treatment, or other factors.

4. No definite conclusions can be drawn from the data as to the relation of sulphur to chlorophyll development in plants. This may come about through the effect of the sulphur in increasing the nitrogen content of the plants.

5. Flowers of sulphur and sodium sulphate, containing the same amount of sulphur as 100 pounds of gypsum per acre, and gypsum at rate of 500 pounds per acre, caused increased dry weights of sweet corn of 35–66 per cent. Larger amounts of flowers of sulphur and sodium sulphate gave no increases. The corn fertilized with sulphur had a higher moisture content than the controls. In the case of the gypsum series this amounted to 5 per cent.

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LITERATURE CITED

1. Ames, J. W., and Boltz, G. E., Sulphur in relation to soils and crops. Ohio Agric. Exp. Sta. Bull. 292. 221–256. 1916.
2. Brown, P. E., and Kellogg, E. H., Sulphur and permanent soil fertility in Iowa. Jour. Amer. Soc. Agronomy **7**:97–108. 1915.
3. Carrier, Lyman, and co-workers, Alfalfa experiments. Va. Agric. Exp. Sta. Bull. 207. 1–20. 1914.
4. Crocker, William, History of the use of gypsum as a fertilizer (unpublished).
5. Demolon, M. A., Sur l'action fertilisante du soufre. Compt. Rend. Acad. Sci. **154**:524–526. 1912.
6. Duley, F. L., Relation of sulphur to soil productivity. Jour. Amer. Soc. Agronomy **8**:154–160. 1916.
7. Grantham, A. E., Alfalfa in Delaware. Del. Agric. Exp. Sta. Bull. 110. 1–42. 1915.
8. Hart, E. B., and Peterson, W. H., Sulphur requirements of farm crops, in relation to the soil and air supply. Wis. Agric. Exp. Sta. Research Bull. 14. 1–21. 1911.
9. Hart, E. B., and Tottingham, W. E., Relation of sulphur compounds to plant nutrition. Jour. Agric. Res. **5**:233–250. 1915.

10. HILLEBRAND, W. F., The analysis of silicate and carbonate rocks. U.S. Geol. Survey Bull. 422. 1–239. 1910.
11. JARDINE, W. M., and CALL, L. E., Alfalfa in Kansas. Kan. Agric. Exp. Sta. Bull. 197. 575–610. 1914.
12. KOCH, F. C., Lecture and laboratory notes in tissue analysis. Course 37 in Department of Physiological Chemistry at the University of Chicago.
13. KRAUS, E. J., and KRAYBILL, H. R., Vegetation and reproduction with special reference to the tomato. Oregon Agric. Exp. Sta. Bull. 149. 1–90. 1918.
14. LYON, T. L., and BIZZELL, J. A., Lysimeter experiments. Cornell Agric. Exp. Sta. Memoir 12. 1–115. 1918.
15. MACINTIRE, W. H., WILLIS, L. G., and HOLDING, W. S., The divergent effects of lime and magnesia upon the conservation of soil sulphur. Soil Science **4**:231–235. 1917.
16. Mass. Agric. Exp. Sta. Rep. 1914 (pp. 157, 158).
17. Methods of Analysis of the Association of Official Agricultural Chemists. Washington. 1920.
18. MILLER, H. G., Relation of sulphates to plant growth and composition. Jour. Agric. Res. **17**:87–102. 1919.
19. OLSON, G. A., and ST. JOHN, J. L., An investigation of sulphur as a plant food. Wash. Agric. Exp. Sta. Bull. 165. 1–69. *figs. 11.* 1921.
20. PITZ, W. J., Effects of elemental sulphur and of calcium sulphate on plant life. Jour. Agric. Res. **5**:771–780. 1916.
21. REIMER, F. C., and TARTAR, H. V., Sulphur as a fertilizer for alfalfa in southern Oregon. Oregon Agric. Exp. Sta. Bull. 163. 1–40. 1919.
22. ROBINSON, W. O., The inorganic composition of some important American soils. U.S. Dept. Agric. Bull. 122. 1–27. 1914.
23. ROBINSON, W. O., STEINKOENIG, G. A., and FRY, W. H., Variation in the chemical composition of soils. U.S. Dept. Agric. Bull. 551. 1–16. 1917.
24. SHEDD, O. M., The sulphur content of some typical Kentucky soils. Ky. Agric. Exp. Sta. Bull. 174. 213–250. 1913.
25. ———, The relation of sulphur to soil fertility. Ky. Agric. Exp. Sta. Bull. 188. 595–630. 1914.
26. SCHERTZ, F. M., A chemical and physiological study of mottling of leaves. BOT. GAZ. **71**:81–131. 1921.
27. SWANSON, C. O., and MILLER, R. W., The sulphur content of some typical Kansas soils and the loss of sulphur due to cultivation. Soil Science **3**:139–148. 1917.
28. TOTTINGHAM, W. E., Effect of level of sulphur supply on plant growth. Wis. Agric. Exp. Sta. Bull. 228. 26. 1912.

EFFECT OF AUTOLIZED YEAST AND PEPTONE ON GROWTH OF EXCISED CORN ROOT TIPS IN THE DARK[1]

WILLIAM J. ROBBINS

(WITH EIGHT FIGURES)

In a previous paper[2] the writer described a simple method by which the excised root tips and stem tips of higher plants can be cultivated under sterile conditions, and some experiments in which this method was used. These experiments showed that the excised root tips of corn would make considerable growth in the dark in a sterile nutrient solution containing mineral salts and glucose, and but little when no carbohydrate was present. It was found, however, that the amount of growth which a corn root tip would make under these conditions was limited. If the original root tips were grown for ten days or two weeks in the dark in the nutrient solutions containing glucose, and then their tips cut off and transferred to fresh nutrient solutions, the amount of growth and production of secondary roots was decidedly less in the second period than in the first, and ceased entirely in the third. The following causes for this cessation of growth were suggested: (1) an unbalanced condition of the nutrient solution, (2) a deficiency of oxygen, (3) incompleteness of the nutrient solution, that is, the lack of some constituent necessary for continued growth. A consideration of the conditions of the experiments and the results obtained suggested that the third possibility should be used as a working hypothesis. This assumption does not eliminate the possibility that the cessation of growth is due to one or both of the other two factors, or that it is due to some factor not considered. It is merely used as the basis for further experimentation.

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[1] Published with permission of the Director of the Agricultural Experiment Station, University of Missouri. The writer acknowledges the kindness of Dr. J. K. WILSON in supplying the Longfellow Flint corn used in this investigation, and the assistance of Dr. W. E. MANEVAL in making the transfers involved in experiments 12 and 14.

[2] ROBBINS, W. J., Cultivation of excised root tips and stem tips under sterile conditions. BOT. GAZ. **73**:376–390. 1922.

Growth of roots attached and detached from grain

That the seed supplies material different either in kind or in quantity from that present in the culture solution is evidenced by the following experiment, in which a comparison was made of the growth of roots attached to the grain and detached from the grain. In this experiment corn grains were sterilized by WILSON'S method as before, and germinated on sterile agar in Petri dishes. After germination, and when the roots were about 3 cm. long, some of the grains were placed in 50 cc. of sterile modified Pfeffer's solution plus 2 per cent glucose in 125 cc. Erlenmeyer flasks, and some of the root tips were cut off and transferred to the same kind of solution and culture flasks. All were placed in the dark at room temperature. At the end of twelve days the roots attached to the grains had gained 26.6 cm., had produced 102 secondary roots on the average, and weighed per ten roots 0.5120 gm. Those detached from the grain had gained 12.4 cm., had eighty-three secondary roots, and weighed per ten roots but 0.1138 gm. (table I).

TABLE I

GROWTH IN DARK OF ROOTS ATTACHED TO GRAIN AND DETACHED FROM GRAIN, IN PFEFFER'S SOLUTION PLUS 2 PER CENT GLUCOSE

Condition	Average original length (cm.)	Gain in length 12 days (cm.)	Average no. secondary roots	Dry weight per 10 roots (gm.)
Attached to grain	2.9	26.6	102	0.5120
Cut off	1.7	12.4	83	0.1138

In tubes of 1 per cent agar a similar difference in the growth of roots attached to the grain and detached from the grain was noted. In fig. 1 the growth of an excised root tip at the end of two weeks in modified Pfeffer's solution plus 2 per cent glucose containing 1 per cent agar, and the growth in the same period of time of a root which was left attached to the grain are shown. Comparing the latter root with the excised root, its greater length, greater size of secondary roots, and greater diameter of root tip are clearly evident.

The effect of peptone and autolized yeast

If we assume that the stoppage in growth of an excised corn root tip on continued transfers is because glucose, the mineral salts of

Pfeffer's solution, oxygen, and water are insufficient for continued root growth, the natural place to look for the materials lacking is the grain or the plant. Extracts of the young embryos including both roots and tops, and of young seedlings a week or ten days old did not benefit the excised root tips. In fact, these extracts used in the proportion of the extract of one embryo or seedling to one root tip showed a slight injurious effect upon the growth of the root.

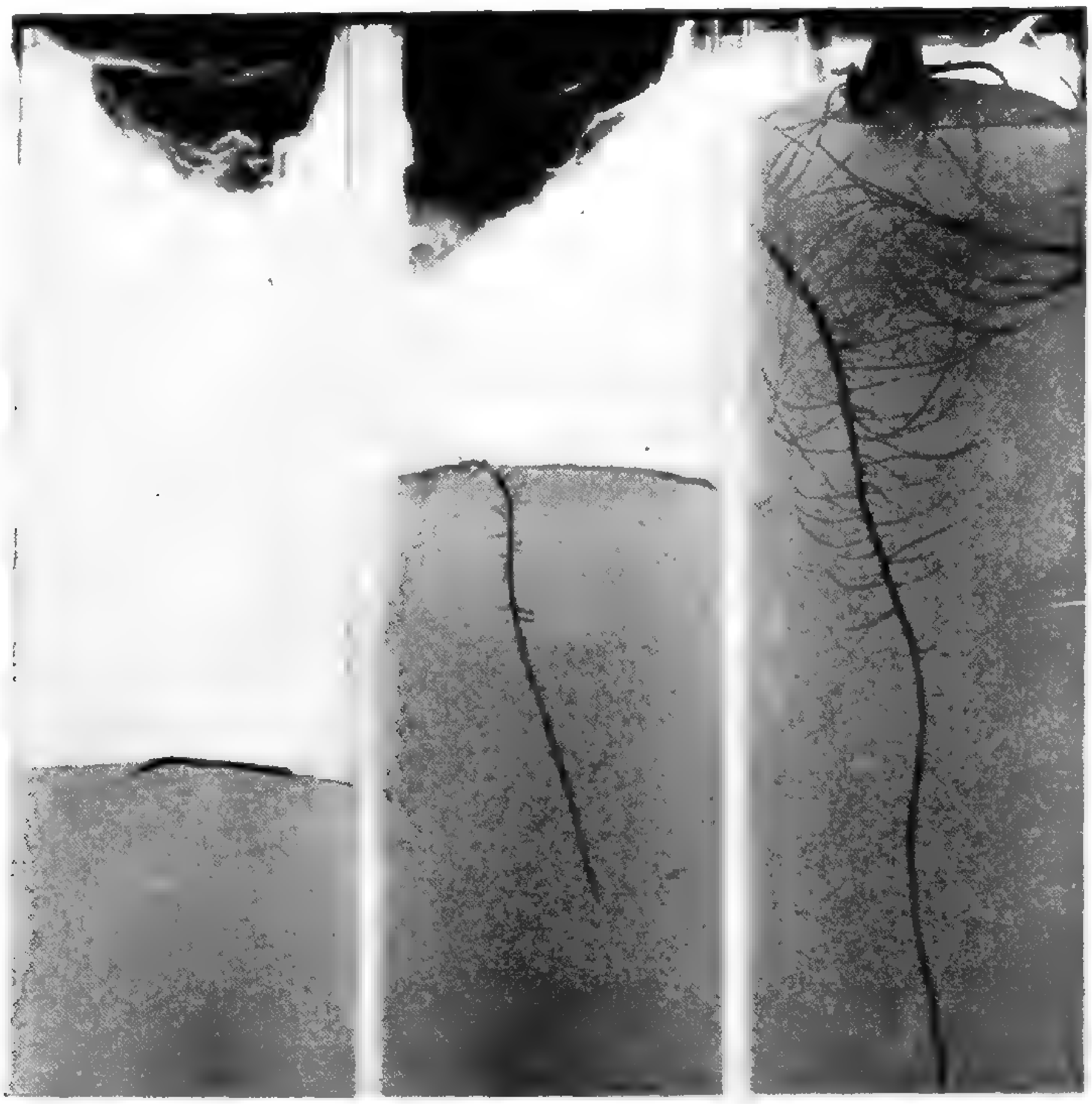

FIG. 1.—Corn root tips originally about 2 cm. long: (1) excised root tip in Pfeffer's solution plus 1 per cent agar; (2) excised root tip in Pfeffer's solution plus 1 per cent agar and 2 per cent glucose; (3) root tip of root attached to grain in Pfeffer's solution plus 2 per cent agar.

Neither did creatinine, gylcocoll, or asparagin used at the concentration of 100 ppm, 79 ppm, and 50 ppm respectively produce any better growth. The first two substances were slightly injurious, the last one no better than the check. Two experiments in which the $Ca(NO_3)_2$ and KNO_3 of Pfeffer's solution were replaced by $CaCl_2$ and KCl, and in which the roots were carried through three periods of culture, showed that the lack of nitrate somewhat decreased the total amount of growth in length instead of increasing it, as generally occurs in water cultures lacking nitrogen when the root is attached to the grain. Peptone and autolized yeast, however, were found to be distinctly beneficial. These two substances were selected because they contained a variety of comparatively simple organic nitrogenous materials, and because they are known to be beneficial to the growth of lower plants, such as the bacteria or the yeasts.

TABLE II

EFFECT OF PEPTONE AND AUTOLIZED YEAST ON GROWTH IN DARK OF CORN ROOT TIPS CUT OFF AND TRANSFERRED

Addition to PFEFFER's solution +2% glucose	No. roots	Average original length (cm.)	Average gain in length (cm.)	Average no. side roots	Dry weight per 10 roots (gm.)	Original P_H	Average final P_H
			1. June 21–July 4				
None	8	1.7	11.6	68	0.0487	4.5	6.8
None	8	1.7	12.7	68	0.0560	4.5	6.5
None	8	1.9	12.8	63	0.0485	4.5	6.3
			2. July 4–July 22				
None	8	1.6	2.5	8.0	0.0030	4.5	5.04
0.04% peptone	8	1.7	4.3	16.0	0.0283	6.5	6.5
0.02% yeast	8	1.7	2.2	10.0	0.0371	5.2	5.3
			3. July 22–August 20				
None	8	1.5	0.05	0	....	4.5	4.6
0.04% peptone	4	1.8	4.1	11.0	....	6.5	6.42
0.02% yeast	2	1.5	5.2	21.0	....	5.2	5.45
			4. August 20–November 12				
None	....	....	....	....	....	....	....
0.04% peptone	3	2.0	1.6	4.5	....	....	....
0.02% yeast	1	2.0	3.5	4.0	....	....	....

The first experiment with peptone and autolized yeast was begun June 21, 1920, and completed November 12 of the same year. The second experiment extended from December 13, 1920, to February 5, 1921. Both experiments demonstrated that excised corn roots whose tips were severed and transferred about every two weeks would grow in the dark in sterile solutions containing peptone or autolized yeast, glucose, and the salts of Pfeffer's solution for four to six two-week periods, while without the peptone or autolized yeast growth stopped in the third two-week period. Autolized yeast appeared to be somewhat more favorable than peptone. The details of these two experiments are as follows.

Experiment 5.—In this experiment the method of culture was as previously described. Grains of Longfellow flint corn were sterilized by WILSON's hypochlorite method. The excised tips of the original primary roots were grown in the modified Pfeffer's solution plus 2 per cent glucose. The root tips of these excised roots were cut off after eleven days and transferred to the same solution, to the same solution plus peptone, or to the same solution plus autolized yeast. Further transfers were made as indicated in table II. Erlenmeyer flasks of 125 cc. capacity containing 50 cc. of solution were used. One cc. of a sterile 2 per cent solution of Difco peptone was added to each flask containing peptone, and 1 cc. of a sterile 1 per cent autolized yeast suspension was added to each flask containing autolized yeast. This produced solutions containing approximately

FIG. 2.—Effect of peptone at end of second period on growth of corn root tips in dark; two root tips on left in Pfeffer's solution plus 2 per cent glucose, two on right in same solution plus about 400 ppm peptone.

400 ppm of peptone and 200 ppm of autolized yeast. Both the yeast and peptone were sterilized intermittently at 100° C. The root tips were grown in the dark at room temperature.

The beneficial effect of the peptone and the autolized yeast began to be evident toward the end of the second period of growth. From the data in table II it can be noted that the root tips in the peptone solution show distinctly greater growth in length, production of secondary roots, and greater increase in dry weight. The maximum increase in this period in the check was 3.2 cm. with eight secondary roots, in the peptone solution 3.6 cm. with twelve secondary roots, and in the yeast 5.5 cm. with nineteen secondary roots. The appearance of two of the best root tips in the Pfeffer's solution plus glucose and in the same solution plus peptone at the end of the second period is shown in fig. 2. A number of the roots in the peptone solution, however, and still more in the autolized yeast solution developed very abnormally. They became swollen, translucent, water-soaked, and extremely brittle, and growth was stopped. From later experiments the large number of these abnormalities is believed to be due to the combined action of the peptone or yeast and the comparatively high room temperature of July (about 30° C.).

The dry weights of the root tips in the yeast and peptone solutions at the end of the second period were comparatively high, as can be noted from later experiments. These high dry weights were due to the large number of thickened and abnormal roots which developed in this period in the yeast and peptone solutions. Of the eight roots in the peptone only four were transferred, and of the eight roots in the yeast only two were transferred. These grew in the third period, however, while the check root tips did very little, as can be noted in table II. The maximum growth in the third period in the check was 0.3 cm. with no secondary roots, in peptone 7.8 cm. with twenty-nine secondary roots, in the yeast solution 9.4 cm. with forty secondary roots. The tips of three of the four roots in the peptone solution were transferred and one of the two roots in yeast. These grew in the fourth period, the maximum in the peptone being 4.5 cm. with no secondary roots, and in the yeast 3.5 cm. with four secondary roots. The one root in the yeast solution was lost by contamination. The results of this experiment, so far as length increase is concerned, are presented graphically in fig. 3.

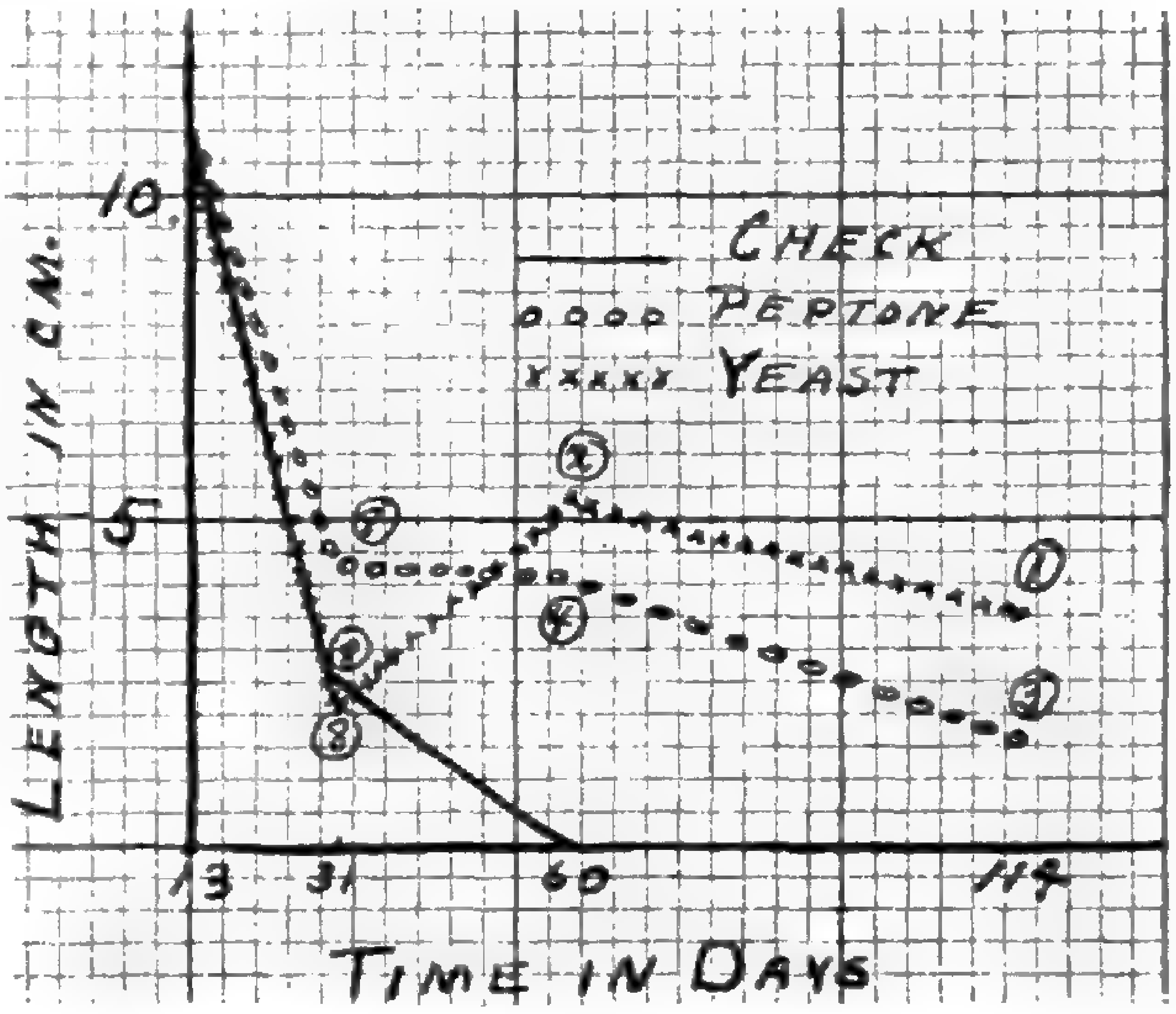

FIG. 3.—Growth in length of root tips of corn in dark for four periods; solutions used were modified Pfeffer's solution plus 2 per cent dextrose (check), and same solution plus approximately 200 ppm yeast or 400 ppm peptone; numbers within the circles represent number of root tips still growing in each period.

Determinations of the H-ion concentration of the solutions used in this experiment were made by GILLESPIE'S[3] method at the beginning and at the end of the first, second, and third periods. The modified Pfeffer's solution at the beginning of each period had a P_H of 4.5. The addition of the quantity of peptone used made the solution more alkaline, raising the P_H to 6.5. The autolized yeast had a similar though less marked effect, raising the P_H to 5.2. The growth of the roots made the check solution more alkaline (table II), raising the P_H in the first period to 6.3–6.8, in the second to 5.04, and barely affecting it in the third period. Both the yeast and the peptone exerted a buffer action, and little change in the reaction was produced in these solutions by the growth of the roots. It was noted that in the Pfeffer's solution containing glucose the change in reaction was roughly proportional to the amount of growth which the roots made. Root tips which grew but little raised the P_H from 4.5 to 5.1 in the first period; those which made the most growth raised it as high as 6.8. Higher values than this have been obtained. In one case root tips of Silver Mine corn grown for three months in the modified Pfeffer's solution plus 1 per cent cane sugar (inverted in sterilizing) changed the P_H to as high as 8.4.

Experiment 10.—In this experiment the methods followed were as in the first experiment, but two concentrations of peptone were

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[3] GILLESPIE, L. J., Colorimetric determination of H-ion concentration without buffer mixtures. Jour. Amer. Chem. Soc. **42**:742–748. 1920.

used instead of one. The excised primary roots of the germinated grains were grown eleven days in the modified Pfeffer's solution plus 2 per cent glucose. The tips were then cut off and transferred to the same solution, or to the same solution plus autolized yeast or peptone. Further transfers at intervals of two weeks were made, as indicated in table III.

TABLE III

EFFECT OF PEPTONE AND AUTOLIZED YEAST ON CONTINUED GROWTH OF EXCISED CORN ROOT TIPS IN DARK

Addition to PFEFFER'S solution+2% glucose	No. roots tips	Average original length (cm.)	Average gain in length (cm.)	Average no. secondary roots	Dry weight per 10 roots (gm.)
	1. December 13–December 24				
None	9	2.2	14.0	95	....
None	13	1.9	14.2	82	....
None	10	1.95	12.9	98.8	0.1020
None	18	1.7	12.1	72	....
	2. December 24–January 8				
None	9	2.1	2.46	12	0.0058
200 ppm autolized yeast	13	1.9	4.8	11	0.0087
200 ppm peptone	9	2.05	2.9	16	0.0064
400 ppm peptone	14	2.1	3.7	16.7	0.0097
	3. January 8–January 22				
None	7	1.8	0.3	1.0	....
200 ppm autolized yeast	10	2.05	2.9	4.36	0.0032
200 ppm peptone	6	1.7	0.3	2.0	....
400 ppm peptone	12	2.0	1.4	1.6	0.0035
	4. January 22–February 5				
None	....	....	....	....	....
200 ppm autolized yeast	9	1.6	0.83	0.65	....
200 ppm peptone	6	1.3	0.50	0.6	....
400 ppm peptone	11	1.7	0.28	1.0	....

Again, toward the end of the second period of growth, the beneficial effect of the peptone and yeast was evident. In this case, however, with the lower winter temperature (ranging around 22° C. in the dark room used for incubation) the abnormalities so evident in the first experiment were not so numerous, and most of the roots were carried over into the third period. Here the check root tips again stopped growth, while those root tips in the yeast and peptone continued to grow. They also grew in the fourth period, and of seven root tips transferred from the solutions containing autolized yeast, one grew from an original length of 1.8 cm. to 2.6 cm. in the fifth period.

It can be noted in table III and in fig. 4, where the average gains in length of the root tips in this experiment are represented graphically, that there is a continued decrease in the amount of gain in length in each successive period in both the yeast and peptone solution. The decrease which occurs in the first period is the most marked. The same holds true for the production of secondary roots and for the dry weight produced in each period. The decrease is most marked in the dry weight. The dry matter produced in the second period, even in the presence of yeast or peptone, is only $\frac{1}{11}$ or $\frac{1}{12}$ of that produced in the first period. A consideration of the data of both experiments shows that autolized yeast is superior in its effect to peptone.

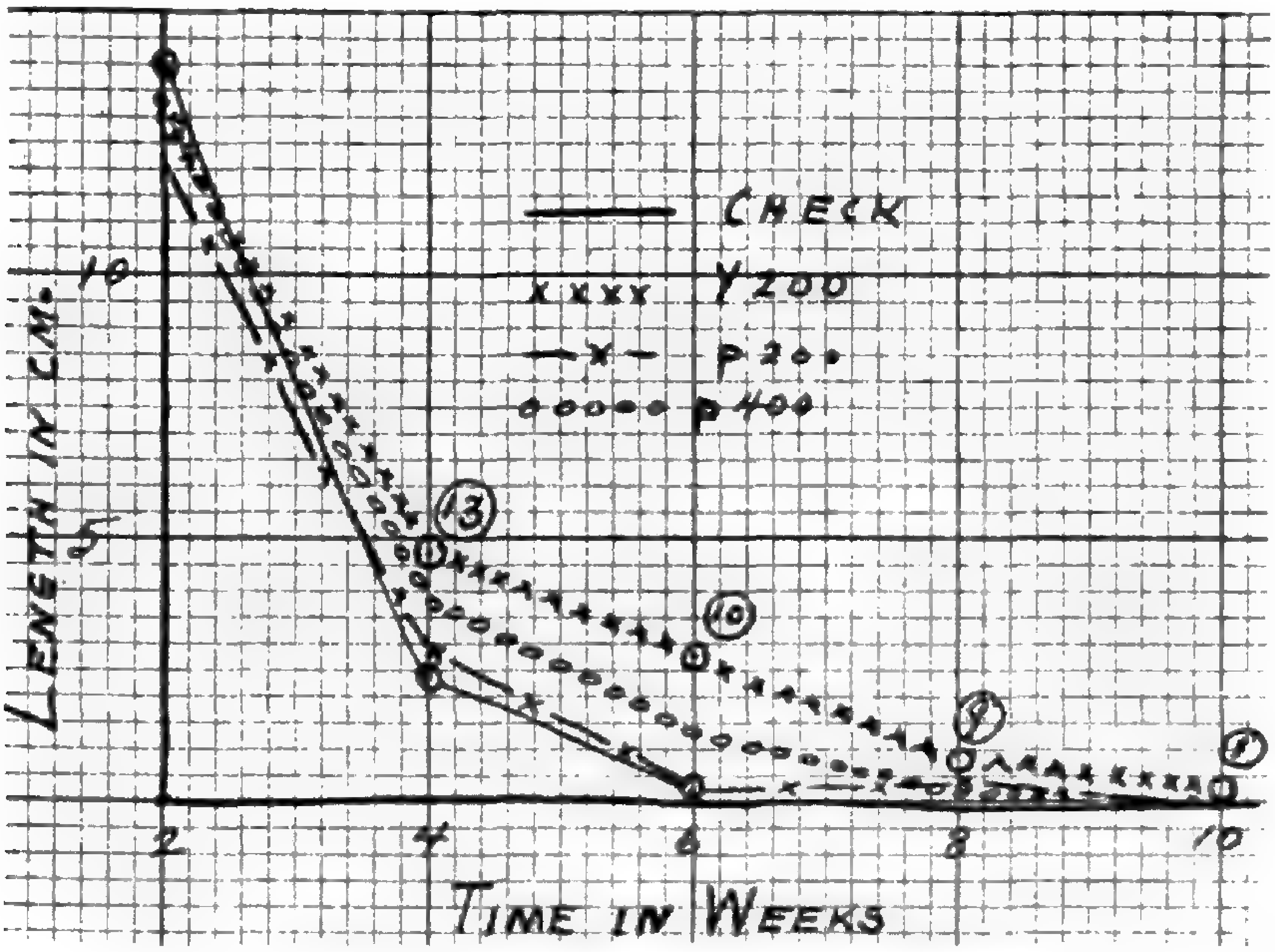

FIG. 4.—Growth in length of excised corn root tips in dark for five periods; solutions used were modified Pfeffer's solution plus 2 per cent glucose (check), same solution plus approximately 200 ppm or 400 ppm peptone (p 200, p 400) or 200 ppm of autolized yeast (Y 200); numbers within circles represent number of root tips still growing in each period in yeast extract.

An observation made in this experiment as well as generally in others should be recorded. The root tips of some of the excised roots in any series always develop abnormally. The root tip becomes clear and glassy, sinks to the bottom of the solution, and almost ceases growth. When this occurs in the early periods of an experiment the secondary roots become unusually long and well developed, frequently attaining a length three or four times that of the secondary roots of an excised root whose tip is developing normally. The appearance of a root whose primary tip became abnormal and whose secondary roots show marked development is shown in fig. 5. A normal root is shown in fig. 6. The latter figure also illustrates the fact noted before that the secondary roots develop from the convex side of the main root.

Effect of different concentrations of autolized yeast

While autolized yeast and peptone exert a favorable influence on the continued growth of the excised root tips in solution cultures containing glucose and the mineral salts of Pfeffer's solution, nevertheless the increase in length and the production of secondary roots and dry matter continuously decrease, until eventually growth stops. If we are to determine the complete nutrient requirements of a root by the method used, it will be necessary to find a set of conditions in which the excised root tips will grow continuously and produce approximately the same increase in length, number of secondary roots, and dry matter period after period as the root tips are cut off and transferred.

It was hoped that increasing the concentration of the favorable factor already found would accomplish this. Experiments were therefore carried out in which concentrations of autolized yeast varying from 10 ppm to 800 ppm were used. The results of these experiments indicated that while 400 ppm were somewhat more favorable than any of the other concentrations used, there was no marked difference in the effects of concentrations as low as 10 ppm and as high as 800 ppm so far as the growth in the early periods was concerned. In the later periods, however, the higher concentrations of yeast were more beneficial. Two experiments were performed dealing with this phase. The first extended from February 5 to April 16, the second from April 3 to May 17, 1921.

FIG. 5.—Excised corn root whose tip has developed abnormally; note development of secondary roots.

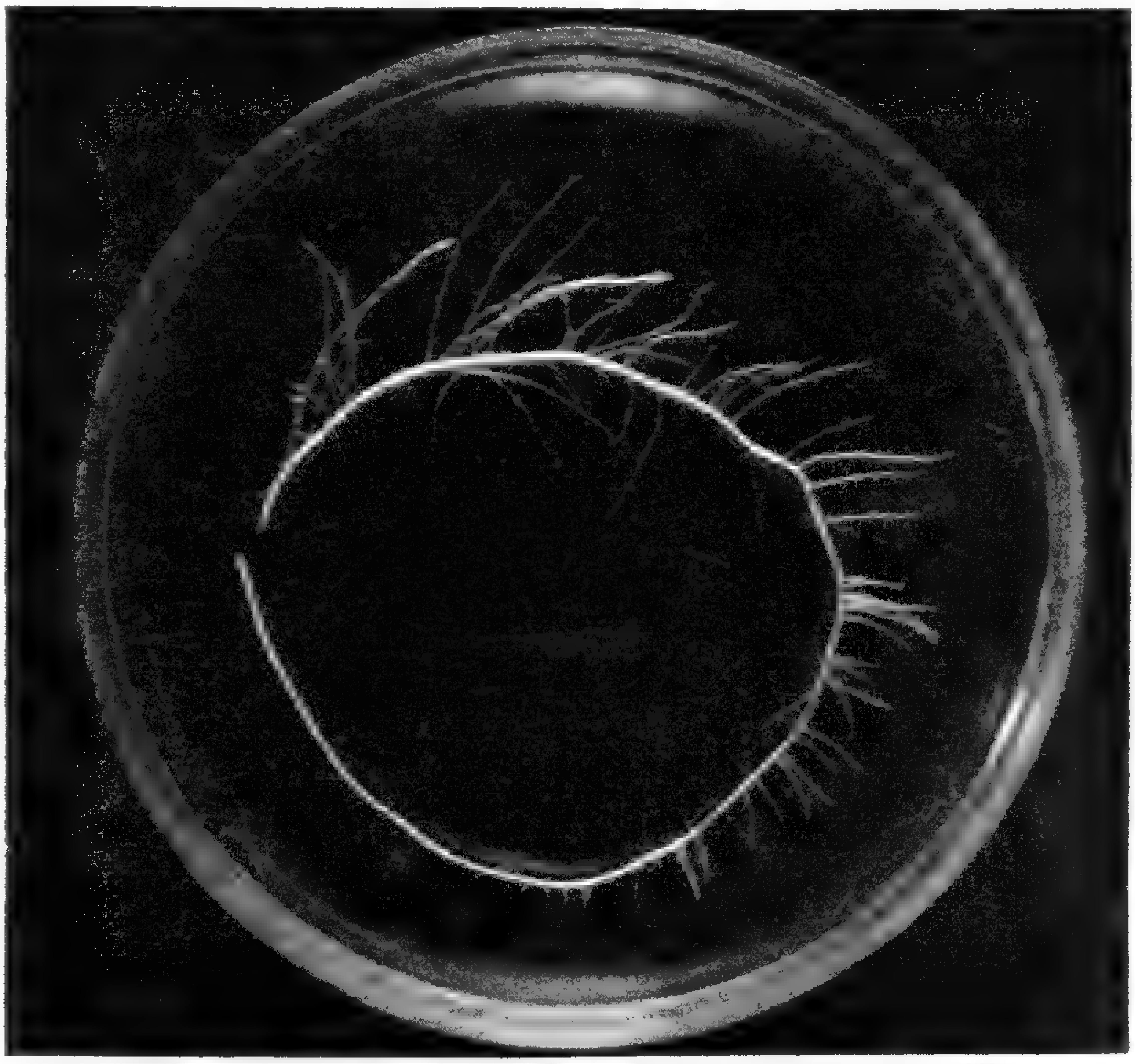

FIG. 6.—Normal excised corn root tip; compare development of secondary roots with those of root in fig. 5.

Experiment 12.—The methods used in this experiment were similar to those used in the previous experiments. The root tips were grown at room temperature in the dark in 125 cc. Erlenmeyer flasks of Pyrex glass containing 50 cc. of solution. The original excised primary roots were grown for the first period in the modified Pfeffer's solution plus 2 per cent glucose, and in the same solution plus 80, 200, 400, or 800 ppm of autolized yeast or 200 ppm of Gold Label gelatine, and transferred to fresh solutions of the same composition as in the first period. Further transfers were made as indicated in table IV. In addition, one set of root tips was grown for six weeks in the Pfeffer's solution containing 2 per cent dextrose without transferring, and one set was grown in the same solution plus 400 ppm of autolized yeast for six weeks without severing the tips, and transferring.

The autolized yeast extract used in experiment 12 was prepared by thoroughly boiling 2 gm. of autolized yeast with 100 cc. of distilled water, filtering, and making up to the original volume with distilled water. This 2 per cent extract of the autolized yeast actually contained 0.3366 gm. of dry matter per 50 cc., of which 0.0525 gm. was ash. An extract prepared in the same way was also used in experiment 14. The actual concentration of autolized yeast in the nutrient solutions to which yeast was added was about one-third of the concentrations given, which are based on the weight of the dry yeast as it came from the bottle. Table V indicates the approximate amounts of dry material, organic matter, and ash added to the culture flasks in the form of the yeast extract.

The data in table IV and the graphic representation of the lengths in fig. 7 show that during the first period the autolized yeast exerted no favorable influence on the growth of the roots. It was not until near the end of the second period that the beneficial effect of the yeast became evident. As was noted earlier, growth in the Pfeffer's solution plus 2 per cent dextrose fell off very markedly in the second period, and stopped in the third. In the presence of autolized yeast, however, the roots grew in the third and fourth periods. The number which could be transferred was decidedly reduced in the fourth period, however, but those which were transferred made some growth in the fifth period, and some of the roots

TABLE IV

EFFECT OF DIFFERENT CONCENTRATIONS OF AUTOLIZED YEAST ON CONTINUED GROWTH OF EXCISED CORN ROOTS IN STERILE CULTURE IN DARK

Addition to PFEFFER'S solution+2% glucose	No. root tips	Average original length (cm.)	Average gain in length (cm.)	Average no. secondary roots	Dry weight per 10 roots (gm.)
	1. February 5–February 19				
None	10	1.7	12.6	67	0.0940
80 ppm yeast	10	1.8	12.0	62	0.0840
200 ppm yeast	10	1.8	11.1	60	0.0730
400 ppm yeast	10	1.9	13.0	72	0.0820
800 ppm yeast	10	1.9	11.0	56	0.0630
200 ppm gelatine	10	1.7	10.4	50	0.0624
	2. February 19–March 5				
None	9	2.3	3.0	9.6	0.0051
80 ppm yeast	10	1.9	5.97	6.1	0.0056
200 ppm yeast	10	2.16	5.71	14.0	0.0116
400 ppm yeast	10	2.0	5.4	13.2	0.0096
800 ppm yeast	10	2.1	5.5	9.0	0.0104
200 gelatine	10	1.85	2.12	3.7	0.0044
	3. March 5–March 19				
None	9	1.9	0.2	2.9	0.0015
80 ppm yeast	8	1.8	4.0	6.5	0.0035
200 ppm yeast	8	1.7	4.2	7.4	0.0043
400 ppm yeast	8	1.9	3.6	9.6	0.0055
800 ppm yeast	6	1.6	4.4	10.7	0.0110
200 gelatine	7	1.5	0.1	0	....
	4. March 19–April 2				
None	....	....	....	....	....
80 ppm yeast	8	2.0	1.15	2.2	0.0030
200 ppm yeast	8	1.9	1.60	0.4	0.0050
400 ppm yeast	8	1.6	1.95	3.5	0.0040
800 ppm yeast	5	1.46	2.60	5.4	0.0046
200 gelatine	....	....	....	....	....
	5. April 2–April 16				
80 ppm yeast	3	1.5	0.7	0	....
200 ppm yeast	4	1.35	0.5	0	....
400 ppm yeast	2	1.65	3.3	8.5	....
800 ppm yeast	4	1.55	0.8	3.0	....
	February 5–March 19				
None	9	2.0	20.4	84	0.0898
400 ppm yeast	13	1.93	20.57	97	0.0635

grew in the sixth period. All the roots still growing were accidentally lost in the sixth period.

An examination of the data in table V and the curves in fig. 7 shows that none of the yeast concentrations prevented the gradual decrease in length gain, secondary root production, and dry matter elaboration. The rate of decrease was very rapid at first, but became less so as the periods passed. The increase in the rate of growth in 400 ppm of autolized yeast in the fifth period is apparent only. It is due to the fact that two roots only out of the eight of the fourth period grew in the fifth period. Their growth was excellent throughout the experiment, but decreased continuously. The

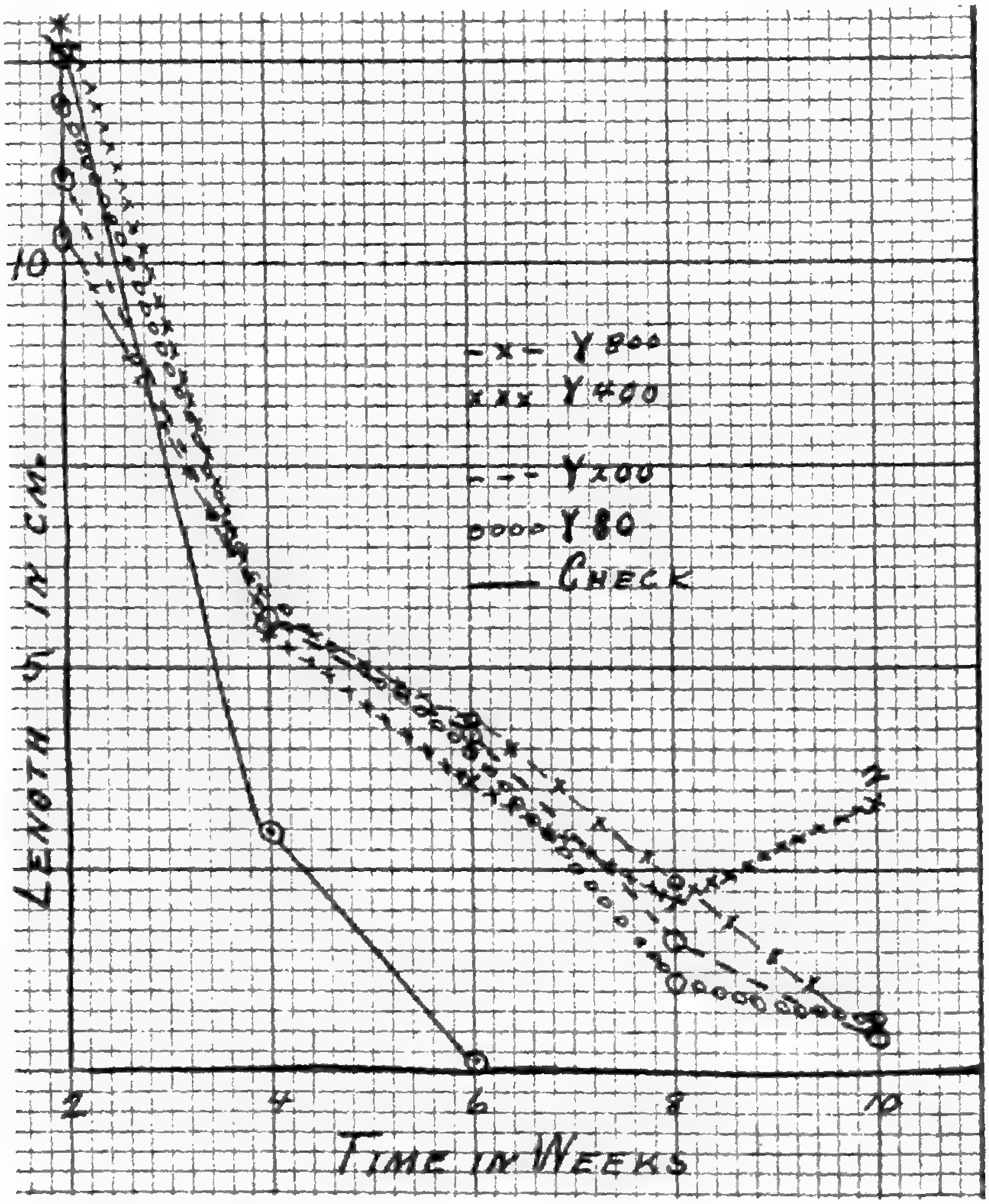

Fig. 7.—Growth in length of excised corn root tips in dark for five periods of transfers; solutions used were Pfeffer's solution plus 2 per cent glucose (check), and same solution plus 80, 200, 400, or 800 ppm autolized yeast.

increases in length for one of these roots, no. 32, for the five periods were as follows: 11.2, 6.5, 4.9, 4.5, and 1.3 cm., and the secondary root production was 58, 12, 3, 4, and 0. For the other, no. 39, the increases in length were 13.0, 11.5, 8.3, 6.9, and 5.4 cm., and the secondary root production was 82, 51, 25, 16, and 17. These figures show a continuous decrease in length. They also emphasize the fact, which has been observed continuously in this work, that the root tips act consistently as individuals period after period. A root tip which develops well in the first and second periods usually continues to show a superior growth in the later periods.

TABLE V

APPROXIMATE ACTUAL MATERIAL, ASH, OR ORGANIC MATERIAL ADDED IN AUTOLIZED YEAST EXTRACT TO CULTURE SOLUTIONS IN EXPERIMENTS 12 AND 14

Concentrations of autolized yeast in culture solutions expressed as bottle-dry yeast (ppm)	Total dry matter (ppm)	Ash (ppm)	Organic (ppm)	Total dry matter added per 50 cc. of culture medium (gm.)
10	3.7	0.5	3.0	0.00016
40	13.5	2.0	11.5	0.00065
80	27.0	4.0	23.0	0.0013
200	67.0	10.5	56.5	0.0032
400	134.0	21.0	113.0	0.0067
800	268.0	42.0	226.0	0.0134

The difference in the effect of the various concentrations of yeast was not great, and appeared most sharply in the later periods. Thus, so far as length is concerned, the gain in 80 ppm of yeast was as great in the second and in the third period as in any of the other concentrations. The secondary root production and dry weight produced, however, were greater in the higher yeast concentrations even in the second period. It was not until the fifth period that 200 ppm of yeast showed its inferiority to 400 ppm; 800 ppm of yeast apparently was somewhat too concentrated. The addition of 200 ppm of a colloidal material like gelatine to the culture solution did not favorably affect the development of the roots. It should be noted that root hairs were found on the root tips in the gelatine solution at the end of the third period. Excised roots which did not have their root tips severed and transferred at intervals (table VI) made just as much growth in length in a period of six weeks in Pfeffer's solution plus 2 per cent glucose as in Pfeffer's solution plus 2 per cent glucose and 400 ppm of autolized yeast.

TABLE VI

Growth of excised corn roots in sterile nutrient solutions in dark; in one case original excised root tips not transferred; in other tips cut off and transferred to fresh nutrient solutions at intervals of two weeks; compiled from data in table IV

Addition to Pfeffer's solution	Transfers	No. roots	Average original length (cm.)	Total average gain in length (cm.)	Average no. secondary roots	Dry weight per 10 roots (gm.)
	February 5–March 19					
None	None	9	2.0	20.4	84	0.0898
400 ppm autolized yeast	....	13	1.93	20.57	97	0.0635
	February 5–March 19					
None	3	9–10	1.7	15.8	79.5	0.1006
400 ppm autolized yeast	....	8–9	1.9	22.0	94.8	0.0971

TABLE VII

Effects of 10, 40, and 80 ppm of autolized yeast extract on growth of excised corn root tips in sterile nutrient solutions in dark

Addition to Pfeffer's solution + 2% glucose	No. roots	Average original length (cm.)	Average gain in length (cm.)	Average no. secondary roots	Dry weight per 10 roots (gm.)
	1. April 3–April 17				
None	10	1.57	11.51	65	0.1054
None	10	1.68	11.12	64	....
None	10	1.75	11.25	65	....
None	10	1.68	12.22	71	0.0826
	2. April 17–April 30				
None	8	1.8	1.25	7.5	0.0027
10 ppm yeast	5	1.96	3.48	9.0	0.0047
40 ppm yeast	3	1.9	2.5	6.0	....
80 ppm yeast	3	2.0	2.8	2.0	....
	3. April 30–May 14				
None	8	1.4	0.13	0	....
10 ppm yeast	5	1.8	1.5	1.2	....
40 ppm yeast	2	1.8	1.5	0	....
80 ppm yeast	2	1.6	1.1	0	....

The secondary root production in the autolized yeast culture was somewhat greater and dry matter somewhat less in this case.

If we compare the growth of roots which had their tips severed and were transferred twice to fresh solutions during a six weeks' period with those which grew continuously undisturbed in the solution, we find (table VI) that in Pfeffer's solution plus 2 per cent glucose the total increase in length and production of secondary roots when the tips were severed and transferred was less than when they were left undisturbed; the dry matter was greater. When yeast was present the length and secondary root production were not affected by the transfers; the dry weight was greater.

The original P_H of the Pfeffer's solution plus 2 per cent glucose was 4.5. The roots which grew for six weeks without transfer changed the reaction to P_H 6.3. The original reaction of the Pfeffer's solution plus 2 per cent glucose and 400 ppm of yeast was P_H 5.2. The roots which grew for six weeks without transfer changed the reaction to a P_H of 6.24.

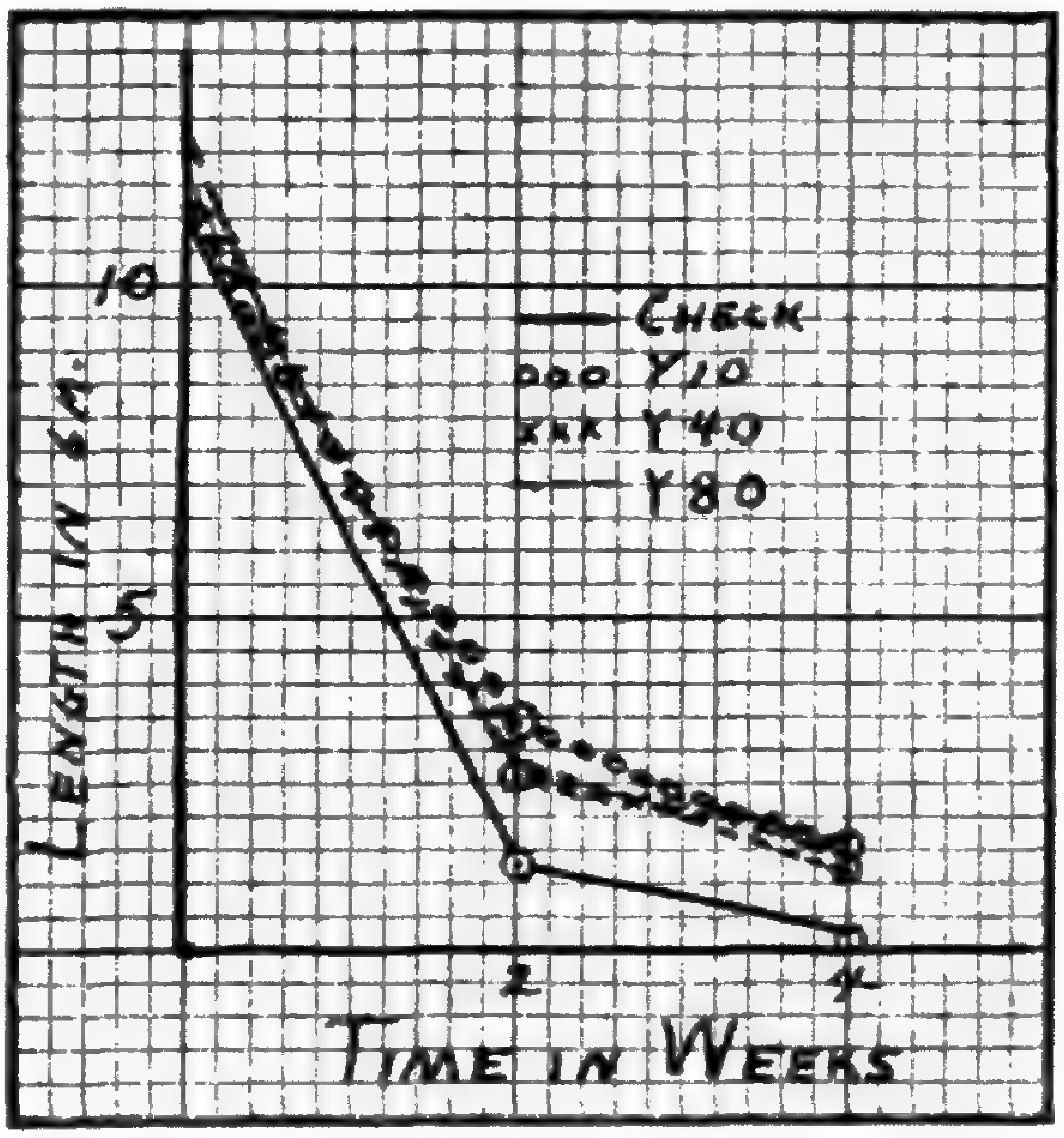

FIG. 8.—Growth in length of excised corn root tips in dark for three periods of transfers; solutions used were Pfeffer's solution plus 2 per cent glucose, and same solution plus 10, 40, or 80 ppm of autolized yeast.

Experiment 14.—The methods and general conditions were the same in this experiment as in experiment 12. In this case, however, concentrations of yeast of 10, 40, and 80 ppm were used. The root tips were grown for the first period with no addition to the nutrient solution, the yeast being added in the second period. Unfortunately one of the flasks containing the concentrated yeast extract was contaminated with bacteria, and most of the cultures were lost in the second period due to the contamination, leaving but two root tips in each of the concentrations, 40 ppm and 80 ppm. From the data in table VII and the graphic representations in fig. 8 it can be noted that the growth in 10 ppm of autolized yeast in the second period compares very favorably with that in 40 ppm or 80 ppm of yeast in the same experiment. In the third period the development in the solution containing 10 ppm of autolized yeast was better than in either 40 or 80 ppm of yeast. If, however, we compare it with the previous experiment in which a larger number of root tips were grown in 80 ppm yeast, we can see that the development was considerably less in 10 ppm than in 80 ppm of yeast. This substantiates the general expression of the effect of autolized yeast in these experiments, namely, that in the first period it has no beneficial effect, in the second period there is little difference between the effects of 10, 40, 80, 200, 400, and 800 ppm; but the later the period the more evident does the beneficial action of the higher concentrations appear.

Discussion

While excised corn root tips which are grown in the dark for about two weeks in Pfeffer's solution plus 2 per cent glucose, and which have their root tips cut off and transferred to fresh solutions at intervals of two weeks, show a continued decrease in the rate of growth and stop growth in the third period, the addition of small amounts of peptone or autolized yeast permits them to grow for as long as six periods before growth ceases.

Two possible explanations for the action of the peptone and autolized yeast suggest themselves: (1) the autolized yeast or the peptone supplies something which the root requires for its continued growth and which is not included in the salts of Pfeffer's solution, water, glucose, and free oxygen; (2) the autolized yeast or peptone balances the solution, performing a function which could be accomplished by a readjustment of the relative quantities of the salts of Pfeffer's solution or their equivalents, of water, of glucose, and of free oxygen. A definite decision between these two possibilities cannot be made from the data at hand. The majority of the results reported in this paper, however, would seem to be explained best on the assumption that the mineral salts of Pfeffer's solution, glucose, free oxygen, and water are insufficient for the continued growth of the root cells of corn, and that this deficiency can partially be supplied by autolized yeast or peptone.

That the effect of the peptone or autolized yeast is not protective is indicated by the fact that a colloidal material like gelatine is not beneficial, and by the fact that the beneficial action of the yeast does not appear in the first period of two weeks, or even in six weeks when the excised roots do not have their tips cut off and transferred to fresh solutions. The assumption, however, that the seedling root contains some substance or substances derived from the grain which are not contained in the basic nutrient solution and which are supplied by the peptone or autolized yeast would explain: (1) That root tips transferred show less total growth than those not transferred. The hypothetical materials are fractionated in the transfers, and the transferred root is limited in the second and third periods by a deficiency of these materials, which can partially be supplied by the peptone and autolized yeast. (2) That in the presence of autolized yeast root tips transferred show as much growth as those not transferred. The hypothetical substances fractionated in the transfers are supplied by the autolized yeast. (3) The fact that with the cessation of growth of the root tip of an excised root there occurs an excessive development of secondary roots. The hypothetical materials which are not used in the growth of the main root tip are utilized for growth by the meristematic tissue of the secondary roots.

Whatever the cause of the beneficial action of the autolized yeast,[4] its effect is limited in some way, as is evidenced by the fact that the various concentrations of yeast produce no correspondingly increased benefits, and the Pfeffer's solution containing peptone or autolized yeast and glucose will not permit continued growth.

Summary

1. Corn roots attached to the grain grow much more rapidly under sterile conditions in the dark in Pfeffer's solution plus 2 per cent glucose than do root tips detached from the grain.

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[4] Lack of space precludes a summary at this time of the voluminous literature dealing with this general problem. Reference should be made to the publications of SACHS, HABERLANDT, HANNIG, BOTTOMLEY, WEBER, BACHMAN, LOEB, MOCKERIDGE, FULMER, NELSON and SHERWOOD, WILLIAMS, and others who have dealt previously with the problem of the necessity of accessory food substances for plant growth, or who have attempted to cultivate single cells or isolated parts of higher plants. Some of the more recent papers on this subject not cited in the earlier publication by the writer are given at the end of this paper. A review of the subject indicated is planned for a later publication.

2. When excised root tips of corn are grown under sterile conditions for about two weeks in the dark in Pfeffer's solution plus 2 per cent glucose, and their root tips are severed and transferred to fresh solutions at intervals: (*a*) growth stops in the third period; (*b*) the addition of peptone or autolized yeast permits the root tips to grow for 4–6 periods; (*c*) a concentration of 200 ppm of gelatine, 100 ppm of creatinine, 79 ppm of glycocoll, 50 ppm of asparagin, or the corn embryo extract used show no beneficial effect; (*d*) in Pfeffer's solution lacking nitrates and containing 2 per cent glucose a little less total growth is made than in Pfeffer's solution containing nitrates and 2 per cent glucose; (*e*) approximately 400 ppm of peptone is more efficient than 200 ppm; (*f*) autolized yeast is more beneficial than peptone; (*g*) the beneficial effect of the autolized yeast does not appear in the first period of growth; (*h*) concentrations of 10, 40, 80, 200, 400, and 800 ppm of autolized yeast (equivalent in dry matter to about one-third the concentrations given) show no marked difference in their beneficial effect, especially in the early periods; (*i*) the higher concentrations of yeast evidence a somewhat greater beneficial effect in the later periods than the lower concentrations.

3. When the total growth of excised corn root tips whose tips are cut off and transferred twice in a six weeks' period is compared with that of root tips left undisturbed, then (*a*) in Pfeffer's solution plus 2 per cent glucose in the dark the total growth in length and production of secondary roots are less, the dry weight is greater; (*b*) in Pfeffer's solution plus 2 per cent glucose and 400 ppm of autolized yeast there is no difference in the growth in length or secondary root production; the dry weight is greater.

4. When the growth of excised root tips left undisturbed for six weeks in Pfeffer's solution plus 2 per cent glucose is compared with the growth in the same solution to which 400 ppm of autolized yeast is added, there is no difference in total length. The secondary root production is somewhat less and dry weight somewhat greater.

5. Excised corn root tips act as individuals.

6. The growth of the secondary roots of an excised root is much greater when the primary root tip stops growth than when it continues to grow normally.

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LITERATURE CITED

1. Bachman, Freda W., Vitamine requirements of certain yeasts. Jour. Biol. Chem. **39**: 235–258. 1919.
2. Bottomley, W. B., The significance of certain food substances for plant growth. Ann. Botany **28**: 531–539. 1914.
3. Fulmer, E. I., Nelson, V. E., and Sherwood, F. F., The nutritional requirements of yeast. I. The rôle of vitamines in the growth of yeast; II. The effect of the composition of the medium on the growth of yeast. Jour. Amer. Chem. Soc. **43**: 186–199. 1921.
4. Gillespie, L. J., Colorimetric determination of hydrogen-ion concentration without buffer mixtures. Jour. Amer. Chem. Soc. **42**: 742–748. 1920.
5. Loeb, J., Rules and mechanism of inhibition and correlation in the regeneration of *Bryophyllum calycinum*. Bot. Gaz. **60**: 249–276. 1918.
6. MacDonald, M. B., and McCollum, E. V., The cultivation of yeast in solutions of purified nutrients. Jour. Biol. Chem. **45**: 307–311. 1921.
7. Mockeridge, F. A., XXXVII. The occurrence and nature of plant growth-promoting substances in various manurial composts. Biochem. Jour. **14**: 432–450. 1920.
8. Robbins, W. J., The cultivation of excised root tips and stem tips under sterile conditions. Bot. Gaz. **73**: 376–390. 1922.
9. Williams, R. J., Vitamines and yeast growth. Jour. Biol. Chem. **46**: 113–118. 1921.
10. Weber, F., Hormone in Pflanzenreich. Naturwiss. Wochenschr. **35**: 241–253. 1920.

LEAVES OF THE FARINOSAE[1]

AGNES ARBER

(WITH PLATES I–III)

Introduction

In papers published in this and other journals during the last few years (ARBER **1–10**) I have dealt with the results of the application of the phyllode theory to the leaves of various groups of monocotyledons. The present paper discusses, from this standpoint, the leaf structure of the families associated by ENGLER (**11**) in the cohort Farinosae. Examination of this group is a matter of some difficulty to a British botanist, since the eleven families which it includes are represented in Europe by one species alone, *Eriocaulon septangulare* With., and even in cultivation comparatively few genera belonging to these families are to be found. I have thus been unable to carry this study so far as I should have wished, because it has been necessary to rely almost entirely on limited quantities of herbarium material, which, in the case of the many fibrous-leaved members of the Farinosae, is peculiarly intractable to sectioning. MCLEAN'S (**14**) method of preparing dried material has proved invaluable, however, even in the case of plants which have lain in herbaria for many years. The specimen of *Cephalostemon affinis* Koern., for instance, sections of which are represented in fig. 26 *A–C*, was collected by SPRUCE in South America as long ago as 1853.

I am indebted for material to the Director of the Royal Botanic Gardens, Kew; the Keeper of the Department of Botany, British Museum (Natural History); Mr. L. RODWAY, of Hobart, Tasmania; and Professor A. C. SEWARD.

I propose in the first place, taking the families in the order in which they appear in ENGLER'S *Pflanzenfamilien*, briefly to describe the principal types of leaf met with in the cohort, and then to discuss their interpretation.

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[1] This paper represents part of the work carried out during the tenure of a Keddey Fletcher-Warr Studentship of the University of London.

Flagellariaceae

I hope to describe the leaves of this family in a later paper, so I will omit all discussion of them here; their most unusual feature is that, in the genus *Flagellaria*, they have tendril apices.

Restionaceae

In this family, which consists of nineteen genera, plants with radical leaves are rare. The genus *Anarthria*, however, forms a notable exception, for it has basal leaves recalling those of *Iris*, which may either be ensiform or "radial." Fig. 3 *A* represents the transverse section of the limb of *Anarthria scabra* R.Br. It will be seen that it has a type of anatomy resembling that of an *Acacia* phyllode, the bundles, which are in two series lying to right and left of the median plane of the leaf, having their xylems directed inward. The palisade parenchyma is interrupted at very short intervals by bands of fibers, one of which occurs between each of the main bundles and the epidermis, while others are associated with the smaller bundles, or occur independently. Fig. 3 *B* shows the margin of such a leaf, with the median bundle, on a larger scale. Two of the fibrous bands (*f*) are visible, and it will be seen that, in the marginal region, the palisade parenchyma passes over into thick-walled elements without contents. The epidermal cells also increase in size and in the thickness of their sclerised walls as the margin is approached. Fig. 4 *A–C* shows the leaf of *Anarthria gracilis* R.Br., which, instead of being ensiform like that of *A. scabra*, is radial. The limb (*C*), in which the fibrous sheaths of the bundles with their extensions to the epidermis form a conspicuous feature, is almost cylindrical in section. The leaves of *Anarthria*, however, are not typical for this family, in which it is usual to find cauline leaves alone. Such leaves generally have a well developed sheath, succeeded by a relatively unimportant limb, which may be flattened or cylindrical, but is often reduced to a mere point (examples occur in *Dovea*, *Elegia*, *Lepyrodia*, and a number of other genera). Fig. 1 *A* shows the appearance of the leaf of *Restio tremulus* R.Br. It has a sheathing base (*s*) which more than surrounds the axis, forming a "wrap-over." The same peculiarity in other members of this family may be carried to a further point; in a species of

Thamnochortus, the sheathing base of the scale leaf is described by VELENOVSKÝ (**18**) as surrounding the axis spirally, and, according to his fig. 358, its attachment forms more than two complete turns round the axis. Both sheath and narrow, flat limb of *Restio tremulus* are fibrous (fig. 1 *B*, *C*), and toward the apex of the limb there is a marked increase in the lignified and sclerised tissue (fig. 1 *D*). The leaf of *Leptocarpus peronatus* Mast., with its sheathing base and limb reduced to a mucro, is represented in fig. 2. In the case of *Elegia deusta* Kth. the leaf apex is more solid, and includes a ring of bundles (**10**, fig. 5 *A*, *B*).

Centrolepidaceae

The leaves of the Centrolepidaceae are usually small, with a sheathing base and an awl-like or threadlike limb. *Gaimardia australis* Gaudich. (fig. 5 *A–C*) may be taken as an example. There is a sheathing leaf base and an awl-like limb, traversed by three bundles inclosed in fibrous sheaths. The leaves of this family may be of a more reduced type, however, as in *Centrolepis aristata* R. and S. In this plant I have found one bundle, traversing both sheath and limb (fig. 11 *B*, *C*). GOEBEL (**13**), on the other hand, describes and figures the leaf anatomy of this species as belonging to an extremely reduced ensiform type, with a second bundle above the median bundle, resulting from the fusion of two laterals. Although I have not found this structure in the only two foliage leaves which I was able to examine, I have seen it in two of the bracts from the base of the inflorescence (*b*, fig. 11 *A*). These bracts are well developed structures with a sheath and limb, closely resembling the foliage leaves.

Mayacaceae

The very delicate leaves of the single aquatic genus *Mayaca*, of which this family consists, are traversed by a single vascular strand (fig. 27 *A*). Several species figured in the *Flora Brasiliensis* (**17**) have a bifid leaf apex, such as is shown here for *M. fluviatilis* Aublet (fig. 27 *B*). It will be seen that the vascular bundle (*vb*) takes no part in the bifurcation, but terminates below the fork.

Xyridaceae

This family consists of two genera, *Xyris* and *Abolboda*. *Xyris*, which includes about forty species, has a leaf with a sheathing base and an ensiform limb, recalling that of many members of the genus *Iris*. In *Xyris Wallichii* Kth. (figs. 12 *A–C*) and *X. brevifolia* Mich. (fig. 18 *A*, *B*) the single bundles alternate to right and left in the flattened limb. Fig. 18 *B* shows the marginal strand of *Xyris brevifolia*, which is peculiar in the possession of a conspicuous mass of fibers adjoining the bundle on the xylem side, whereas such a fibrous strand is more usually developed outside the phloem. As POULSEN (**16**) has already shown, the vascular bundles of the leaves of *Xyris* may either be single or associated in groups of two or more. Figs. 13 and 14 show bundle groups in the case of *X. asperata* Kth. (*trachyphylla* Mart.) and *X. anceps* Lam. These bundle groups may attain considerable complexity; that represented in fig. 13 *B* (*X. asperata*) consists of nine strands imbedded in a common fibrous sheath. Although the flattened ensiform leaf type is usual in *Xyris*, it is not universal. POULSEN (**16**) has figured a species (*X. teretifolia* Poulsen) in which the transverse section of the leaf limb is oval, the distance between the adaxial and abaxial margins being only about half as much again as the width.

In *Xyris* the leaf epidermis is generally thick-walled. It may retain much the same character at the two margins as on the flanks of the leaf (*X. Wallichii*, fig. 12 *C*), or the elements at the margins may be considerably elongated, forming a fibrous border to the leaf (*X. anceps*, fig. 14 *B–D*). In this case the marginal elements, instead of standing out horizontally, slope downward, with the result that they are cut obliquely in transverse sections passing through the leaf border, which at first glance thus suggest that the epidermis is multi-layered in this region (fig. 14 *D*).

Xyris gracilis R.Br. is significant as possessing sheathing leaves, in which the limb may be reduced to a mere point, and in which the leaf base forms practically the whole organ (fig. 17). These leaves may be compared with those of the second genus of Xyridaceae, *Abolboda*. I have examined the leaves of *A. grandis*

Griseb. var. *minor*, and find them to be traversed by a single series of normally orientated bundles, corresponding to those in the sheath leaves of *Xyris gracilis* (fig. 17 *C*). *A. Poarchon*, as figured by SEUBERT (**17**), has an acuminate apex to the foliage leaf, while it also has bracts which terminate in a "cornet," recalling that described by HALLIER in the case of the sepals of certain dicotyledons (see reference and discussion, **10**). It is probable that the apex in both foliage leaf and bract of *A. Poarchon* Seub. is a vestigial petiole, and is morphologically equivalent to the ensiform limb of *Xyris*.

Eriocaulaceae

The Eriocaulaceae have both cauline and radical leaves of a simple type. The two principal genera are *Eriocaulon* and *Paepalanthus*, both of which include more than a hundred species. Fig. 19 *A* shows the general internal structure of the British species of *Eriocaulon*, *E. septangulare* With. A large proportion of the leaf is occupied by lacunae, separated by lamellae, each of which includes a single normally orientated bundle. Fig. 19 *B* shows one lamella with its vascular strand on a larger scale. A fragment of a diaphragm (*d*) with intercellular spaces between its cells is seen attached to the lamella on one side. In *E. cuspidatum* Dalz. the limb terminates in a mucro (fig. 19 *D*), while in *E. Wallichianum* Mart. f. *submersa* the tip of the ribbon leaf is minutely truncate (fig. 19 *C*). In this aquatic species, as in so many water plants, there is an increase of the tracheal tissue near the leaf apex, and there are indications of water stomates and possibly an apical opening, but in herbarium material it is difficult to identify these structures with certainty. The leaves of certain species of *Eriocaulon* may be much reduced; in the aquatic *E. setaceum* L. the fragile linear leaf, traversed by a single bundle, recalls that of *Mayaca*. In the case of *Paepalanthus* I have examined one species, *P. speciosus* Gardn. Here, as in *Eriocaulon*, there is one series of normally orientated bundles in the leaf. The large epidermal cells are a striking character.

Rapateaceae

The leaves of this family are much larger and more complex than those of the Eriocaulaceae; they may show a definite differentiation into sheath, petiole, and limb (*Rapatea longipes*, fig. 24). A curious feature common to the leaves of various members of the family is their tendency to asymmetry. The leaf sheath, as ENGLER points out in the *Pflanzenfamilien*, is folded, but the median bundle does not occupy the trough of the fold, and the limb correspondingly is not of equal width on either side of the midrib. These features are shown in fig. 24, *Rapatea longipes* Spr.; fig. 25 *A*, *B*, *R. angustifolia* Spr.; and fig. 26 *A*, *B*, *Cephalostemon affinis* Koern. In the last species the sheath is peculiar, since it thins out markedly at the fold, which is quite remote from the median bundle, whose position is marked externally by a ridge of fibrous tissue (*f*) adjacent to the lower surface (fig. 26 *A*). This ridge can also be recognized in the limb (fig. 26 *B*). The lacunate character of the leaf tissue in *Rapatea angustifolia* Spr. is indicated in fig. 25 *C*, which also shows the median bundle with its fibrous sheath, and the small fibrous strands which run beneath the epidermis. The details of the median bundle of the limb of *Cephalostemon affinis* are seen in fig. 26 *C*.

Bromeliaceae

The leaves of the Bromeliads are often of a simple type, with a broadly sheathing base prolonged into a linear to ovate limb. In some cases, however (*Tillandsia usneoides* L. fig. 20 *A*, *B*), there is a marked distinction between the sheathing leaf base and the limb, which has a definitely petiolar character. In other cases the main part of the leaf suggests a leaf base, but there is a solid apical region which may correspond to the limb of *Tillandsia usneoides* on a reduced scale (**10**). The figures of *Aechmea gamosepala* Wittm. in the *Flora Brasiliensis* (**15**) show that the foliage leaves in this case have an acuminate apex, while the outer perianth members terminate in an elongated mucro, which is probably equivalent to the "cornet" in the case of *Abolboda Poarchon* Seub., discussed in a preceding paragraph.

Commelinaceae

The leaves of this family differ from most of those hitherto considered in their very complete differentiation. They usually have a conspicuous sheath, sharply marked off from a limb, from which it may or may not be separated by a distinct petiolar region. Fig. 9 represents the leaf of *Streptolirion volubile* Edgw., in which all these parts are well developed. In *Commelina*, *Aneilema*, and *Tradescantia* there is a striking range of form in the limb, which in different species shows (within each genus) gradations from linear to ovate.

Pontederiaceae

Fig. 8 *A* represents what is perhaps the most complex type of leaf met with in the Farinosae, that of *Eichhornia speciosa* Kth. (*Pontederia crassipes* Mart.). The ligular sheath (*lig. s*, fig. 8 *A*, *B*) with its lobed apex, almost suggesting a second leaf blade, to which attention has been drawn by GLÜCK (**12**), is, as he points out, unparalleled among monocotyledons; it may perhaps be remotely compared with the curious frill-like top of the ochrea of a *Polygonum* from Java, figured by VELENOVSKÝ (**18**, fig. 277). The petiole of *E. speciosa* is dilated, and terminates in a limb, which, as shown in a previous paper (**1**), possesses both normal and inverted bundles (fig. 8 *C*). In this paper it was recorded that inverted bundles occurred in the limb of *Eichhornia*, *Pontederia*, and *Heteranthera*. The family also contains three other genera, *Monochoria*, *Reussia*, and *Hydrothrix*. In *Monochoria plantaginea* Kth. I have now been able to observe that inverted as well as normal strands occur, and in a very small fragment of the leaf of *Reussia subovata* Solms, the only material available from this genus, I again found both types of strand. It has thus been possible to establish the occurrence of inverted strands in five genera of the Pontederiaceae; the sixth, *Hydrothrix*, is an aquatic plant in which it is useless to look for this anatomical peculiarity, since, as GOEBEL has shown, the leaf is so much reduced as to be traversed by one vascular strand alone (**1**).

The shape of the limb in the Pontederiaceae ranges from the narrow, almost linear form sometimes met with in *Monochoria plantaginea*, to broader types with a cordate base, such as *Pontederia nymphaefolia* Kth., or with an auricled base, such as that illustrated in *M. hastaefolia* Presl (fig. 7).

Philydraceae

This small family consists of four species assigned to three genera. Sections were secured of three of these species, one belonging to each genus. The monotypic *Philydrum lanuginosum* Banks (fig. 22) has an ensiform leaf, whose shape and plan of vascular anatomy recall *Anarthria scabra* R.Br.; the leaf, however, is conspicuously lacunate. *Pritzelia pygmaea* F. Muell., representing the second of these monotypic genera, is related to *Philydrum* in its leaf structure, very much as *Anarthria gracilis* is related to *A. scabra*. The leaf of *Pritzelia* has a sheathing base (fig. 21 *A*) and a limb, which so closely approximates to radial structure that it is not possible to determine from internal evidence which is the median bundle (fig. 21 *B*). The leaf contains a number of large, solitary, acicular crystals (*c*, fig. 21 *C*). The leaves of the third genus, *Helmholtzia*, have been described as equitant and ensiform, but Dr. Stapf has been so kind as to inform me that in both the two species of which the genus consists, *H. acorifolia* F. Muell. and *H. glaberrimum* Hook. f., the vaginal portion of the leaf is strongly keeled, and that this keel runs as a midrib throughout the "vertical" limb. The limb is thus expanded in a plane at right angles to the truly ensiform limb of *Philydrum*. In accordance with this difference of construction, the leaf anatomy of the species which I have been able to examine, *H. acorifolia*, proves to be dorsiventral, with palisade parenchyma on the adaxial surface (fig. 23 *A*, *B*). The most striking feature of the leaf from the present standpoint is that, despite its dorsiventrality, it is characteristically phyllodic in structure, containing, besides normally orientated bundles (*nb*), others that are inverted (*ib*). Fig. 23 *C* shows the inverted group from the upper side of the midrib, in greater detail.

Conclusions

CLASSIFICATION OF LEAF TYPES IN FARINOSAE

In the preceding pages the treatment of the leaves of this cohort has been almost exclusively descriptive, but I propose now to consider the interpretation of their morphology. The most concise way is probably by means of a classification, based on the phyllode theory, of the principal types of leaf enumerated in dealing with the various families. On this basis the leaves of the Farinosae fall into the following six groups:

1. Phyllodes consisting of a sheathing base, and an ensiform limb equivalent to a petiole flattened in the vertical plane; for example, *Anarthria scabra* R.Br. (Restionaceae), fig. 3; *Xyris* (many species) (Xyridaceae), figs. 12, 14, 18; *Philydrum* (Philydraceae), fig. 22.

2. Phyllodes consisting of a sheathing base, and a limb departing little in character from a normal petiole and containing an arc or ring of bundles; for example, *Anarthria gracilis* R.Br. (Restionaceae), fig. 4; *Elegia deusta* Kth. (Restionaceae) (**10**, fig. 5 *A*, *B*); *Gaimardia australis* Gaudich. (Centrolepidaceae), fig. 5; *Xyris teretifolia* Pouls. (Xyridaceae) (**16**, fig. 3); *Pritzelia pygmaea* F. Muell. (Philydraceae), fig. 21.

3. Phyllodes essentially similar to (2), but in which the petiolar limb is reduced to a mere point; for example, *Leptocarpus peronatus* Mast. (Restionaceae), fig. 2; *Eriocaulon cuspidatum* Dalz (Eriocaulaceae), fig. 19; some Bromeliaceae.

4. Phyllodes similar to (3), but further reduced until they consist of leaf bases alone; for example, many Eriocaulaceae and Bromeliaceae.

5. Phyllodes in which the whole or the distal region of the petiole is flattened in the horizontal plane into a pseudolamina, containing inverted as well as normal bundles; for example, Pontederiaceae (figs. 7, 8); *Helmholtzia acorifolia* F. Muell. (Philydraceae), fig. 23.

6. Phyllodes in which the whole or the distal region of the petiole is expanded in the horizontal plane to form a pseudolamina without inverted bundles; for example, many Commelinaceae; *Rapatea* (Rapateaceae), figs. 24, 25.

COMPARISON WITH LEAVES OF OTHER COHORTS

One of the most striking results elicited by a general study of monocotyledonous leaves is the way in which certain leaf types recur again and again in this group, among plants by no means closely allied to one another. The leaves of the Farinosae afford many examples of these parallelisms. I will confine myself to an attempt to trace some of the relations between the leaf types of the Farinosae and those of two other cohorts, Helobieae and Liliiflorae; the Liliiflorae include those monocotyledons most nearly related to the Farinosae (11), while the Helobieae are somewhat more remote.

The ribbon leaves of certain aquatic species of *Eriocaulon* recall the leaves of a corresponding form met with so frequently among the Helobieae (7), although, if my interpretation be correct, the ribbon leaves of Helobieae are of petiolar nature, while in those of *Eriocaulon* merely the leaf base is represented. Although the proportion of the parts is so different, it is such a leaf as that of *Restio tremulus* (fig. 1 *A*), rather than that of a submerged *Eriocaulon*, which is equivalent to the ribbon leaf of, for instance, *Cymodocea nodosa* of the Potamogetonaceae (7). On the other hand, those leaves of the Farinosae in which the limb, although linear, is rather awl-like than ribbon-like, such as *Tillandsia usneoides* (Bromeliaceae, fig. 20), and *Gaimardia australis* (Centrolepidaceae, fig. 5), may be closely compared with *Cymodocea manatorum* (7). The venation of the limb of *Streptolirion volubile* of the Commelinaceae again (fig. 9) resembles that of *Alisma parnassifolium* (7). A contrast with the Helobieae, however, is furnished by *Monochoria hastaefolia* (fig. 7), whose venation is essentially different from those of the species of *Sagittaria*, which it recalls in the outline of its pseudolamina (7). Turning to more detailed structure, the inverted bundles in the leaf limb of the Pontederiaceae and of *Helmholtzia* find their analogue in those of certain Hydrocharitaceae.

The occurrence of the ensiform leaf in the Farinosae, both in the Restionaceae, Xyridaceae, and Philydraceae, is another instance of the widespread distribution of this leaf type among the monocotyledons. It is known from the Helobieae, Spathiflorae, Liliiflorae, and Microspermae, as well as in the Farinosae. Not only in form, but also in internal structure, this type of leaf shows remarkable uniformity in the different groups. *Xyris Wallichii* (fig. 12 *A*) and *X. brevifolia* (fig. 18 *A*), with their alternating bundles, can be paralleled in *Iris* (1, 6). The fibrous margins, also, which are so marked a feature of the ensiform leaves of many Liliiflorae (6), reappear in *Xyris*. For comparison with the leaves of the Farinosae, fig. 6 *A* and *B* show a section of the ensiform limb of *Hewardia tasmanica* Hook. (Liliaceae). It will be seen that in the distribution of the marginal fibers it resembles both *Tritonia* (fig. 10), and *Xyris Wallichii* (fig. 12 *C*) and *X. anceps* (fig. 14 *D*). This similarity is also carried into the details of the vascular system. Fig. 6 *A* shows examples from the limb of *Hewardia* of opposite bundles imbedded in a common fibrous sheath, and thus resembling the pair of bundles from the limb of *Xyris montivaga* Kth., shown in fig. 15. With this may also be compared the paired bundles of *Tofieldia* of the Liliaceae (**1**), *Tetroncium* of the Juncaginaceae (**7**), and *Tritonia* (fig. 16 *A*, *B*) and *Moraea Robinsoniana* (**6**). These bundle pairs are not only characteristic of the ensiform leaves of monocotyledons, but may be found also among the *Acacia* phyllodes, with which I believe these leaves to be homologous; they occur, for instance, in *Acacia neurophylla* (**6**). In addition to these bundle pairs, which clearly originate by more or less complete fusion of strands belonging to the opposite sides of the phyllode, *Xyris* also shows bundle groups of a different nature, illustrated here in the case of *Xyris asperata* (fig. 13 *A*–*C*) and *X. anceps* (fig. 14 *A*). These find their parallel in the tribe Johnsonieae of the Liliaceae (**5**). In the limb of *Arnocrinum Drummondii* Endl. there are bundle groups imbedded in fibers, which may be compared with those of *Xyris asperata*. It is true that they do not, as in the case of *Xyris*, occur in an ensiform leaf, but the ensiform leaf type is found in *Johnsonia*, to which *Arnocrinum* is probably more nearly related than it is to the five other members of the tribe.

The Farinosae furnish additional evidence for the close relationship of the ensiform and "radial" types of leaf. This relation, to which attention has already been called, both in the case of the leaves of the Liliiflorae and of the phyllodes of *Acacia*, is displayed with special clearness in *Iris*, among whose species there are examples of both forms of leaf, and also of intermediate types. In the Restionaceae, Xyridaceae, and Philydraceae there are comparable cases. Within the genus *Anarthria* both types are found (cf. figs. 3 and 4), and the same is true of *Xyris* (cf. figs. 12, 14, 18 with POULSEN'S fig. 3 of *X. teretifolia*, **16**); while the ensiform leaf of *Philydrum* (fig. 22) may be compared with the "radial" leaf of the related genus *Pritzelia* (fig. 21).

The type of leaf consisting of a sheathing leaf base terminating in a more or less cylindrical apex, which I interpret as a reduced petiole, occurs both in the Farinosae and Liliiflorae. For instance, the leaf of *Elegia deusta* Kth. (Restionaceae) is closely similar to that of *Distichia clandestina* Buch. of the Juncaceae (**10**). The apical tendril of *Flagellaria* (Flagellariaceae) also recalls that of *Gloriosa* and other Liliaceae. I hope to discuss the morphology of these leaf tendrils in a later paper.

To complete the parallel between the leaves of the Farinosae and those of the Liliiflorae, it may be noted that the limbs of the Pontederiaceae and of *Helmholtzia*, with their inverted bundles, to some extent approach those of certain species of *Allium* (**5**), and of such Amaryllids as *Zephyranthes* (**8**); while the similarity in shape of the cordate leaf limb of the Commelinaceous climber, *Streptolirion volubile* (fig. 9), and that of various Dioscoreaceae and Liliaceae is of wider interest, since it is an example of the recurrence of a form which appears again and again among monocotyledons. In former papers (**1, 4**) I have brought together a number of instances, from this class, of leaves with a cordate base; the list of families in which leaves of this type are found may now be increased to ten by the addition of the Stemonaceae, Amaryllidaceae, and Hydrocharitaceae. This comparison between the leaves of the Farinosae and those of the Helobieae and Liliiflorae emphasizes again the important part which parallelism of development has played in the evolution of the monocotyledonous leaf. The tendency for related stocks (even those whose affinity is far from close) to progress along corresponding lines is no doubt a very general feature of evolutionary history, although its prevalence is only gradually receiving full recognition. The fact that, in the monocotyledonous leaf, such parallelisms are displayed to an almost exaggerated degree, becomes to some extent explicable, if this organ is regarded as a phyllode consisting of leaf base and petiole alone. The loss of the lamina would inevitably impose restrictions upon the further evolution of the leaf, by confining its potentialities within a narrowed boundary. It might thus induce a tendency to the repetition of a definite series of forms, monotonous in the basic features of their construction, but endlessly varied within their given limits.

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LITERATURE CITED

1. ARBER, AGNES, The phyllode theory of the monocotyledonous leaf, with special reference to anatomical evidence. Ann. Botany **32**:465–501. 1918.
2. ———, The vegetative morphology of *Pistia* and the Lemnaceae. Proc. Roy. Soc. B. **91**:96–103. 1919.
3. ———, Leaf-base phyllodes among the Liliaceae. BOT. GAZ. **69**:337–340. 1920.
4. ———, Tendrils of *Smilax*. BOT. GAZ. **69**:438–442. *pl. 22.* 1920.
5. ———, On the leaf structure of certain Liliaceae, considered in relation to the phyllode theory. Ann. Botany **34**:447–465. 1920.
6. ———, The leaf structure of the Iridaceae, considered in relation to the phyllode theory. Ann. Botany **35**:301–336. 1921.
7. ———, Leaves of the Helobieae. BOT. GAZ. **72**:31–38. *pl. 1.* 1921.
8. ———, Leaves of certain Amaryllids. BOT. GAZ. **72**:102–105. 1921.
9. ———, On the development and morphology of the leaves of palms. Proc. Roy. Soc. B. **93**:249–261. 1922.
10. ———, On the leaf-tips of certain monocotyledons. Linn. Soc. Jour. Bot. **46**:467–476. 1922.
11. ENGLER, A., Die systematische Anordnung der monokotyledoneen Angiospermen. Abhandl. Akad. Wiss. Berlin. Abhandl. II. 1–55. 1892.
12. GLÜCK, H., Die Stipulargebilde der Monokotyledonen. Verhandl. Nat.-Med. Vereins Heidelberg. N.F. **7**^{1}:1–96. *pls. 1–5.* 1901.
13. GOEBEL, K., Organographie der Pflanzen. Zweite Auflage. Teil 1. Allgemeine Organographie. 1913.
14. MCLEAN, R. C., The utilization of herbarium material. New Phytol. **15**:103–107. 1916.
15. MEZ, C., Bromeliaceae, in MARTIUS, C. F. P. VON, Flora Brasiliensis **3**: part 3. 1892.
16. POULSEN, V. A., Anatomiske Studier over Xyris-Slaegtens vegetative Organe. Videnskab. Meddel. Naturhist. Forening Kjøbenhavn. pp. 133–152. *pls. 2, 3.* 1892 for 1891.
17. SEUBERT, M., Xyridaceae and Mayaceae, in MARTIUS, C. P. F. VON, Flora Brasiliensis. **3**: part 1. 1855.
18. VELENOVSKÝ, J., Vergleichende Morphologie der Pflanzen. Teil 2. 1907.

EXPLANATION OF PLATES I–III

Throughout, xylem (*xy*) shown in black; phloem (*ph*) in white; and fibers (*f*) dotted; *lac*, lacuna; *pp*, palisade parenchyma; *mb*, median bundle;

ep, epidermis; *s*, sheath; *pet*, petiole; *l*, limb; *n*, node; *ax*, axis; in all sections of equitant leaves, the identity of abaxial and adaxial margins has had to be decided on internal evidence, owing to fragmentary character of herbarium material available.

PLATE I

FIG. 1 *A–D.—Restio tremulus* R.Br.: *A*, axis bearing leaf (×½); *B*, transverse section sheath of leaf (×14); *C* and *D*, transverse section limb of another leaf (×23); *D* nearer apex than *C*.

FIG. 2.—*Leptocarpus peronatus* Mast., axis bearing leaf (×½).

FIG. 3 *A*, *B.—Anarthria scabra* R.Br.: *A*, transverse section leaf limb (×14), section slightly reconstructed at upper margin; *B*, dorsal (abaxial) margin of *A* (×77).

FIG. 4 *A–C.—Anarthria gracilis* R.Br., transverse section one leaf: *A*, sheath (section slightly reconstructed at margins of sheath); *B*, basal part of limb; *C*, limb (×23).

FIG. 5 *A–C.—Gaimardia australis* Gaudich.: *A*, leaf (×3½ circa); *B* and *C* transverse section of one leaf (×77); *B*, sheath; *C*, limb.

FIG. 6 *A*, *B.—Hewardia tasmanica* Hook. (for comparison): *A*, transverse section leaf limb (×23); *B*, upper margin of *A* (×77).

FIG. 7.—*Monochoria hastaefolia* Presl, limb of leaf to show venation (×½).

FIG. 8 *A–C.—Eichhornia speciosa* Kth.: *A*, small leaf to show dilated petiole and ligular sheath (*lig.s*) (×½); *B*, top of sheath from *A*, viewed from adaxial side to show three ligular lobes (×½); *C*, transverse section through margin of leaf limb in *A*, in direction of arrow (×14); *nb*, normally orientated bundles; *ib*, inverted bundles.

FIG. 9.—*Streptolirion volubile* Edgw., axis bearing leaf (×½).

FIG. 10.—*Tritonia*, garden hybrid (for comparison), margin of ensiform leaf (×193).

FIG. 11 *A–B.—Centrolepis aristata* R and S.: *A*, axis of small plant bearing inflorescence inclosed in bracts (*b*, *b*), foliage leaf to right (×½); *B*, transverse section sheath foliage leaf (×23); *B*, transverse section limb (×77); *bs*, bundle sheath, consisting of one inner thick-walled layer, and one outer layer of larger cells with thinner walls.

PLATE II

FIG. 12 *A–C.—Xyris Wallichii* Kth.: *A*, transverse section leaf limb (×23); *B*, stomate from *A* (×198); *C*, margin of *A* (×198).

FIG. 13 *A–C.—Xyris asperata* Kth. (*trachyphylla* Mart.): *A*, transverse section transition region between sheath and limb (×23) to show bundle groups; *B*, bundle group marked *x* in *A* (×77); group of four bundles (b_1, b_2, b_3, b_4), in common fibrous sheath, from transverse section of leaf limb (×318).

FIG. 14 *A–D.—Xyris anceps* Lam.: *A*, transverse section limb of leaf (×23); b_1, single bundle; b_2, group of two bundles; b_3, group of three bundles; *B*, apex of leaf limb viewed as solid object (×23); *C*, fibrous margin of leaf viewed as solid object (×77); *D*, transverse section margin of *A* (×77).

FIG. 15.—*Xyris montivaga* Kth.: pair of opposite bundles (xy_1 and ph_1, xy_2 and ph_2) in common fibrous sheath (×318); similar to b_2 in fig. 14 *A* (*X. anceps*).

FIG. 16 *A*, *B*.—*Tritonia* (garden hybrid) for comparison, lettering as in fig. 15; fibers represented in black (instead of white with double lines indicating thickness of walls, as in other figures): *A*, pair of opposite bundles from transverse section of leaf limb (×318); *B*, similar pair of bundles, but with xylems fused (×318).

FIG. 17 *A–C*.—*Xyris gracilis* R.Br.: *A*, leaf consisting mainly of leaf sheath, with reduced limb (*l*); *B*, apex of another reduced leaf in which limb is absent (both natural size); *C*, transverse section of leaf shown in *B* (slightly enlarged).

FIG. 18 *A–B*.—*Xyris brevifolia* Mich.: *A*, transverse section leaf limb (×23); *B*, marginal bundle such as that marked *x* in *A* (×193).

FIG. 19 *A–D*.—*Eriocaulon: A* and *B*, *E. septangulare* With.; *A*, transverse section of limb of leaf (×23); *B*, lamella marked *x* in *A* (×77); *ue*, upper epidermis, *le*, lower epidermis; *d*, fragment of diaphragm seen in surface view; *C*, *E. Wallichianum* Mart. f. *submersa*, apex of leaf (×½); *D*, *E. cuspidatum* Dalz., apex of leaf to show mucro (×½).

FIG. 20 *A*, *B*.—*Tillandsia usneoides* L., transverse section leaf (×23): *A*, sheath; *B*, limb.

PLATE III

FIG. 21 *A–C*.—*Pritzelia pygmaea* F. Muell., transverse section leaf: *A*, sheath; *B*, limb (×23); orientation of *B* uncertain; *C*, margin of sheath in *A* (×77) to show stomate (*st*) and crystals (*c*).

FIG. 22.—*Philydrum lanuginosum* Banks, transverse section limb of leaf (×14).

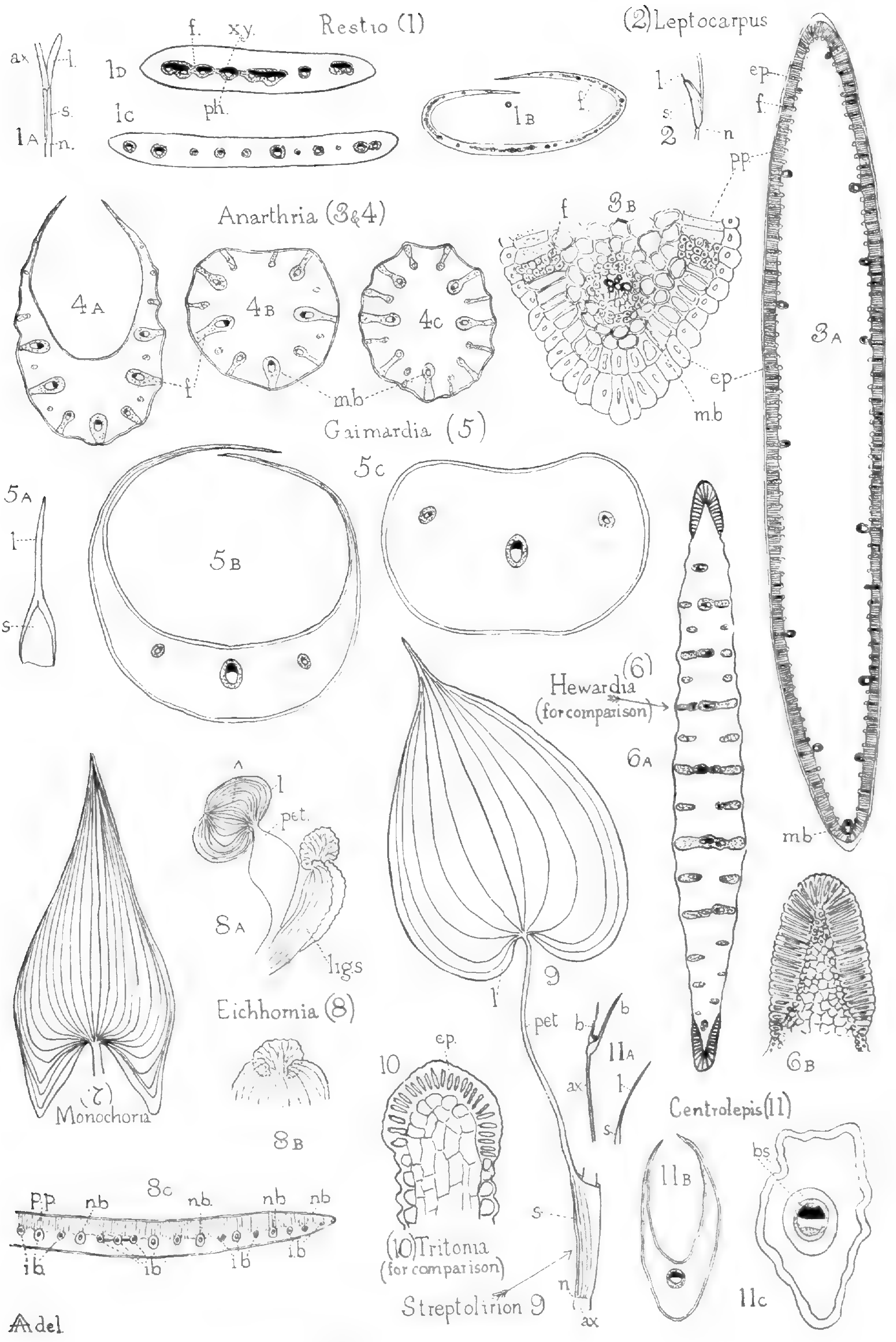
FIG. 23 *A–C*.—*Helmholtzia acorifolia* F. Muell., *A* and *B*, transverse section limb of leaf (×23): *A*, midrib region (*mr*); *B*, margin, to show normally orientated bundles and bundle groups (*nb*) and inversely orientated bundles and bundle groups (*ib*); *C*, inverted bundles of midrib from section similar to *A* (×77).

FIG. 24.—*Rapatea longipes* Spr., small leaf (×½).

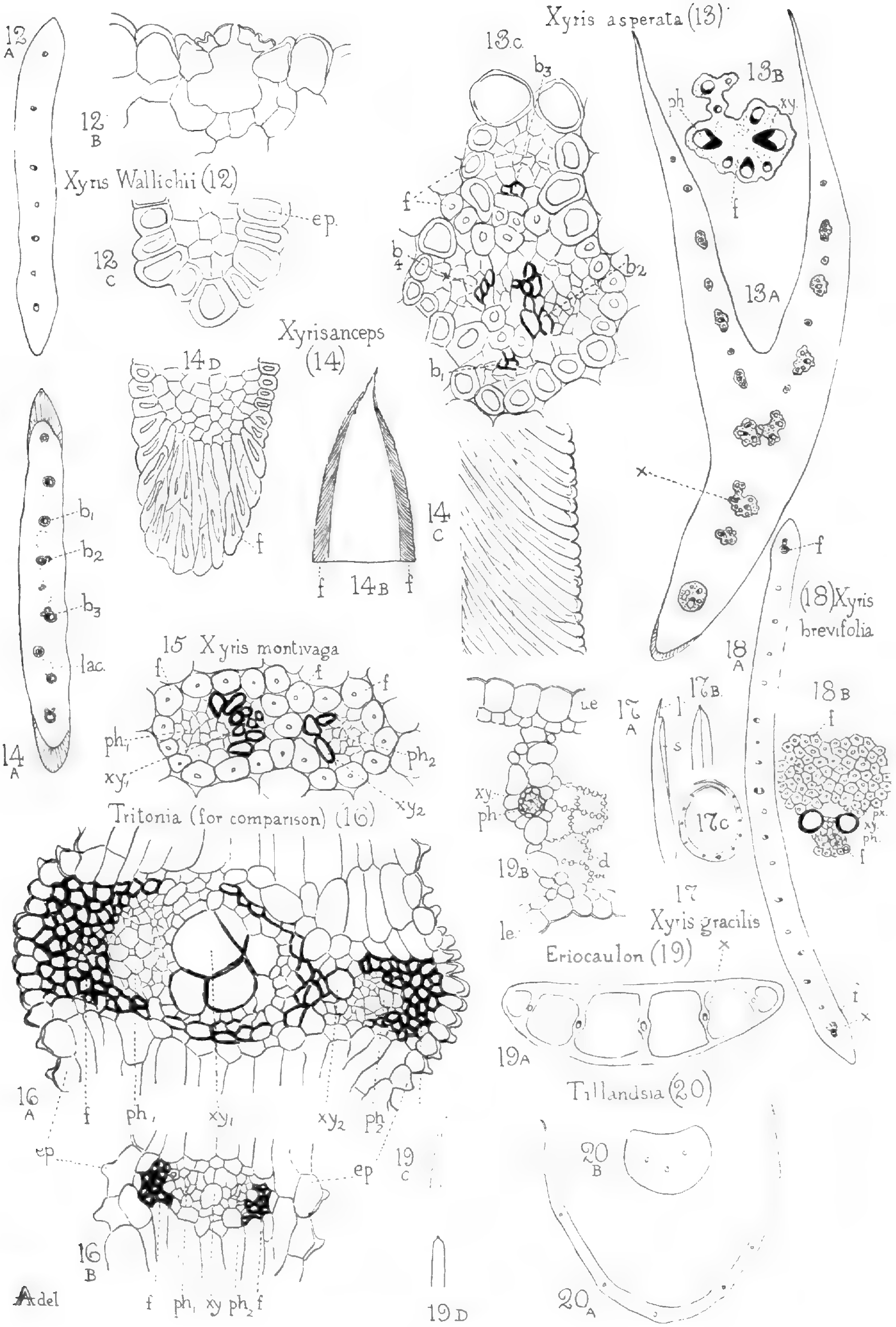
FIG. 25 *A–C*.—*Rapatea angustifolia* Spr.: *A* and *B*, transverse section leaf (×14); *A*, sheath; *B*, limb; *C*, median bundle (*mb*), of limb in *B* (×77).

FIG. 26 *A–C*.—*Cephalostemon affinis* Koern: *A*, *B*, transverse section leaf (×14); *A*, sheath; *B*, limb; *C*, median bundle of limb (×77).

FIG. 27 *A–B*.—*Mayaca fluviatilis* Aubl.: *A*, leaf (×½); *B*, leaf apex (×14); *vb*, vascular strand.



ARBER on FARINOSAE



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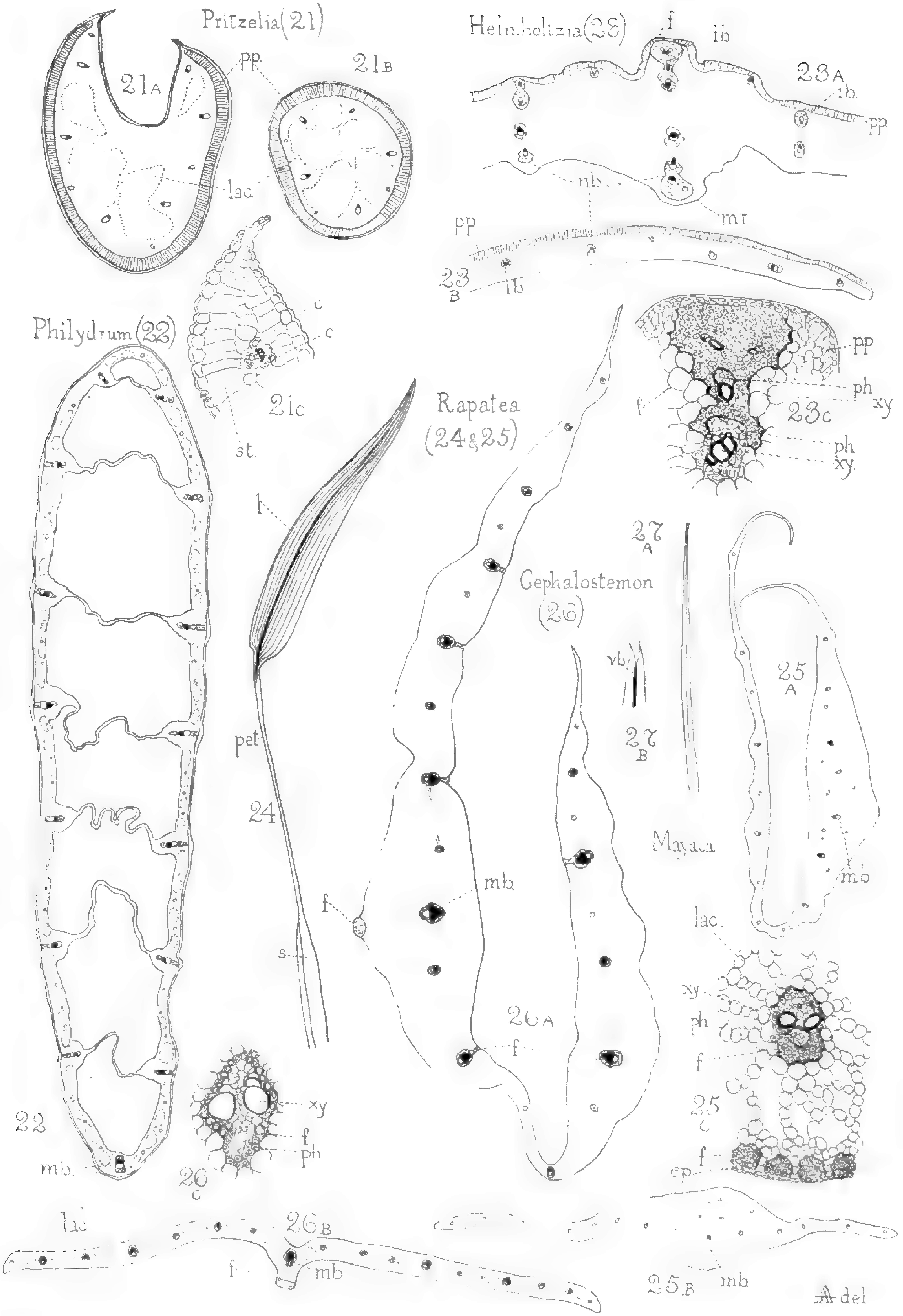

ARBER on FARINOSAE

INHERITANCE OF FRUIT SHAPE IN CUCURBITA PEPO. I

EDMUND W. SINNOTT

(WITH THREE FIGURES)

During the past few years much progress has been made in our knowledge of the inheritance of quantitative characters. We know much less, however, about the factors which control the interrelationships between these various size characters, and which thus determine the shape of the organism. The present paper is a preliminary report on some investigations dealing with certain phases of shape inheritance in the summer squash.

For the past six seasons the writer has been carrying on some breeding work with a number of the common types of *Cucurbita Pepo*. Commercial material of this species is apt to be very much hybridized and consequently to yield a remarkable variety of forms. Many strains are also self-sterile, or become so after a year or two of inbreeding. A considerable variety of types was obtained from four leading seed firms in the spring of 1916, and an attempt was made, by persistent self-fertilization, to establish from this highly heterozygous material types which would be essentially pure. Of course the majority of plants refused to set seed under these conditions, but about twenty-five were found which were self-fertile, and the offspring of these (except for a number of lines which have since been lost through sterility) have been continuously inbred for six generations. In most cases sterility disappeared by the fourth generation, and by this time such a high degree of uniformity was shown in all plant characters as to make it clear that a fairly close approach to homozygosity had been attained. In 1919 a number of crosses involving all the more notable character differences were made among these various pure types, and the uniformity of the F_1 in every case gave further assurance that the parent types were approximately pure. An F_2 generation from each of these crosses was made during the past season (1921).

A number of the pedigrees involved rather radical shape differences in the original types crossed. Several pure strains of the ordinary "scallop" or "pattypan" type were obtained (fig. 1) with fruit essentially disclike in shape, being from two to four times as broad as thick, with the teeth or scallops around the edge varying in number, shape, and development. In one race of white discs there appeared very early several plants in which the fruit differed markedly from that of the disc parent, being nearly spherical in shape, approximately as thick as broad, and with a rather weak development of teeth. Intermediate forms did not appear, and the spheres when inbred never produced any other shape. Several pure lines with spherical fruits were thus obtained (fig. 1).

Fig. 1

A single plant of one of these spherical-fruited lines was crossed in 1919 with a plant from each of three disc-fruited lines. These three lines differed somewhat in fruit shape, one having a relatively flat disc and the others relatively deep ones, one being somewhat flatter than the other. The F_1 generation in each case showed complete dominance of the disc shape, and the type of disc (deep or flat) was essentially like that of the particular disc parent used. In the F_2 generation there was a sharp segregation into approximately three-quarters disc and one-quarter sphere. It is evident that the chief difference between these two shapes is caused by a single factor, and that this shows complete dominance. F_2 counts for the three pedigrees, together with the results expected on the single factor hypothesis, are shown in table I.

The problem is not quite so simple as this, however, for the F_2 segregates do not resemble exactly the original types, it being especially noticeable that the sphere segregates differ in shape, those coming from the crosses where the flattest disc was used being noticeably flatter than those coming from the crosses where the thickest disc was used.

TABLE I

FRUIT SHAPE IN F_2 OF THREE CROSSES OF DISC×SPHERE

Pedigree	Disc	Sphere
15	83	18
1	79	29
19	41	13
Total	203	60
Expectation, 3:1	197	66

To bring out more clearly the various shape differences, a study of the relative sizes of the two major dimensions, width and length, as represented by an index, is useful. This shape index for a given fruit is its width (the dimension at right angles to the fruit axis) divided by its length or thickness (dimension parallel to the fruit axis). The means for the shape index of parents, the F_1, and the two extracted types in the F_2 for the three pedigrees, are set forth in table II. The frequency distribution of the individuals in the three pedigrees with respect to this shape index is also given in fig. 2.

TABLE II

MEANS FOR FRUIT SHAPE INDEX (WIDTH÷LENGTH) IN THE FOUR PURE LINES STUDIED, IN F_1 AND THE TWO TYPES SEGREGATING IN F_2, FOR THE THREE PEDIGREES; FIGURES IN PARENTHESES ARE INDICES EXPECTED ON BASIS OF TWO-FACTOR HYPOTHESIS

PURE LINES	OBSERVATION	EXPECTATION	F_1	OBSERVATION	EXPECTATION	F_2	DISC		SPHERE	
							Observation	Expectation	Observation	Expectation
103 (sphere)	0.97	......								
15 (disc)	2.48	(2.47)	15	2.59	(2.47)	15	2.51	(2.47)	0.86	(0.97)
1 (disc)	2.50	(2.72)	1	2.91	(2.72)	1	2.61	(2.66)	1.20	(1.16)
19 (disc)	3.28	(3.22)	19	3.34	(3.22)	19	2.94	(3.03)	1.50	(1.53)

Records for a comparatively small number of individuals of the four pure types are here presented, since only a few plants are grown of each of the various pure lines in any one year. The F_1 generations were also small, but the F_2 generations were considerably larger. Cultures involving much greater numbers are now being grown.

From a study of these few individuals, however, certain points stand out clearly. The F_1 shows complete dominance of the disc shape. In fact, in every case the F_1 is even a little flatter than the parent disc. This is notably the case in pedigree 1, but there is reason to believe that the pure type of this line is probably a little flatter than is shown by the individuals here portrayed.

In pedigree 15, which involves the deepest disc, both the sphere and disc F_2 segregates are essentially like the original types, being a little deeper in each case. A single factor difference between disc and sphere is apparently sufficient to account for these facts. It will be noted that there is a very sharp segregation between the two shapes in the F_2. In pedigree 19, however, which involves the flattest disc, the F_2 spheres are decidedly flatter than the parent sphere type (1.50 as compared with 0.97), and the F_2 discs distinctly deeper than the parent disc type (2.94 as compared with 3.28). Pedigree 1 is somewhat intermediate between these, the F_2 spheres being somewhat flatter than the original sphere type, and the F_2 discs being about the same as the original disc type, but considerably deeper than the F_1 discs.

In pedigrees 19 and 1 there is evidently something more than a single disc or "flattening" factor at work. This coincident flattening of the spheres and deepening of the discs may readily be explained by assuming that there is a second dominant flattening factor, considerably weaker in its effect than the major one already discussed, and segregating independently of it. The parent disc type would possess both of these and the parent sphere type lack them both. The major difference between disc and sphere would still be caused by the larger factor, the "spheres" lacking it and the "discs" possessing it. In the F_2, however, three-quarters of the spheres would possess the smaller flattening factor, and the average shape of the whole sphere group would thus be flatter than that of the pure type; and a quarter of the discs would lack this smaller factor and thus be less flat than the original discs in which both flattening factors are present. This would tend to bring the two shape types somewhat nearer together in the F_2 than in the pure types, a condition which evidently obtains in these two pedigrees.

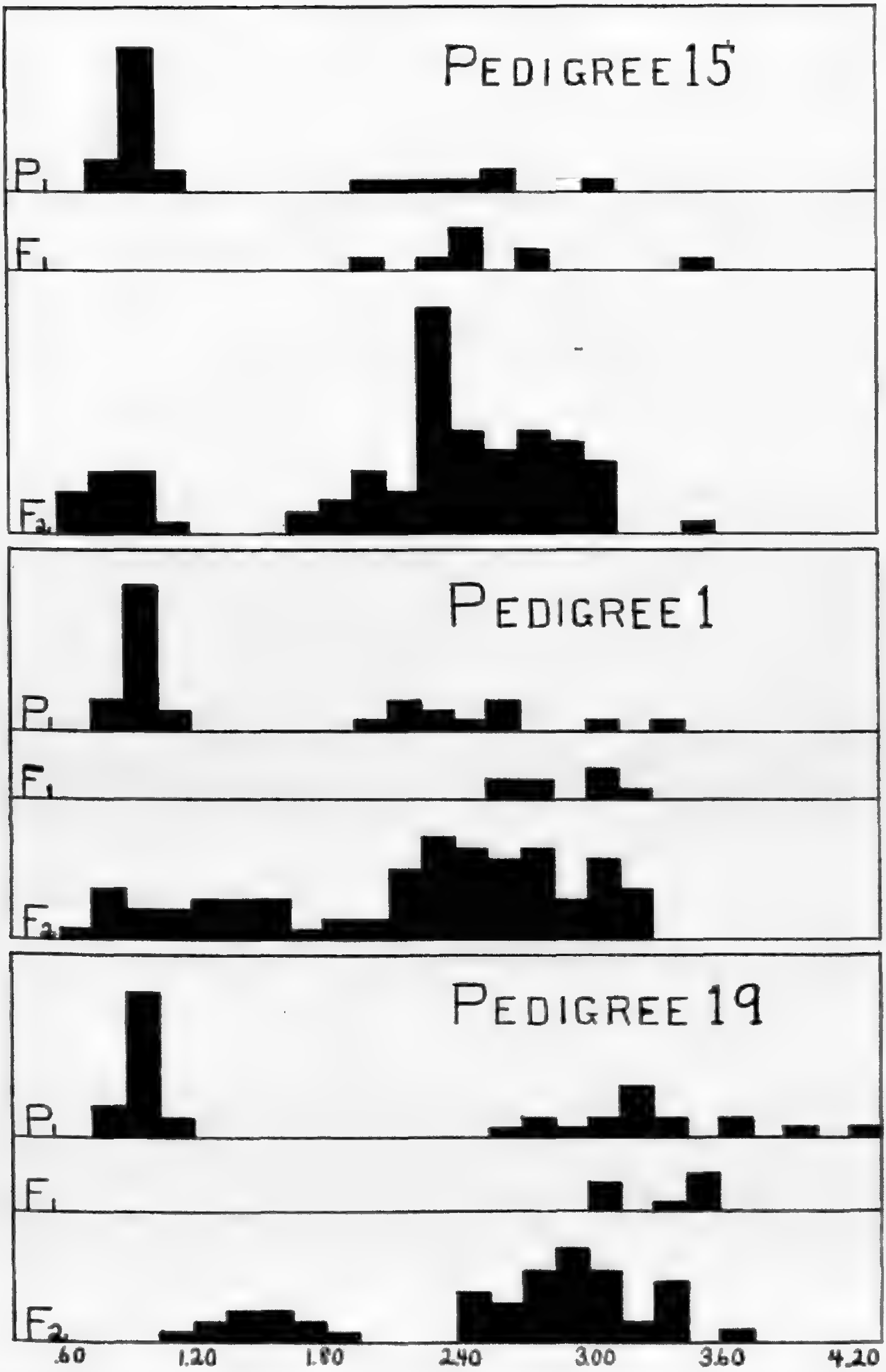

FIG. 2.—Fruit shape index (width/length) in parents, F_1 and F_2 of three pedigrees

If we assume that the discs used in pedigree 15 differ from the spheres by the possession of a single dominant flattening factor *A*, which increases the index by 1.50, that in pedigree 19 the disc type possesses in addition to this a second dominant flattening factor *B*, which increases the index by 0.75, and that in pedigree 1 the supplementary flattening factor (which we may call *C*) is smaller and can increase the index by only 0.25, we would obtain approximately the shapes which we actually find in the F_2. The original sphere type has an index of 0.97. The disc in pedigree 15 would thus have an index of 0.97 plus 1.50, or 2.47, as compared with the 2.48 which was found. The F_1 and F_2 types would be expected to repeat these indices, a condition which they come reasonably close to doing. In pedigree 19, however, the parent disc (*AB*) would have an index of 0.97 plus 1.50 plus 0.75, or 3.22, as compared with the 3.28 observed. The F_1 should be approximately the same. In the F_2 three-quarters of the spheres would be *aB*, 0.97 plus 0.75, and the other quarter *ab*, 0.97 only, giving a mean for the whole sphere group of 1.53. Similarly, three-quarters of the discs would be *AB* or 3.22, and one-quarter *Ab* or 2.47, giving a mean for the disc group of 3.03. These indices are close to the actual figures. The same general situation would occur in pedigree 1, factor *C* being present instead of *A*, and the original disc type being *AC*. In table II the actual indices and the theoretical expectations are both presented.

It will be noted that this hypothesis does not explain the fact that the F_1 discs are in every case flatter than the pure types. The numbers involved are also much too small to prove such a hypothesis. They certainly seem to indicate, however, that in two of the disc types more than one factor is responsible for the shape difference between it and the sphere, and that these two factors are of unequal effect.

This is probably a rather simple case of shape inheritance. Indeed, other pedigrees at present in process of completion indicate that shape inheritance in squashes is often much more complex, involving a considerable number of factors, some of which also show lack of dominance. In GROTH'S[1] work with tomatoes, a

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[1] GROTH, B. H. A., Some results in size inheritance. N.J. Agric. Exp. Sta. Bull. 278. pp. 92. *pls. 34.* 1915.

study of the F_2 in a number of crosses involving differences in fruit shape shows that a distinct bimodal curve is present, thus suggesting a segregation something like that here reported in squashes. In many other shape crosses studied by this investigator, however, the F_2 showed a wide range of shapes with no clear cut segregation of types, thus suggesting the operation of a larger number of factors. EMERSON's[2] work with squashes also shows a great increase in the variability of shape in the F_2 and (apparently) no clear cut segregation. The parental types used by EMERSON, however, were not inbred homozygous strains.

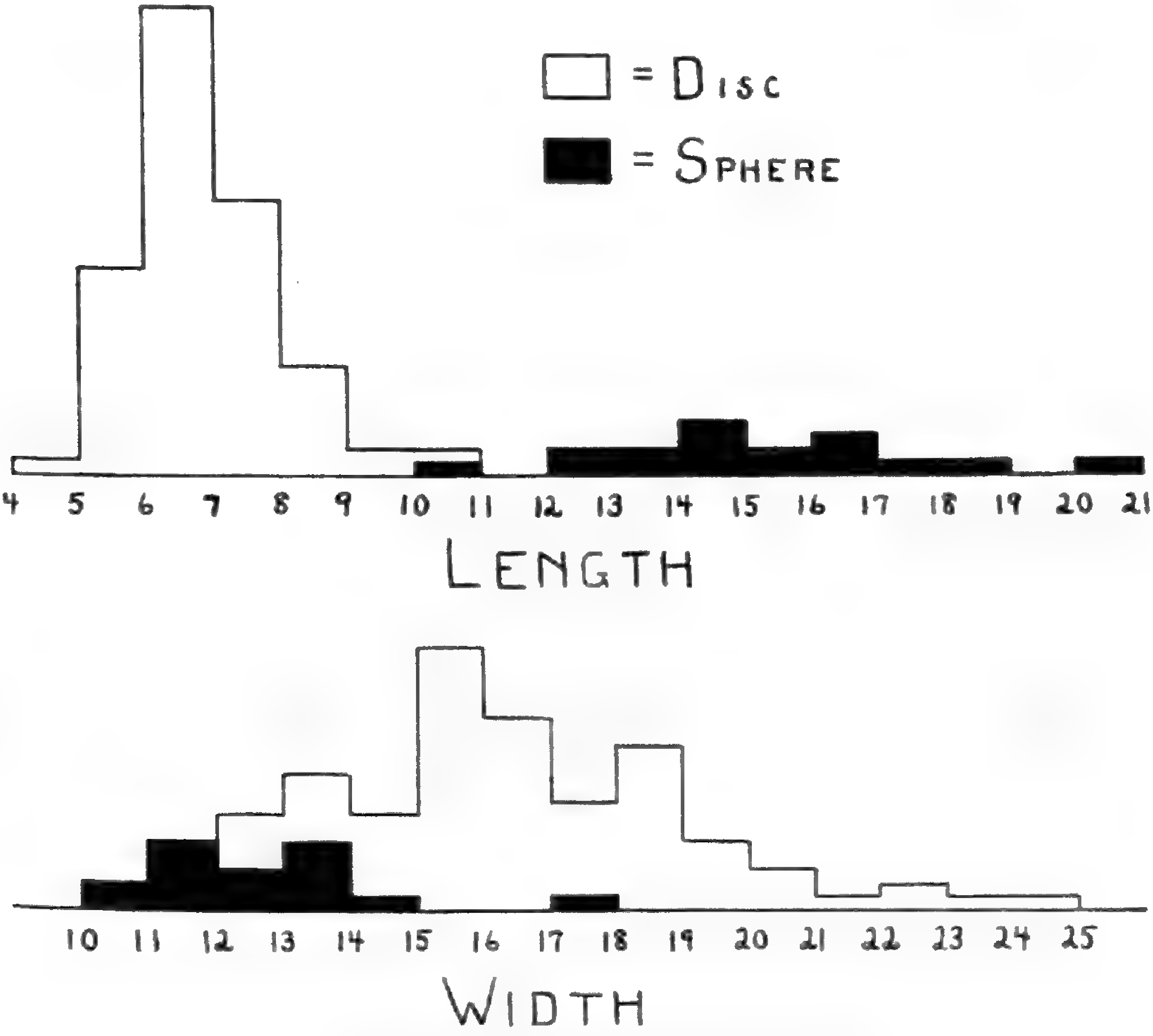

FIG. 3.—Length and width of F_2 fruits, pedigree 19

It is assumed that in the present case such things as "shape factors" exist in the germ plasm and are operative. It may be objected

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[2] EMERSON, R. A., The inheritance of sizes and shapes in plants. Amer. Nat. **44**:739–746. 1910.

that the results are due merely to a segregation of those size factors which control fruit thickness and fruit width. That such an explanation is scarcely tenable, however, is indicated by a study of the actual dimensions in the F_2, for here we find that the squashes which are the longest (thickest) are also those which are narrowest (thus producing the sphere types), and that the squashes which are thinnest are also those which are widest (thus producing the disc types). The frequency distribution of the actual lengths and the actual widths of the F_2 fruits in pedigree 19 are set forth in fig. 3. That part of each curve which consists of individuals which are spherical in shape is shaded, the discs remaining white. In fruit length there is evidently a fairly clear segregation into long and short, but no segregation is apparent in width. It is noteworthy, however, that the long fruits are not scattered irregularly through the various widths, as would be the case if length and width segregated independently, but that in practically all cases these long fruits are considerably narrower than the average, and the short fruits wider than the average, so that instead of a great variety of types in two rather vague groups, two very distinct shapes result.

There is evidently something controlling the dimensional proportions which the individual exhibits, and thus determining its shape. The relation between these shape factors and those which control size is a matter of considerable interest. The suggestion is perhaps worth considering that the ordinary "size" factors govern in some way the total amount of growth attained, and that the shape factors control the distribution and proportions of this growth.

Summary

1. Pure lines of summer squashes differing in fruit shape have been isolated.

2. In crosses between a type with approximately spherical fruits and three different races having "scallop" or "disc" fruits, the disc shape showed complete dominance in the F_1 in every case, and in the F_2 there was a sharp segregation into three-quarters disc fruits and one-quarter sphere. A single, large, dominant flattening factor thus seems to distinguish these disc types from the spherical ones.

3. In two of these crosses, the extracted spheres are distinctly flatter than the pure types, and the extracted discs distinctly deeper than the pure discs. This can be explained by assuming the operation, in each case, of a second flattening factor, also dominant, but showing a much smaller effect than the first.

4. Evidence is brought forward that shape-determining factors actually exist, and that the facts here set forth are not due merely to the segregation of size factors.

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BIOCHEMISTRY OF PLANT DISEASES[1]

IV. PROXIMATE ANALYSIS OF PLUMS ROTTED BY SCLEROTINIA CINEREA

J. J. Willaman and F. R. Davison

(WITH TWO FIGURES)

In the third paper of this series (**6**) the literature bearing on the chemistry of plant diseases, especially the effect of disease on the composition of plants and the chemical differences between resistant and non-resistant varieties of the same species of plant, was reviewed in some detail. Since the present work is a continuation of the former, it will not be necessary to make reference again to the results of other investigations, except incidentally during the discussion.

In the previous work on the brown rot of plums, caused by the fungus *Sclerotinia cinerea,* it was found that during the progress of the rotting the H-ion concentration of the sap increases markedly; that oxalic acid is produced, but hardly in sufficient quantity to account for the increased acidity; that tannin decreases during the rotting; that protein nitrogen increases, due probably to the protein formed in the fungus mycelium. Resistant varieties did not differ conspicuously from non-resistant varieties, so far as the analyses showed, except that the resistant varieties usually had a more acid sap, and that more oxalic acid was produced in them. The tannin content of green plums usually increases after picking from the tree, but infection by *Sclerotinia* entirely inhibits this increase.

Experimentation

In the present work the ordinary proximate analyses, together with the determination of calcium, were made, using four varieties of plums at three stages of maturity, grown at the University Fruit

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[1] Published with the approval of the Director as Paper no. 272, Journal Series, Minnesota Agricultural Experiment Station. Presented at the meeting of the American Chemical Society, September 9, 1921.

Breeding Farm at Excelsior in 1918.[2] In table I are given the data concerning the samples.

PREPARATION OF MATERIAL.—The plums were halved or quartered, freed from the pits, and placed in ovens at about 90°–95° C. until dry enough to grind. It was not thought necessary to use special precautions in drying, considering the nature of the analyses to be made. The finely ground material was stored in bottles. The plums were rotted by sterilizing in mercuric chloride solution, and then inoculating with a suspension of spores by means of a hypodermic syringe, plunging the needle right to the pit. It required from eight to fourteen days for complete rotting, as shown by the tissue becoming dark brown throughout.

TABLE I

DATA ON PLUM SAMPLES, 1918

Lab. no.	Variety	Abbreviation	Date picked	Stage of growth	Condition of fruit	No. of days required for rotting	Texture of rotted fruit
	Resistant						
79...	Burbank × Wolf 9	B × W 9	July 2	I	Green, half grown	9	Firm
87...	Burbank × Wolf 9	B × W 9	Aug. 21	II	Fully grown, not ripe	12	Firm
91...	Burbank × Wolf 9	B × W 9	Sept. 3	III	Ripe	10	Fairly firm
80...	Abundance × Wolf 18	A × W 18	July 2	I	Green, half grown	12	Firm
88...	Abundance × Wolf 18	A × W 18	Aug. 21	II	Fully grown, not ripe	9	Hard
92...	Abundance × Wolf 18	A × W 18	Sept. 3	III	Ripe	11	Firm
	Non-resistant						
81...	Compass	C	July 2	I	Green, half grown	6	Soft
89...	Compass	C	Aug. 2	II	Fully grown, not ripe	7	Soft and watery
93...	Compass	C	Aug. 8	III	Ripe	6	Soft
82...	Sand cherry × Formosa	SC × F	July 2	I	Green, half grown	6	Somewhat soft
90...	Sand cherry × Formosa	SC × F	Aug. 2	II	Fully grown, not ripe	7	Soft
94...	Sand cherry × Formosa	SC × F	Aug. 8	III	Ripe	6	Soft

METHODS OF ANALYSIS.—The moisture content of each sample was carefully determined at the time the other analyses were made, by drying to constant weight in a vacuum at 60° C. For the ash determination, 2 gm. samples were incinerated in platinum dishes, and then heated in a muffle at 550° C. until constant in weight. The calcium was determined in the ash by the McCRUDDEN method (3), which has proved to be very simple and accurate. The Official Methods (1) were followed for total nitrogen and ether extract,

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[2] The writers' thanks are due to Dr. M. J. DORSEY for his courtesies in furnishing the samples.

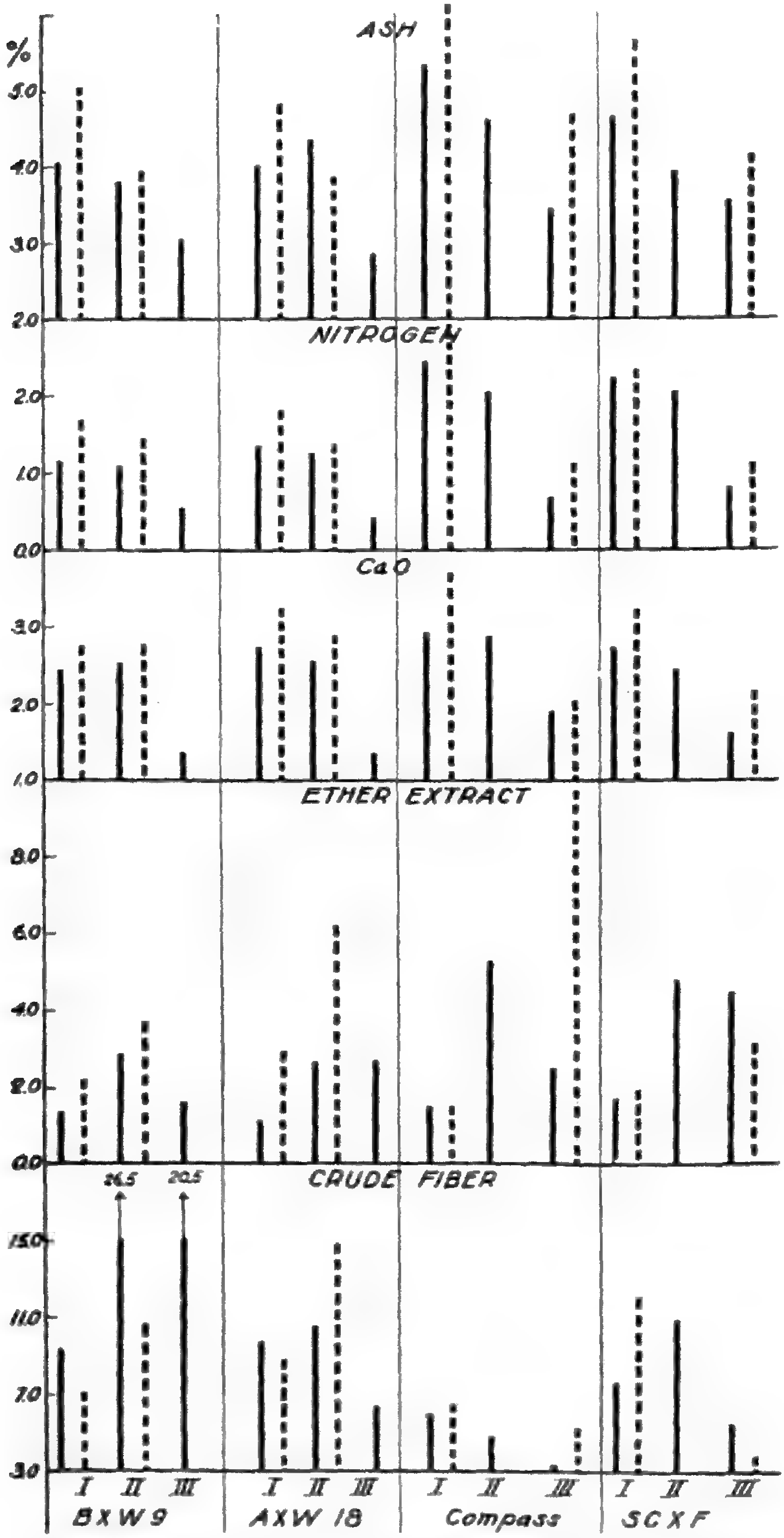

FIG. 1.—Composition of sound and of rotted plums of various varieties at three stages of growth: solid line, sound samples; dotted line, rotted samples; see table I for stages of growth and names of varieties.

and the KENNEDY (2) modification of the SWEENEY method was used for crude fiber.

Results

The data obtained in these analyses are shown graphically in figs. 1 and 2. All values are calculated to a moisture free basis. In fig. 1 the sound and rotted portions of each sample are placed side by side, so as to show the change in composition during rotting. It will be seen that in almost all cases the rotted portion shows a higher content of all constituents except crude fiber; the latter shows no general tendency in either direction, sometimes being greater and sometimes less in the rotted than in the sound material. The increase in these constituents is probably due to loss of dry matter through respiration. Previous work has shown that respiration is higher in infected than in sound plums, and this results in a relative increase in the substances determined. The figures for ether extract and for crude fiber are erratic in their changes, which is in keeping with the empirical nature of the methods of analysis.

In fig. 2 the same data are used, but they are arranged so as to give more direct comparison of varietal characteristics. The first

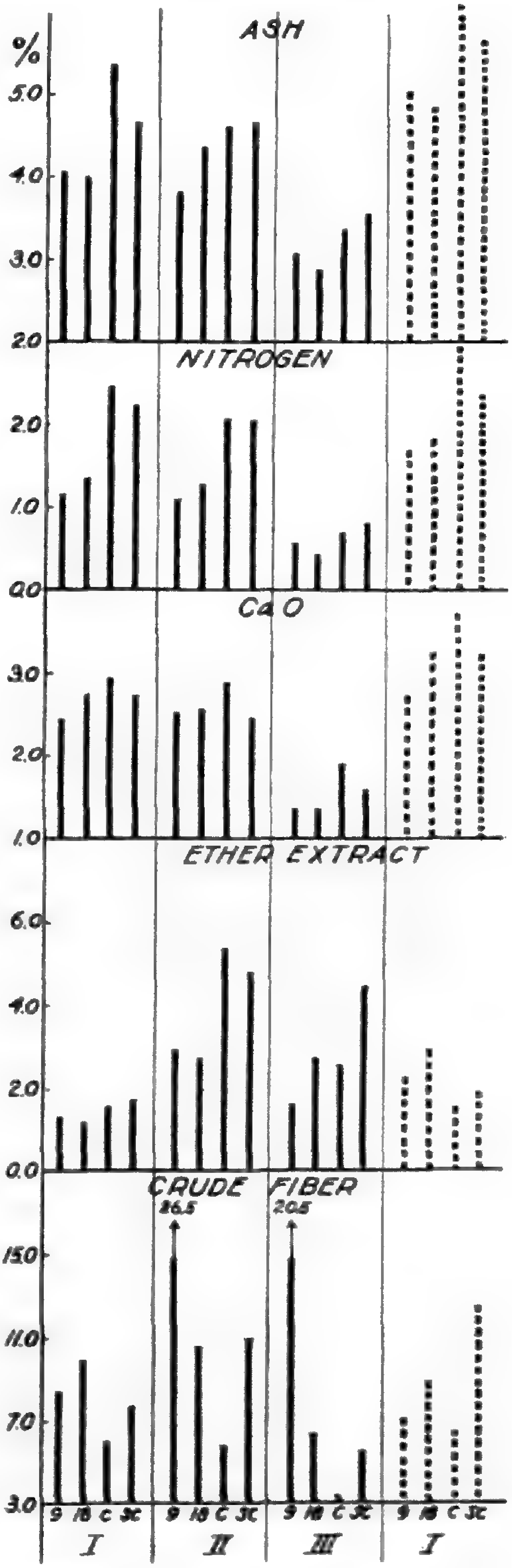

FIG. 2.—Composition of sound and of rotted plums, grouped to bring out varietal differences: solid line, sound samples; dotted line, rotted samples; first two varieties in each group are resistant to brown rot; second two are susceptible; see table I for stages of growth and names of varieties.

two varieties in each section, indicated by 9 and 18, are resistant; the next two are non-resistant. Some of the rotted samples are missing, due to loss by contamination. It will be seen that the crude fiber is markedly higher in the resistant than in the non-resistant varieties, but all other constituents are lower. This relation holds in both the sound and the rotted samples, although data for the latter are available for the first stage only. VALLEAU (4) reports a positive correlation between firmness of the plum flesh and its resistance to brown rot. The firmness is due to the structural elements of the tissue, the cellulose cell walls and the pectic middle lamellae. Only the former are represented in the determination of crude fiber. From the results at hand it appears quite probable that the quality and quantity of cellulose material are important factors in resistance properties, although the pectic substances also play a part in the metabolism of this fungus, as was pointed out in a previous paper of this series (5). In what ways the middle lamella may play a part in resistance properties is not known. Since it is a compound of pectin with calcium, it was thought that determinations of calcium in the present samples might throw some light on the question, but the data show that calcium is higher in the susceptible varieties in about the same magnitude as the ash and nitrogen, and no special significance can be seen in it.

In fig. 2 can be seen certain changes in the composition of plums during the course of ripening. As maturation progresses, the ash, nitrogen, and calcium steadily decrease. This is probably due to large increases in the soluble carbohydrates and organic acids. The crude fiber is somewhat higher in the second stage, which is just previous to full ripeness. The Compass variety is conspicuously low in crude fiber, and it is very susceptible to rotting by *Sclerotinia*. In table I the data on rate of rotting show the same tendencies as were discussed in the preceding paper of this series, namely, that the resistant varieties succumb to the rot much more slowly, and when rotted have a much firmer texture than the susceptible varieties.

Conclusions

1. Plum tissue that has been rotted by *Sclerotinia cinerea* is consistently higher in ash, CaO, nitrogen, and ether extract than

is the sound tissue. This is no doubt due to loss of dry matter by respiration in the rotted samples.

2. The resistant varieties are conspicuously higher in crude fiber than the susceptible. The quality and quantity of the structural elements of the tissues no doubt are important factors in their resistance properties. The ash, nitrogen, CaO, and ether extract are lower in the resistant varieties, but not sufficiently so to constitute limiting factors in the nutrition of the invading parasite.

3. As the ripening of plums proceeds, there is a decrease in the ash, nitrogen, and calcium content, due probably to storage of carbohydrates and acids.

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LITERATURE CITED

1. Association of Official Agricultural Chemists. Methods of Analysis. Baltimore. 1916.
2. Kennedy, C., A modification of the Sweeney method for crude fiber. Jour. Ind. and Eng. Chem. **4**:600–601. 1912.
3. Mitchell, J. H., Report on inorganic plant constituents. Jour. Ass. Official Agric. Chemists **4**:391–394. 1921.
4. Valleau, W. D., Varietal resistance of plums to brown rot. Jour. Agric. Res. **5**:365–395. 1915.
5. Willaman, J. J., Pectin relations of *Sclerotinia cinerea*. Bot. Gaz. **70**: 221–229. 1920.
6. Willaman, J. J., and Sandstrom, W. M., Biochemistry of plant diseases. III. Effect of the fungus *Sclerotinia cinerea* on plums. Bot. Gaz. **73**:287–307. *figs. 7.* 1922.

"MAGNESIA INJURY" OF PLANTS GROWN IN NUTRIENT SOLUTIONS

W. F. GERICKE

Among the first signs of injury to the tops of wheat seedlings and other cereals grown in nutrient solutions of relatively high (as compared with other salts in solution) concentration of $MgSO_4$, one of the three or more salts commonly used in culture solution experimentation, is that resulting in abscission of the leaf tips of the plants. The progression of this injury consists of wilting, withering, desiccation, and finally abscission. This injury has been found to be most pronounced on young shoots. It also occurs in a much greater degree under conditions conducive for high transpiration of the plants than for low. The term "magnesia injury" has been applied to this peculiar phenomenon of abscission of leaf tips, being so named because usually found associated with comparatively high concentrations of magnesium ion (from $MgSO_4$) in nutrient solutions. Additions of soluble calcium salts to nutrient solutions giving injury were often found to be beneficial. This lent support to the concept that a certain calcium-magnesium ratio, or a range thereof, plays an important part in the physiological balance of nutrient solutions.

Recent experiments by the writer appear to bear on this magnesium question. When wheat seedlings were grown in single salt solutions it was found that some cultures gave the typical symptoms and others did not. Briefly, the tests were as follows. Seedlings 6–8 cm. high, with roots 8–10 cm. long, were fitted into and supported on paraffined corks. These were fitted into one quart containers (Mason jars) filled with the single salt solutions to be tested. The solutions were prepared from Baker's C.P. analyzed salts and distilled water. The salts were tested to see whether they conformed to specifications, but no further purification was made. A small amount of $FeSO_4$ (5 drops of 0.01 mol solution) was added to the cultures at weekly intervals. The cultures were allowed to grow four weeks. Table I gives the experimental data.

The injury sustained by the seedlings grown in the solutions of potassium salts differed with the salts used, being least in the KH_2PO_4 set and most marked in that of K_2SO_4. Considerable abscission of the leaf tips occurred in the seedlings grown in the solutions of KNO_3, although these cultures made by far the best growth (total dry matter production) of any of the nine different salt solutions employed. In marked contrast to these are the results obtained from the cultures grown in the solutions of different calcium salts, none of which gave any symptoms of the characteristic injury. Of the cultures grown in the solutions of magnesium salts, those grown in $Mg(NO_3)_2$ and $MgSO_4$ were severely injured, but those grown in $MgHPO_4$ were not, being perfectly normal in this respect. While the P_H values of solutions were not the same, nevertheless it is not evident that this was an important factor to account for the results.

TABLE I

RELATIVE EFFECTS OF SALTS IN PRODUCING ABSCISSION OF LEAF TIPS OF WHEAT SEEDLINGS

Salts	Concentration (mol)	P_H value	Injury
KNO_3	0.01	6.3	Decided
KH_2PO_4	0.01	5.2	Slight (some plants showing none)
K_2SO_4	0.01	7.4	Excessive
$Ca(NO_3)_2$	0.01	5.5	None
$CaHPO_4$ and $Ca(H_2PO_4)_2$	Saturated 0.002	5.0	None
$CaSO_4$	0.01	6.7	None
$Mg(NO_3)_2$	0.01	5.3	Excessive
$MgHPO_4$	0.01	6.7	None
$MgSO_4$	0.01	6.0	Excessive

The injury of abscission to the leaf tips of the cultures grown in the solutions of potassium salts appeared to be similar in every respect to that of the cultures grown in solutions of $Mg(NO_3)_2$ or $MgSO_4$. Whether it was physiologically the same cannot be stated at this time. If the injury to these seedlings was physiologically the same, it appears that there are at least two factors apparent in the data that are related to this injury of abscission of leaf tips, as follows:

1. The lack of or the deficiency of calcium in the nutrient media. It is in this sense that the injury to the seedlings grown in the solutions of potassium salts is to be accounted for, as it was obviously not due to the presence of magnesium. Furthermore, as the cultures grown in the different solutions of calcium salts were not injured, this is suggestive that beneficial results should obtain from additions of calcium salts to nutrient solutions that have shown themselves to be poor media for plant growth because of excessive amounts of potassium or magnesium salts. Numerous tests have proved this to be true. This, however, does not necessarily imply any definite calcium-magnesium ratio or a calcium-potassium ratio as a condition of physiological importance in nutrient solutions. The amount of calcium ions needed to overcome the harmful effects of excessive concentrations of potassium ions or magnesium ions will be determined largely by other conditions or sets of conditions under which the plants grow, of which those affecting the rate of transpiration of the plants are not of least importance.

2. The relation of phosphorus. A relatively high concentration of magnesium ion and the absence of calcium (except a trace as an impurity) in the solutions are conditions supposed to be conducive to injury. As no abscission of the leaf tips appeared in the seedlings grown in solutions of $MgHPO_4$, it appears that in the magnesium-phosphorus relation is a condition that in this case is physiologically important. It is hardly possible that the trace of calcium found in $MgHPO_4$ could account for these results. Equally large traces of calcium were found in the other salts used, especially K_2SO_4, which contained 0.005 per cent. As this amount did not prevent injury, it seems reasonable to discount the effect of a smaller trace of calcium found in $MgHPO_4$ as being the cause for the non-injury to the seedlings. The fact that KH_2PO_4 produced less injury than did any of the other potassium salts used presumably was due to some action involving the H_2PO_4 ion.

As the potassium salts and two of the magnesium salts used gave injury to the wheat seedlings, one can infer that this was due to some action of a positive ion of the salts in solution. On the other hand, certain positive ions, calcium for example, did not produce injury. It does, as has been shown in other experiments, prevent injury where magnesium ions are excessive. The harmful effect of some of the positive ions, however, can also be prevented or greatly mitigated by the presence of certain negative ions. The phosphate ion comes in this category in the cases of the $MgHPO_4$ and KH_2PO_4 in this experiment, the former salt producing no injury and the latter salt only a little. Under certain conditions, therefore, it may be expected that additions of phosphate salts should prove beneficial to nutrient solutions that are toxic due to excessive concentrations of either magnesium or potassium. This the writer has found to be the case in other experiments.

No reference has been made to the literature, as this is to be taken up later when the subject will be discussed in greater detail.

UNIVERSITY OF CALIFORNIA

CURRENT LITERATURE

BOOK REVIEWS

Morphology and cytology of fungi

DEBARY'S great treatise, indispensable as it still is to the working mycologist, has become in many respects so obsolete as to be not only unsatisfactory but even misleading in the hands of less advanced students of the fungi, and a modern, authoritative work in the English language, with the same comprehensive scope, is greatly to be desired. The volume by Dame GWYNNE-VAUGHAN[1] in large measure meets this need, so far as the groups of which it treats are concerned. Presumably another volume in the same series is forthcoming, which will complete the account.

The first part of the book, occupying thirty-four pages, is devoted to general introductory matter, applicable to the fungi as a whole. It contains definitions, a general outline of the classification adopted, and brief discussions of such topics as saprophytism, parasitism, growth reactions, and symbiosis, the last-named term used in a narrowly restrictive sense to apply only to lichens and mycorhizas. The remainder of the book is devoted to a detailed treatment by subdivisions of the particular groups specified in the title.

In the matter of systematic treatment the author has been commendably conservative for the most part, but she has not hesitated to make radical rearrangement of the currently accepted systems where the need has existed. This is strikingly shown in the treatment of the Ascomycetes. The "great group" Ascomycetes is divided into three "groups" (why not classes and subclasses?): Plectomycetes, Discomycetes, and Pyrenomycetes. Of these, the Discomycetes includes all the orders commonly recognized as included in that assemblage; the Pyrenomycetes is made to include, with appropriate reservations, the Laboulbeniales, but does not include the Erysiphales, which are placed in the primitive "group" Plectomycetes, with the Exoascales and the Plectascales. The latter order is expanded to include everything from the Endomycetaceae and the yeasts to the Terfeziaceae. The result is an arrangement simpler and more usable than that of ENGLER, which at the same time does no violence to our present admittedly very imperfect knowledge of the relationships among these primitive forms. Furthermore, it is a pleasure to see in a modern book a reversion to the earlier conception of the Archimycetes, the Oomycetes, and the Zygomycetes as three coordinate groups of the Phycomycetes, rather than an adherence to the more recent but less logical practice of placing all the lower Phycomycetes in the Oomycetes.

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[1] GWYNNE-VAUGHAN, Dame HELEN, Fungi. Ascomycetes, Ustilaginales, Uredinales. Cambridge Botanical Handbooks. pp. xi+232. *figs. 196.* Cambridge. 1922.

The general discussion of each subclass is followed by a key to its orders, and of each order by a key to its families. These keys are clear and simple, and add greatly to the value of the book. After each special topic, or discussion of a family, a bibliography is appended, in which the selection of the titles has been dictated by matured judgment. The illustrations are numerous and appropriate. Many of them are crudely drawn, which detracts greatly from the appearance of the book, but they are always clear and accurate. Much consideration is given to the cytological aspect, which in view of the research interests of the author is not surprising. The frequency with which she emphasizes the points in need of further cytological investigation should prove stimulating to workers in this field.

The implication on page 47 that we must accept two nuclear fusions as the normal situation in all Ascomycetes will be regarded by many mycologists as premature. The entire subject must be carefully reinvestigated in the light of recent parallel studies on the Basidiomycetes before this can be regarded as definitely established. In this connection, the reference to clamp connections as structures which have no significance other than to facilitate the passage of food (p. 1), and as merely vegetative phenomena (p. 188), will have to be revised. Again, the statement that "in *Puccinia*, *Phragmidium*, and other Uredinales . . . . the basidia are developed in chains" seems to stress unduly the cytological conception of the basidium, just as the thought of each cell of the promycelium as a one-spored basidium, as held by other botanists, seems to place undue emphasis on morphological detail. Lacking more convincing evidence than has yet been presented, it would seem better to retain the intermediate and generally accepted view that the teleutospore cell is not a basidium, but is the cell that gives rise to one, either externally, as a promycelium, or internally. In fact, the author is not consistent in her treatment, and seems to recognize the latter view on the same pages on which she gives different interpretations.

The proofreading has been carefully done and typographical errors are few. Fig. 27*e* is a transverse, not a longitudinal section, and a few words, mainly proper names, are misspelled, all of very minor importance. The author is to be congratulated on the effective presentation of a mass of information which has heretofore been scattered and largely inaccessible to the majority of the workers in this field. She has performed an invaluable service in collating this work and in presenting it so clearly and concisely. The book is a necessity for the reference shelf of every laboratory where mycology is taught.—G. W. MARTIN.

Lichens

Miss ANNIE L. SMITH,[2] of the British Museum, is the author of a notable work on lichens, in which are considered the history of lichenology, the morphol-

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[2] SMITH, ANNIE L., Lichens. 8vo. pp. xxvii+464. *figs. 165.* Cambridge University Press, England. 1921.

ogy of the lichen thallus, reproduction in lichens, and the physiology, bionomics, phylogeny, taxonomy, ecology, and economics of lichens.

The history of lichenology is treated in seven chapters, six of which follow KREMPELHUBER in the first volume of his *Geschichte und Litteratur der Lichenologie.* The seventh period extends from 1867 to the present time. Very wisely, little space is devoted to the first six periods, as much of the work of these periods was based on a wholly wrong conception of lichens, and the systematic treatment was largely very poor. In 1867 and the following year DEBARY and SCHWENDENER established the fact that what had been considered constituent parts of lichens were algae, with which the lichens were growing in symbiotic relationship, and made modern lichenology possible. For the six periods, those especially interested can refer to KREMPELHUBER'S extended treatment; but it would seem that the seventh period, which covers all of modern lichenology, might well have received more space.

Preceding the discussion of morphology proper is a chapter in which the algal host cells are treated as constituents of the lichen, under the term "gonidia," which designation, according to the belief of many botanists, should have been consigned to oblivion long since. The treatment of the relationship between the lichen and its algal host contains much information that is valuable, but unfortunately it is all based on the supposition that the lichen is a composite structure, a fungus and many individual algae, and still in some mysterious way a plant. It is the belief of a constantly increasing number of American botanists that such a confusing presentation should never be placed before the student or the botanist.

In the treatment of types of thalli, structures peculiar to lichens, cells and cell products, general nutrition, assimilation and respiration, illumination, and color, the chapters on morphology and physiology carry much that is valuable. All is colored by a phraseology which is confusing to those who believe that lichens are fungi, however, and indeed scarcely comprehensible to them, excepting a few of the older ones, who, like the reviewer, were taught to believe that the lichen should be considered both a colony and an individual.

The chapter on reproduction on the whole is excellent, and is perhaps the most up-to-date and valuable portion of the volume. The discussion takes form under such topics as types of fruits, development of reproductive organs, apogamous reproduction, stages of apothecial development, and spores and asci. The treatment of forms of reproductive organs in lichens is the first adequate presentation to appear in a text. For those of us who believe that lichens are fungi which should be treated like other fungi, and that carpologic development and structure should play a large part in taxonomic disposition, this chapter brings our data together in convenient form so far as lichens are concerned. Unfortunately, the discussion of the matter in other Ascomycetes is given separately toward the close of the chapter.

Rather closely related to the consideration of reproduction stands that of phylogeny in another chapter. This opens with a presentation regarding the algal hosts, which many would omit from a consideration of the phylogeny of lichens. Following this is a valuable treatment of the relationships between lichens and other fungi from the standpoint of evolution. The evolution of the thallus is considered next, then that of the various groups of lichens. The chapter closes with a "scheme of suggested progression in lichen structure."

The chapter on taxonomy considers first the various schemes of arrangement, following which the lichens are arranged according to the system of ZAHLBRUCKNER, as given in ENGLER and PRANTL. Then follow treatments of number and distribution of lichens, with a survey of the lichens of polar and temperate regions, and fossil lichens. Probably ZAHLBRUCKNER would modify the classification outlined fifteen years ago considerably, were he to give his present views; but it is readily conceded that no lichenist, save perhaps WAINIO, has shown so much skill and knowledge in the classification of lichens. For one who believes that lichens are dual organisms, there is no other possibility than to consider them a distinct group of plants, as is done in the volume before us. Also, as a matter of expediency, lichens may be treated separately by those who believe that they are fungi, although at the expense of failing to present adequately the many close relationships between lichens and other fungi. In any flora which covers all fungi, distribution should be made somewhat as is done in papers by the reviewer and several other American botanists.

The chapter on bionomics contains a valuable discussion of the growth and duration of lichens, the season of fruit formation, dispersal and increase, parasitism, and the diseases of lichens. Closely related to this is the chapter on ecology, which brings together for the first time a considerable portion of the material which deals with this subject. Although the research on lichen ecology is mainly pioneer work and much of it faulty, the worker in this phase of ecology must refer to the chapter, which will be bewildering to many who have concluded that lichens are fungi.

While SCHWENDENER and other Europeans have held that lichens are fungi, they have not been able to treat lichens as fungi after the method of mycology in general. This has been left to America, where the more rational treatment of these plants began to take form a generation ago and is now taught in a considerable number of institutions and believed by many teachers and other botanists. The reviewer does not relish attacking the conception of lichens held by the author of a work of great merit. The issue is unavoidable, however, and although American botanists may get much of value from the volume, it is to be hoped that students will not be taught that lichens are not plants, but yet in some mysterious way are plants, and that maturer botanists may be able to get that which is valuable from the work and not fall into the confusing phraseology regarding the nature of lichens.—BRUCE FINK.

NOTES FOR STUDENTS

Vegetation of British Guiana.—HITCHCOCK[3] has given an interesting sketch of the conditions of plant life in British Guiana, with notes on the flora. The climate shows great uniformity of temperature, the mean maximum having the narrow variation of 83° to 87° F., and the mean minimum from 74.5° to 76.5° F. The annual rainfall ranges from 150 inches in the north to 50 inches in the south, with a relatively dry period extending from August to November. Virgin forest covers the greater part of the country, showing many of the characteristics of tropical rain forest, such as stratification, abundance of lianas, buttressed trunks for the larger trees, and great luxuriance. Attention is directed to cleanness of the forest floor due to the destruction of fallen trees and branches by decay and by wood ants. This condition is also accentuated by the scanty growth of low vegetation due to the density and uniformity of the shade. Species of the forest trees are not gregarious, but are scattered here and there.

The flora shows some interesting comparisons with that of the United States. Grasses, sedges, and leguminous plants are about equally abundant in the two regions. Some families common in the United States have here few species, as Fagaceae, Cruciferae, Ranunculaceae, Rosaceae, Labiatae, Umbelliferae, and Compositae. Some familiar families are found in Guiana chiefly as trees, as Euphorbiaceae and Solanaceae. On the other hand, certain families sparsely represented in the United States are found in greatly increased numbers. Notable among these are the Araceae, Palmaceae, Piperaceae, Rubiaceae, Myrtaceae, Lauraceae, Sapotaceae, and Melastomaceae. Orchids reach their highest development on the mountains.—GEO. D. FULLER.

Soil moisture.—As a result of recent investigations, PARKER[4] presents evidence that the vapor pressure of different moisture contents, the equilibrium relations with seeds, and the freezing point depression due to solid material do not indicate different forms of soil water such as given by the dilatometer method. This would indicate that the old method of classifying soil moisture as hygroscopic, capillary, and gravitational, although open to certain objections, still remains the best available. The opposing view, presented by BOUYOUCOS, has already been noted in this journal.[5] PARKER'S experiments, however, tend to support the views of such modern authorities as RUSSELL and KEEN that all the water present in the soil is subjected to the same law over the whole experimental range, and the various constant and critical points shown by soils at varying degrees of water content are equilibrium values only, and do not indicate any break or abrupt change in the physical condition of the soil moisture.

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[3] HITCHCOCK, A. S., Floral aspects of British Guiana. Ann. Rept. Smithsonian Inst. for 1919. pp. 293–305. *pls. 12.* 1921.

[4] PARKER, F. W., The classification of soil moisture. Soil Science **13**:43–54. 1922.

[5] BOT. GAZ. **73**:420. 1922.

In this and in a previous paper,[6] PARKER holds that the freezing point depression for the moisture equivalent and probably also for the wilting coefficient is practically a constant for all soils. This may be taken as additional evidence that the moisture equivalent and wilting coefficient are within very considerable limits constants for all soils.—GEO. D. FULLER.

Physicochemical problems relating to soil.—Under this title[7] the Faraday Society has brought together in book form the papers presented before the Society during the general discussion of this topic at its meeting in London on May 31, 1921. The discussion is divided into five sections, and was planned to form a rather complete survey of the subject. The first section is general in nature, and consists of a survey of the whole field by RUSSELL, and a discussion of the physical properties of soils in relation to survey work by ROBINSON. The second section deals with the problems of soil moisture, with papers by KEEN, ODÉN, HOAGLAND, SHULL, and HACKETT. The third section considers the organic constituents of the soil, with papers by PAGE, ODÉN, and SALISBURY. The last two sections are concerned with colloidal properties, the adsorption phenomena of section four being discussed by FISCHER, CROWTHER, and MORRISON. The final section deals with the dispersity, flocculation, and plasticity of clays by ODÉN, COMBER, and MELLOR.

The discussion as a whole forms an important addition to soil literature, and one is impressed by the usefulness of such symposia as the Faraday Society has organized from time to time. The custom of holding such general discussions devoted to an entire survey of some field of investigation is one that might well be established among scientific societies in this country to replace the less valuable type of symposium now in vogue here.—C. A. SHULL.

Photosynthesis control in forest plants.—In investigating the conditions affecting photosynthesis in forest undergrowth, LUNDEGÅRDH,[8] using a new form of assimilation-chamber, found that with variations of carbon dioxide concentration and low light intensities both the light and the carbon dioxide supply are controlling factors. The advantages from a supply of carbon dioxide above normal appear most strongly in low intensities of light. For *Oxalis acetosella* at 0.025 of sunlight, and for *Viola tricolor* at 0.25, an approximately direct proportionality was obtained between the carbon dioxide concentration and the intensity of photosynthesis. In the forest, on account of the production of the CO_2 by the ground, the air (especially that nearest the ground) becomes rich, the CO_2 content often rising to more than twice normal. This increase, least over sandy soil and greatest over the humus of beech woods,

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[6] PARKER, F. W., Methods of studying the concentration and composition of the soil solution. Soil Science **12**:209–232. 1921.

[7] Physicochemical problems relating to the soil. Trans. Faraday Soc. **17**:217–368. 1922.

[8] LUNDEGÅRDH, HENDRIK, Ecological studies in the assimilation of certain forest plants and shore plants. Svensk. Bot. Tidsk. **15**:46–95. *figs. 9.* 1921.

is an important condition for the existence of shade flora. In such plants, in order that the daily products of photosynthesis may compensate the respiration of darkness, there is required at 18° C. an average illumination of little more than 0.01 light; and in order that growth may be maintained, in the case of *Oxalis*, a minimum daily illumination of 0.25 light for somewhat more than an hour. In sun plants an equilibrium between respiration and photosynthesis is reached at about 0.025 light.—GEO. D. FULLER.

Prothallia from sex organs of Polypodium.—STEIL[9] has reported that "in an old culture of *Polypodium irioides* the sterile cells of a large number of antheridia and archegonia became vegetative like ordinary prothallial cells." No case of regeneration from the sex organs of a pteridophyte has been reported previously. The cap cell and ring cells of the antheridia produced prothallial filaments and secondary antheridia; while the cells of neck and venter of the archegonia also produced filaments and antheridia, but in no case secondary archegonia. The antheridia produced in this way reached maturity, developing actively motile sperms. It was impossible to state under what cultural conditions the cells of the sex organs regenerated, but it is suggested that the peculiar behavior was the result of unfavorable conditions which appeared in the old culture.—J. M. C.

Oxalophytes over limestone.—Recent studies by SALISBURY and TANSLEY[10] have shown that *Quercus sessiliflora*, regarded as a decided oxalophyte, occurs in the regions under consideration over limestones. Analyses, however, prove that leaching has so reduced the lime content of the surface soils that they are often really acid in their reactions, and hence the oak seedlings and the accompanying calcifugous herbaceous vegetation really develop in a non-calcareous or even in an acid soil. This adds another to the rapidly accumulating array of facts indicating how dangerous it is to assume that calcareous rocks always give rise to calcareous soils.—GEO. D. FULLER.

Stock-poisoning plants.—LAWRENCE[11] has published an account of the principal stock-poisoning plants of Oregon for the use of "the Oregon farmer or stockman." The statement is made that "the annual toll of the poisonous plants in Oregon is surprisingly heavy." It is of interest to botanists to note that the principal poisonous plants of Oregon are *Delphinium* (6 spp.), *Zygadenus* (2 spp.), *Cicuta* (3 spp.), *Lupinus* (1 sp.), *Astragalus* (1 sp.), and *Pteridium* (1 sp.).—J. M. C.

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[9] STEIL, W. N., The development of prothallia and antheridia from the sex organs of *Polypodium irioides*. Bull. Torr. Bot. Club **48**:271–277. *figs. 4.* 1921.

[10] SALISBURY, E. J., and TANSLEY, A. G., The Durmast oak-woods (*Querceta sessiliflorae*) of the Silurian and Malvernian strata near Malvern. Jour. Ecol. **9**:19–38. *pl. 1.* 1921.

[11] LAWRENCE, W. E., The principal stock-poisoning plants of Oregon. Oregon Agric. Coll. Exper. Sta. Bull. 187. pp. 42. *pls. 2. figs. 10.* 1922.

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HETEROTHECA GRIEVII THE MICROSPORANGE OF HETERANGIUM GRIEVII

MARGARET BENSON

(WITH PLATES IV, V, AND EIGHT FIGURES)

Introduction

The sporangia here described have so far only been recorded from the Pettycur deposits of the Calciferous Sandstone Series of Scotland. They have been found in three blocks, one of which, presented to the writer by Professor BAYLEY BALFOUR in 1905, yielded also some of the specimens of *Sphaerostoma* of which a description has already been published (5); the other two blocks were collected at Pettycur by the writer in 1910. In all three blocks they were found associated with *Heterangium Grievii*. Their somewhat flattened fusiform shape is approximately that of *Telangium Scottii* (4), but the dimensions are less. *T. Scottii* measures about 2×3 mm. in width at its maximum girth and 4 mm. in length, whereas the new sporange only measures roughly 1×2 mm. in width and 3.5–4 mm. in length. A further resemblance to *Telangium* is their possession of longitudinal loculi or series of loculi, and of peripherally placed tracheids. These resemblances do not seem adequate to suggest its inclusion in this form genus, because there are many remarkably distinctive characters in the new form.

In the first place, each peripheral loculus of *Telangium* is represented in *Heterotheca* by a series of relatively small loculi.

Secondly, central loculi as well as peripheral ones occur in the new form. A third outstanding difference is the absence of any evidence that the sporange underwent apical dehiscence, which is almost universally the case in species of *Telangium*, whether Lower Carboniferous (for example, *T. affine*) or Upper Carboniferous (for example, *T. Scottii*, etc.). The only exception known to me is a species[1] described by NATHORST (11). A fourth outstanding difference from *Telangium Scottii* (the only species so far described with structure preserved) is the presence in the new form of water storage tracheids, which, although few in the normal specimens, are very abundant in the partially sterile specimens. Lastly, a character is found which at first sight seems of minor importance. The new sporange retains the twofold cortical sclerotic plates which form so characteristic a feature of the cortex of *Heterangium* stem and petiole. It is this latter feature which the writer regards as irrefutable evidence of the safe attribution of these microsporangia to *Heterangium Grievii*, with which they have invariably been found associated. Although, therefore, the sporange has the same claim to the name of the parent plant as either stem or petiole, it has been found convenient to refer to it under the name of **Heterotheca Grievii.**

DIAGNOSIS.—I have founded the form genus *Heterotheca* for the reception of such microsporangia as resemble *Telangium* in form and possibly in insertion, but possess vertical sclerotic plates in their periphery and horizontal sclerotic plates deeper down in the cortex, like the vegetative organs of *Heterangium Grievii*. The loculi are not confined to the periphery, but central ones also occur.

The specific name of the form now described is that of the parent plant.

Form, anatomical structure, and grouping of specimens

FORM.—The form of the sporange can be deduced from the various series of sections through single specimens. Some of these have been plotted out to scale in text figs. 1 and 2. These show

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[1] This species, of which the type specimen was figured by ARBER (1) under the name of *Carpolithus Nathorstii*, may prove to be a species of *Telangium*, owing to NATHORST'S discovery of its longitudinal loculi filled with microspores. The plate referred to exhibits what was possibly approximately the habit of *Heterotheca Grievii*.

H. Grievii to have had a tapering apex and a maximum girth nearer the base than the apex. The majority of the transverse sections through the upper part are circular, but in the basal region the sectional area is oval (figs. 8, 9), hence there are two aspects of each sporange given in text figs. 1 and 2.

In longitudinal sections the apical part is seen in surface view if present, as indicated in text fig. 3. In the upper third, the surface loculi are only slightly rubbed down in the center, the lateral ones being seen almost in surface view. If two sections are secured longitudinally through a single sporange, they are both somewhat peripheral, as the size of the body scarcely admits of two longitudinal sections. Both transverse series and longitudinal sections show that the sporange was almost always somewhat injured at the base where it has become detached from the parent plant.

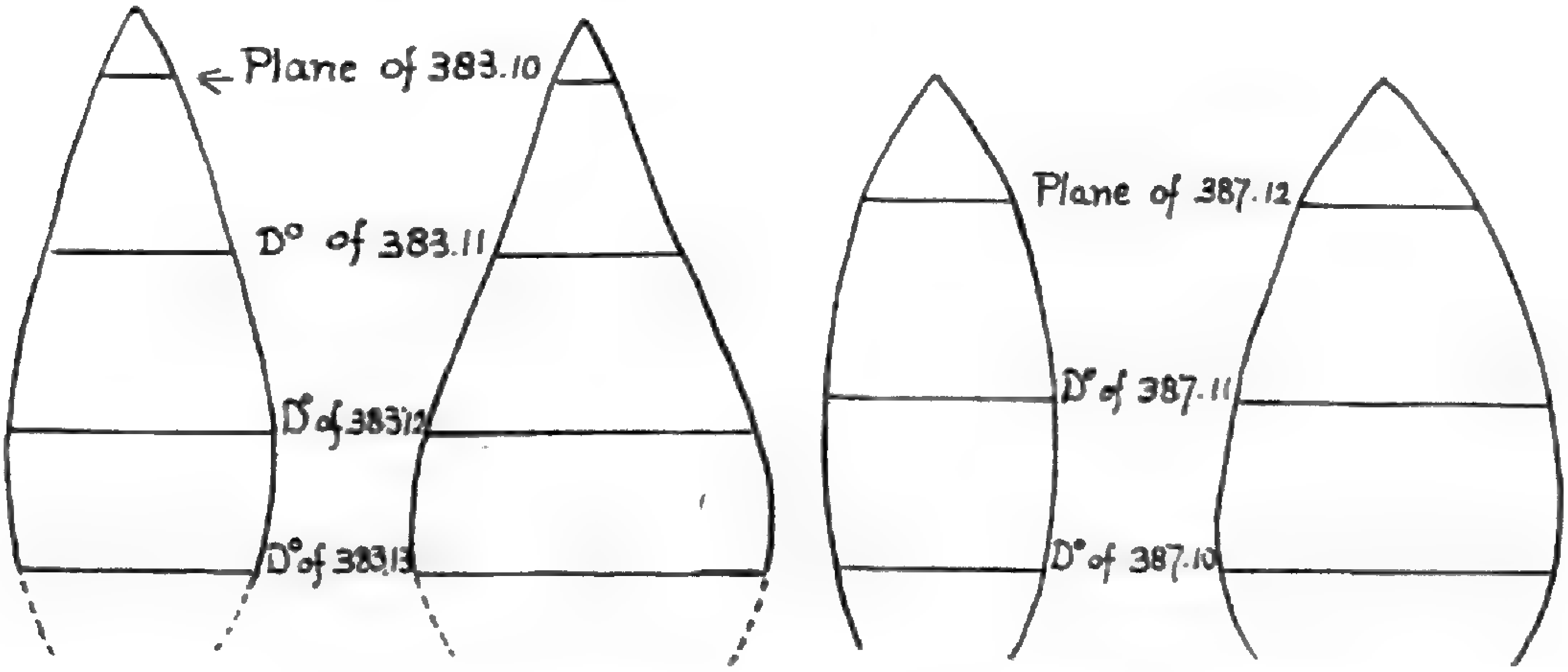

FIG. 1 FIG. 2

FIGS. 1, 2.—Showing slightly bifacial form of sporange as indicated by plotting series of transverse sections to scale on vertical line; fifth section in series (CN.383.14) just included five separated bases of loculi series, a fact on which the length (4 mm.) is deduced.

ANATOMY.—The whole sporange is surrounded by a large-celled epidermis. The contents of the epidermal cells are carbonized, and from the mode of preservation of mucilaginous tissue in the Pettycur deposits, this blackened condition of the epidermis evidently precludes the view that it was mucilaginous (fig. 1). Beneath the epidermis is the hypoderm, composed of about five layers of cells. This tissue is continuous with the interstitial tissue between the loculi. In this cortical region are frequently found, alternating with the peripheral loculi, especially near the apex and base, the characteristic vertical sclerotic plates of *Heterangium Grievii* (figs. 3, 4, 9, 13).

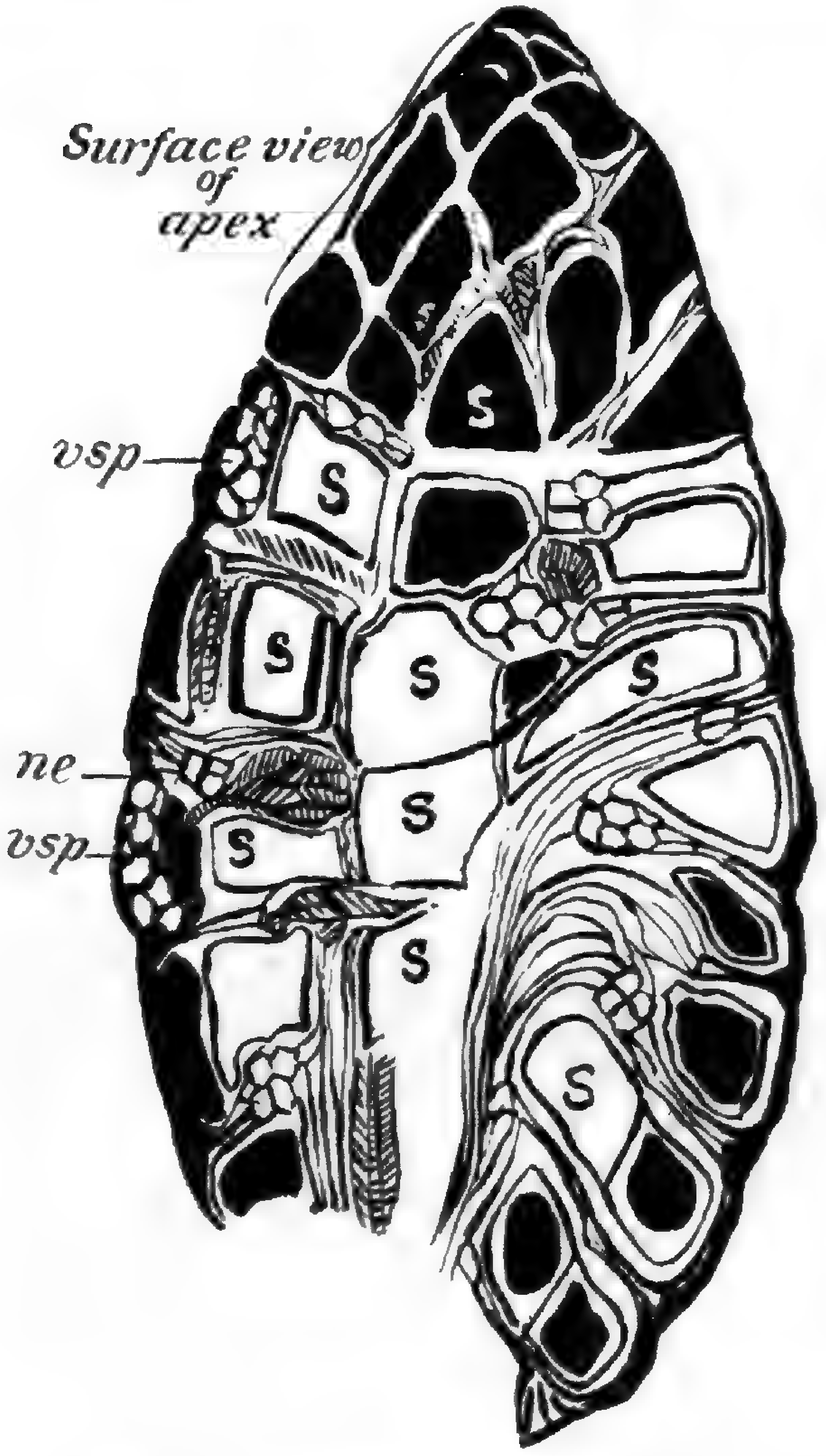

Fig. 3.—Diagrammatic drawing from two slightly oblique longitudinal sections (CN.386.17, 18), latter of which is nearly radial at base, and former superficial for upper third; it illustrates the somewhat spiral direction of locular series, preservation of spores in some but not in all loculi, vertical *Heterangium* sclerotic plates on left, large amount of water storage tissue and connecting tubules which abut on plates as in cortex of *Heterangium* petioles; sections were selected because of presence of ripe spores; central region is injured.

Loculi.—The peripheral series of loculi which run longitudinally and somewhat spirally are more numerous than the loculi of *Telangium Scottii*, numbering in the middle zone of the sporange twelve (figs. 3, 9; cf. also text fig. 4). Their approximation into groups is sometimes seen near the apex (CN.383.10). Immersed in the ground tissue of the central region of the sporange, except at the extreme apex, there are further loculi, of which at least four appear in transverse sections (figs. 3, 12). Text fig. 3 is drawn from longitudinal sections too near the periphery to give the number and disposition of central loculi. The origin of additional central loculi by segmentation of peripheral loculi is suggested in some cases (text fig. 4), and the presence of ripe spores almost uniformly distributed in figs. 11 and 12 (CN.307.17, 18) suggests that the main content of the young normal sporange was potential sporogenous tissue. Each loculus is surrounded by a sheath of small, longitudinally extended, narrow cells. These sheaths, probably as a result of the shrinkage of the tissues due to carbonization, often become separated from the tissues which must have abutted on them in the living state. In the case of the central loculi the sheaths are often ruptured or entirely dissolved away, leaving the spores among the elements of the ground tissue (figs. 5, 11, 12). Small sclerotic plates often become incorporated with the walls of loculi, and when seen in profile are very characteristic (text figs. 4, 6, s^1p^1).

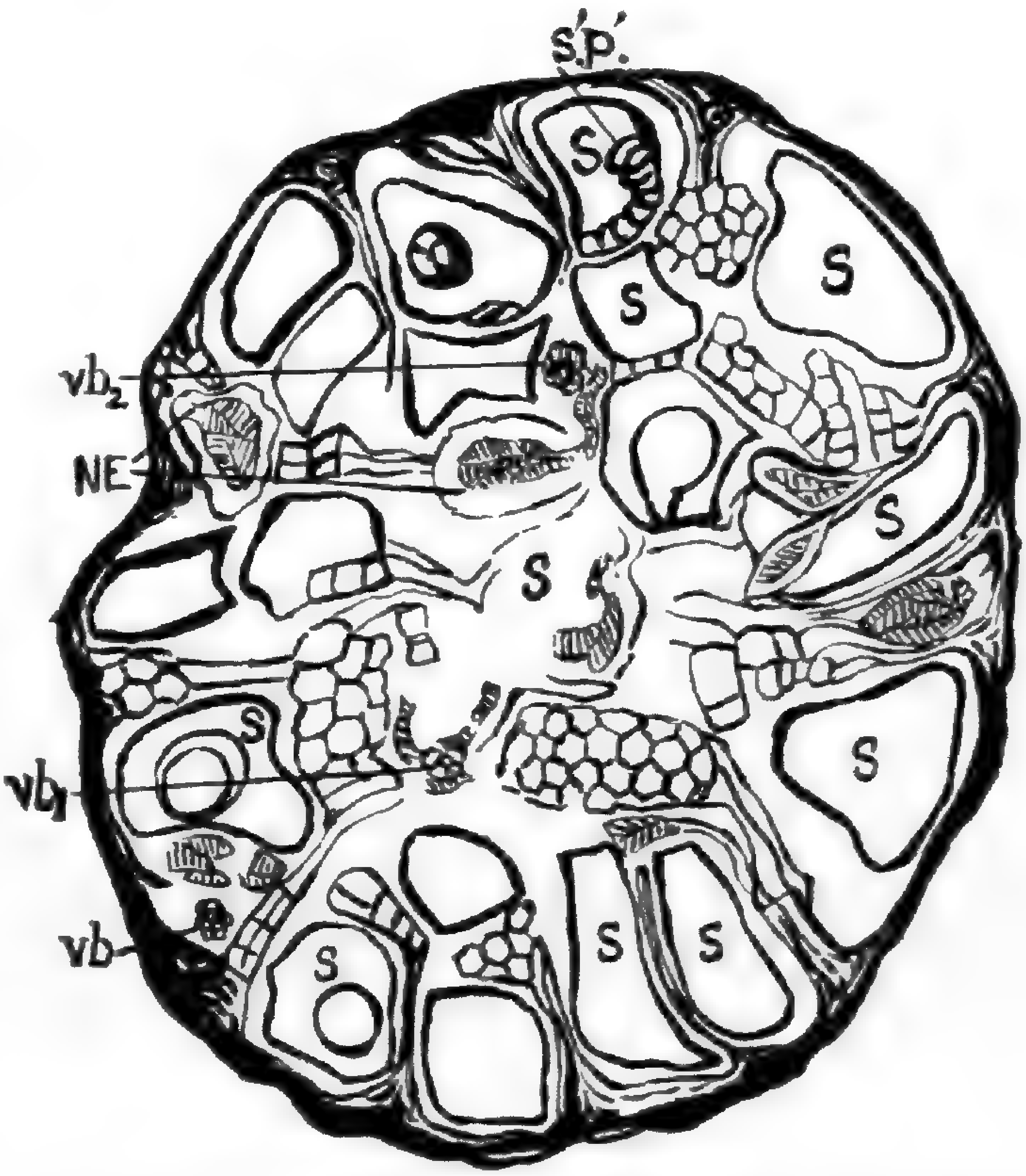

FIG. 4.—Diagrammatic drawing of transverse section of class II synangium (CN.411.31); swollen ground tissue not entered in detail; two deeply lying vascular bundles shown in transverse section (vb_1, vb_2); one peripheral vascular bundle also cut transversely on left, but most are cut so obliquely that they are merely indicated by hatching; nerve ending seen near center with its delicate branch strand; one or two central loculi are broken down, and carbonization of peripheral ones probably explains absence of spores in some (cf. fig. 5); at s^1p^1 a plate occurs attached to a loculus wall and is seen in profile as in CN.307.18, for which consult text fig. 6 and fig. 12.

In one synagium (figs. 11, 12) every loculus contains numerous ripe spores of tetrahedral form, similar in size and character to those in the pollen chamber of *Sphaerostoma ovale* (CN.270.1) among specimens of which it lies (text fig. 5). The spores measure 29 μ in their transverse and 20 μ in their radial extent. In this specimen some of the locular walls have broken down, but many of the peripheral and at least two of the central loculi are still intact. In other cases spores can be demonstrated only in some of the loculi, while others show opaque black or dark brown contents, a condition which is possibly the result of the conditions to which the sporange was exposed during fossilization. As a rule the spores are better preserved in the interior than near the surface, which may be due to the conditions of exposure being slightly different. In a large number of cases the contents of the loculi have entirely been dissolved, or are in an abnormal condition. Such are the series CN.383.10–13 and CN.386.11–12 (figs. 1–4). In the series CN.391.23–27, slide 391.26 shows in two cases a nerve ending in the center of the abortive loculus. In a large number of cases the peripheral loculi have been carbonized, but not the central ones. Swollen nerve endings accompanied by the *Heterangium* sclerotic plates appear to occupy the whole of the core of the sporange in the most advanced phyllodic specimens (text fig. 7). I refer to the partially or entirely abortive specimens as "phyllodic," as their tissues approximate the tissue elements of the ordinary sterile petiole of *Heterangium*. Moreover, they are linked together as in the petiole. There is a considerable range of form and size in the plates; some sporangia even seem to have produced more plates than others (fig. 12).

FIG. 5.—Camera drawings of spores from loculi in CN.386.18 and CN.307.17; tetrad in middle is characteristic and possibly indicates residuum of spore mother still persisted as in recent cycads; spores on left drawn from those in pollen chamber of *Sphaerostoma* for comparison (CN.270.1).

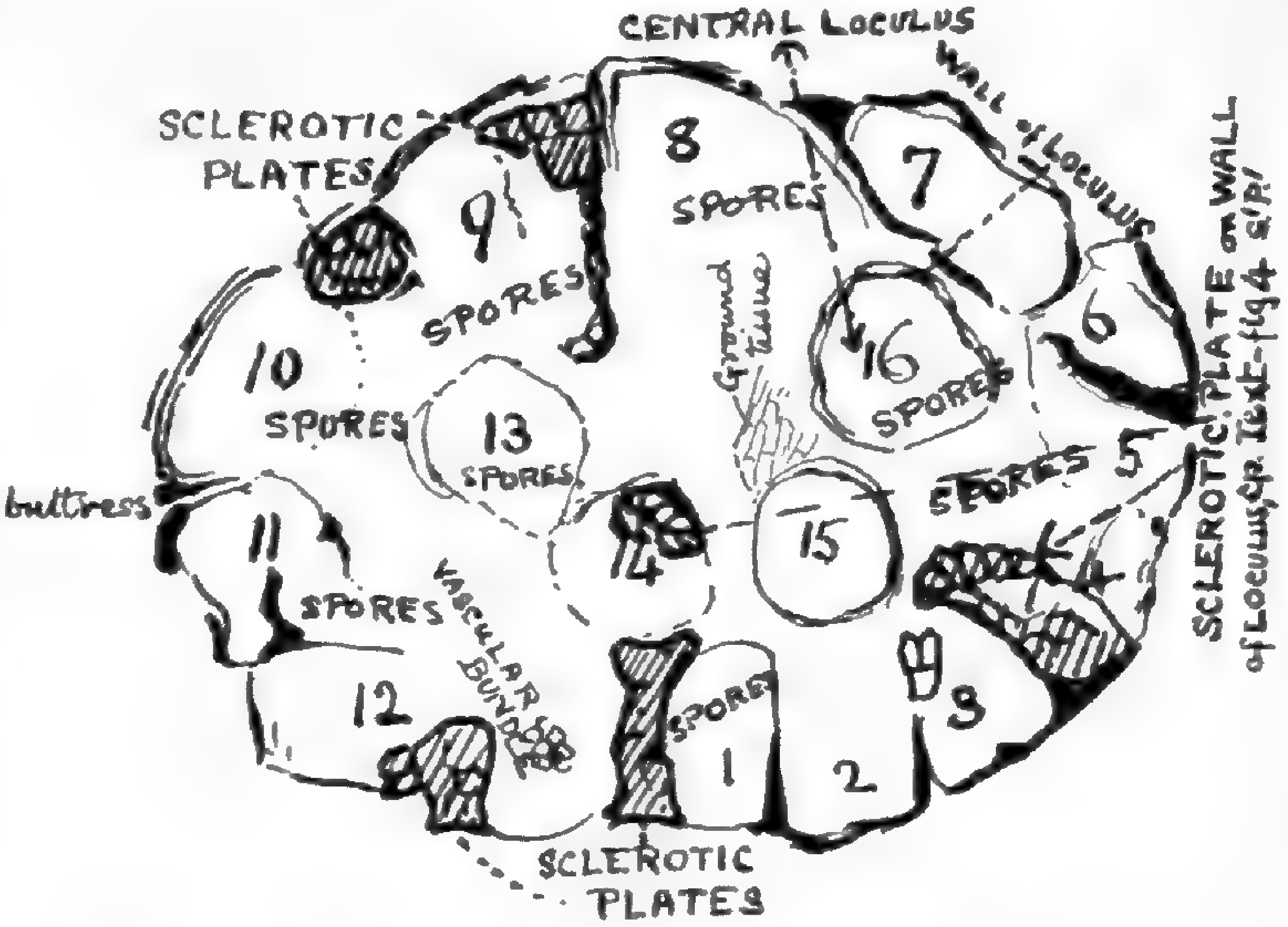

FIG. 6.—Key to fig. 12, showing outline of sclerotic plates, etc., which are clearer in slide than in micrograph.

The vertical plates in the periphery often have their cells thickened with layers of cell wall substance which almost obliterate the lumen of the cell; other plates are small, but most show the hexagonal form of cell indicated in text figs. 3 and 4. Abutting on these are tubular cells which are disposed mainly in the horizontal or transverse plane (text fig. 3). In the fossil these are generally swollen, and owing to their form and pale color have somewhat the appearance of macaroni. Some fibers accompany the delicate branches of the vascular bundles, and are continuous with a parenchymatous hood-like envelope to the nerve ending. Many, perhaps more in some specimens than in others, show a spiral line of thickening which resists imbibition and consequent swelling.

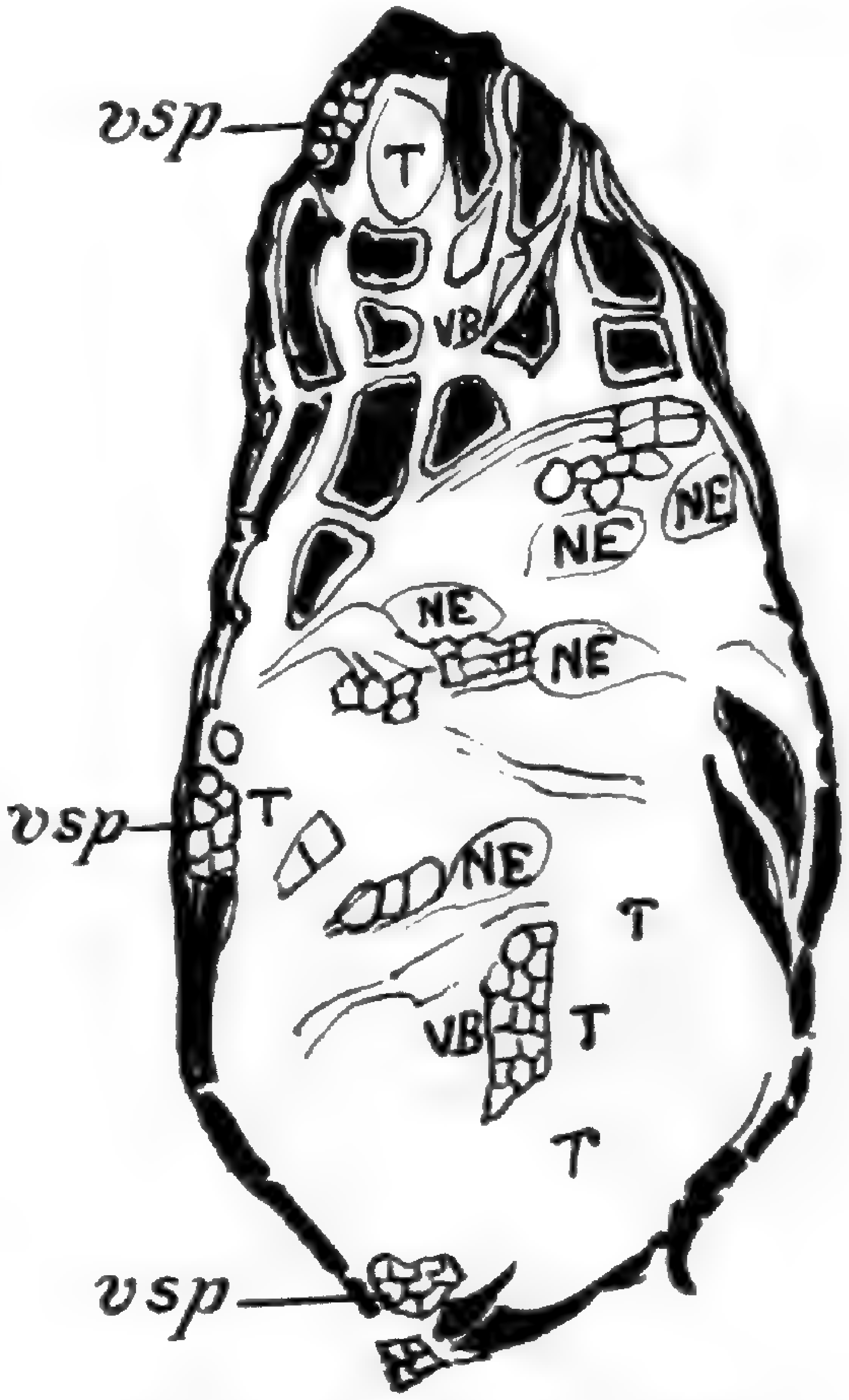

FIG. 7.—Camera sketch of specimen of *Heterotheca* in CN.288.2; no carbonaceous matter is retained in center, and therefore lignified elements of ground tissue appear exceptionally clearly; five nerve endings, many transfusion elements, and two vascular bundles can be distinguished in original; distribution of vertical plates (*vsp*) characteristic; striated elements seen near apex as in many cases (cf. text fig. 3).

The six to eight mesarch strands of primary xylem which travel mainly in the peripheral region give off the delicate centroscopically directed xylem strands, which end in the aqueous storage tissue. The contrast between the short barrel-shaped tracheids of the nerve ending and the slender carriers of the water is quite reminiscent of highly organized water storage organs in recent plants. The majority of the nerve endings in the synangium were directed to its central region. Transfusion elements of varying degrees of specialization are exceedingly abundant in some specimens, and in some phyllodic specimens, such as that outlined in text fig. 7, where the carbonaceous matter is dissolved away in the center, the wide extent of the striated, possibly lignified, transfusion elements of the nerve endings is very clearly seen by the aid of the microscope. The nerve endings appear here to have wholly usurped the position of the central loculi (cf. fig. 6). The enlarged nerve ending is a familiar feature of the sterile pinna of *Lyginopteris*, and probably occurred in that of *Heterangium* also. The macerated condition of some of the specimens may be explained by the conditions to which they were subjected during fossilization. If we accept GORDON'S explanation (**8**) of the mode of formation of the Pettycur plant deposits, we may attribute it partially to the action of thermal springs and warm pools in which the accumulation of the plant remains were deposited and gradually infiltrated.

Text figs. 3 and 4, which illustrate the preceding statements, are both mere diagrams based on definite sections showing ripe spores. The positions in which the latter are clearly present have been indicated by *S*. They were probably present in other loculi, but were welded together into an impenetrable mass, owing probably to the presence of some vestige of the spore mother cell, which, as in recent cycads, appears to have remained long enveloping the tetrad. In the specimen shown in figs. 11 and 12 the spores are free, and there is no welding together of the contents of the loculus. In text figs. 3 and 4 sclerotic plates are indicated by groups of hexagonal cells. Two vertical plates can be seen in text fig. 3 (*sp*), and where plates are incorporated with the wall of a loculus they are indicated by s^1p^1. Larger horizontal plates are also seen in text fig. 4 in the central region. Vascular bundles, their delicate ramifications and large nerve endings, are indicated by hatching. The wall of a loculus has apparently broken down, as shown in the center of both text figs. 3 and 4, and spores are found among the swollen fibers. In both diagrams there is strong suggestion of segmentation of loculi, as Professor BOWER kindly pointed out to me.

Although the distribution of the vascular strands is mainly peripheral, at least two may be found in the foci of the ellipse formed by a cross-section (text fig. 4, vb_1, vb_2), but the number cannot be regarded as determined. The peripheral strands probably alternated with every two series of loculi, but owing to the obliquity of their course (fig. 10 and text-fig. 7) they are often difficult to distinguish. An interesting point in the structure of the sporange in detail is the absence of any evidence of the building up of the synangium by a fusion of sporangia. The whole body strongly suggests its origin from a single sporange which has shared in the same type of skeletal elements as the petiole on which it was borne.

The sporogenous regions were distributed fairly equally throughout the body, except that their disposition in the periphery probably was determined by the approximately dictyoxylon type of cortex. If the loculi appear to be of unequal size, this is due partly to the varying girth of the sporange, and partly to the plane of section through the loculus. Wherever a section occurs in the surface plane, as in the upper third of text fig. 3, the buttresses are seen to lie so obliquely that a transverse section necessarily cuts through the contained loculi at different angles, and explains the fact that a transverse section does not differ much in appearance from a longitudinal one, except in form.

Grouping of specimens.—The available specimens, roughly sixty in number, may be grouped in four classes:

Class I.—Specimens such as that occurring in CN.307.16–19 (figs. 11 and 12), where the vegetative tissue is subordinate in amount to the sporogenous tissue. Such specimens show septa, mesarch vascular strands, and only a few water storage elements. The sclerotic plates characteristic of *Heterangium* in such sporangia are well developed, both in the periphery and in connection with the deeper lying septa.

Class II.—Specimens such as those occurring in CN.411.30, 31 and CN.386.17, 18 (figs. 5, 10). These contain ripe, apparently normal spores and normally disposed loculi, but undoubtedly a larger proportion of vegetative tissue than those in class I (text figs. 3, 4). The following series also belong to this class: CN. 386.7, 8; CN.395.5, 6, 18, 19; CN.396.4, 5.

Class III.—Specimens such as those occurring in CN.383.10–13 and CN.386.11, 12 (figs. 1–4, 8, 9). Although one of these is possibly an immature form showing carbonization of the sporogenous tissue within the loculi, for example, CN.386.11, 12, it is not possible to be sure of the nature of the carbonized contents.

No recognizable spores occur in any of the specimens included in class III, and in several series of sections which exhibit the structure of the body from apex to base, such as that in CN.391.23–27, the loculi seem to be filled up by concentric series of blackened cells, and in some cases have a well defined nerve ending in the center (CN.391.26).[2]

The majority of the available specimens belong to class III, which may be taken to include also specimens such as that in CN.393.6*A* (fig. 13), in which vegetative development obtains to such an extent that the conclusion is obvious that they were abortive or almost completely so. These specimens are of value as demonstrating the extreme condition of the phenomenon, and render probable the suggestion that there was some degree of phyllody (approximation to pinna structure) even in those of class II. The sections showing vegetative development are sometimes slightly larger than those in the normal series CN.307.17–19.

Significance of specimens in classes II and III

The explanation of the state of these sporangia, purely on the assumption of partial sterilization and maceration before impregnation with calcite, is not wholly satisfactory. In recent plants, when sporangia abort, the process affects the sporogenous tissue mainly, as in some specimens of *Angiopteris* sporangia, where the wall and apical crest (annulus) remain unaffected. A mucilage gland results in several familiar instances (**7, 16**). In the specimens under consideration, however, the loculi show no sign of a mucilaginous content, but, together with the septa, are represented by a considerable amount of tissue comparable with that in the vegetative pinnae of *Heterangium*, the parent plant.

KIDSTON and LANG (**10**) point out that the sporange in *Rhynia* and *Hornea* may be regarded as "corresponding to the tip of a branch of a thalloid sporophyte, inclosing sunken tetrasporangia."

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[2] Tracheids possibly formed from degenerating sporogenous tissue of the ovule have been recorded by TREUB (**17**) in *Casuarina*, and by the writer (**2**) in *Castanea*, but it is an unusual phenomenon.

It is not an organ *sui generis*, but partakes of the same potentialities as other branches.

The widely accepted view that the Sphenopterid leaf is a meriphyte, and that the pinna corresponds with the cladodified primary branch axis, makes it easy to conceive of the microsporange showing in some cases the same anatomical elements as the pinna. The Sphenopterid type of frond as recorded by ARBER (2) had already made its appearance in the Devonian, and *Heterangium Grievii* may well have retained some reminiscence of the earlier unspecialized condition from which we conceive the sporange to be evolved. The curious specimens under discussion, which probably owe their preservation in a fossil form to their condition (as only two or three specimens of class I are so far recorded), are thus of considerable interest for the pinna-like character they exhibit. Nor is their more or less sterile condition without significance in any discussion as to the origin of the seed habit in *Heterangium*. Any comparable instability in the homosporous sporangia of the ancestor could well have been the beginning of a differentiation into megasporangia and microsporangia. A plant bearing vascular sporangia which retained but a few tetrads and relatively much surrounding vegetative tissue might well be regarded as on the high road to seed formation.

Evidence of synangial origin of seed

It must have occurred to many morphologists that a seed was a transformed synangium, the nucellus representing the one persisting fertile loculus, and the inner integument the vestigial peripheral part. In 1904, the evidence then available for this theory of the synangial origin of the seed was discussed by the writer (4) in connection with the description of *Telangium Scottii*, a synangium very common in the Coal Measures of the Upper Carboniferous rocks of the north of England, and the first species of that genus to be described showing structure. There were at that time certain difficulties in the general acceptance of the theory, chiefly because the structure of *Telangium* was such that, while explaining the integument, the absence of any central loculus rendered the explanation of the nucellar or central fertile loculus of the seed difficult.

Thus, as the absolute parallel between seed and synangium could not then be demonstrated, there were critics who preferred to lay stress on the possibility of the seed having acquired its characteristic structure independently of its ancestral condition, rather than by a transformation of structures already present. The inner integument, as well as the outer or indusial envelope, was attributed to foliar upgrowths around the nucellus, which alone was regarded as the representative of the ancestral sporange. The views of such critics are summed up by OLIVER (**13**) in his account of *Physostoma elegans* in the following words:

> The synangial theory, though no doubt tenable, presupposes in the ancestor the existence of the exceptional condition of a synangium, in which the peripheral members were ranged symmetrically around a central sporangium, and in which they persist as a sterilized envelope to form the seed coat.

Having at that time already secured several specimens which indicated the existence of a synangium with central loculi surrounded by peripheral ones, the writer took an early opportunity of collecting some more of the Pettycur deposits in which these interesting specimens occurred. By 1911, several hundred sections had been cut from two of the blocks collected the previous year. These blocks contained much *Heterangium Grievii* material, and yielded the large number of specimens now available of the new synangium. Owing to the care with which the stones were cut, many of the synangia, although scarcely 4 mm. in length and 2 mm. in width, are represented by series of three or four sections in the transverse plane or two in the longitudinal plane. The average thickness of the stone involved in the section cutting was 0.8 mm.

As already stated, the series of transverse sections CN.307.17–19, CN.343.10–13, and CN.412.30–31, although cut from different blocks, demonstrate clearly with many others that a synangium was present in these older rocks, associated with and showing the characteristic cortical structures of *Heterangium Grievii*, a synangium in which central loculi, as well as the more commonly occurring peripheral, occur. It is also interesting that even in the microsporangial apparatus an unusual amount of vegetative tissue obtained was also clearly shown for the first time. The publication of the details of the structure of *Sphaero-*

stoma in 1914 was to have been followed by the account of the microsporange *Heterotheca*, but other things have claimed attention, and it has only been since November 1918 that the work has again been taken up. The investigation of a larger number of specimens has thrown new light on the structure of *Heterotheca*, especially on the character and differentiation of the non-sporogenous tissue. With the possible exception of *Crossotheca* (text fig. 8, II), it differs from all other described microsporangia, and approaches what must be assumed to be a synthetic type of seed and microsporange. It is a succulent, sclerotic, vascular synangium, with central as well as peripheral loculi, and exhibits in its own tissues most of those found in the seed.

If we may have even in sporangia coexistent in the same species of plant a large amount of vegetative tissue, it is obvious that there is ground for assuming that in the early phases of evolution of the seed this would have been available as an envelope to the central fertile loculus, if the peripheral ceased to form sporogenous tissue.

A difficulty some have had in accepting the theory of the synangial origin of the seed has been expressed by the statement that "nothing useful has ever been produced by a sterilized sporange." It is granted that abortive sporogenous tissue generally eventuates only in a mucilage cavity, as COULTER and LAND (**7**) and STARR (**16**) have shown in particular cases, but useful structures certainly can be formed by the elaboration of the vegetative tissue surrounding masses of sporogenous tissue, as has recently been demonstrated (**6**) in the megasporange of *Mazocarpon*. It is not altogether sterilized potential sporogenous tissue of the peripheral loculi in *Heterotheca* which formed the inner integument of the ovule (canopy), but mainly an elaboration of the vegetative tissues which originally surrounded those masses of sporogenous tissue and finally supplanted them. For example, *Azolla* and all such leptosporangiate ferns were foredoomed to failure in the construction of a seed on these lines, as their peripheral sporangia had in all probability lost their vascular supply and their skeletal tissue before heterospory was evolved. The parallel to the seed habit which may be noted in *Azolla* is limited to the fact that the megasporange is one-spored, and occupies a central position in the sorus.

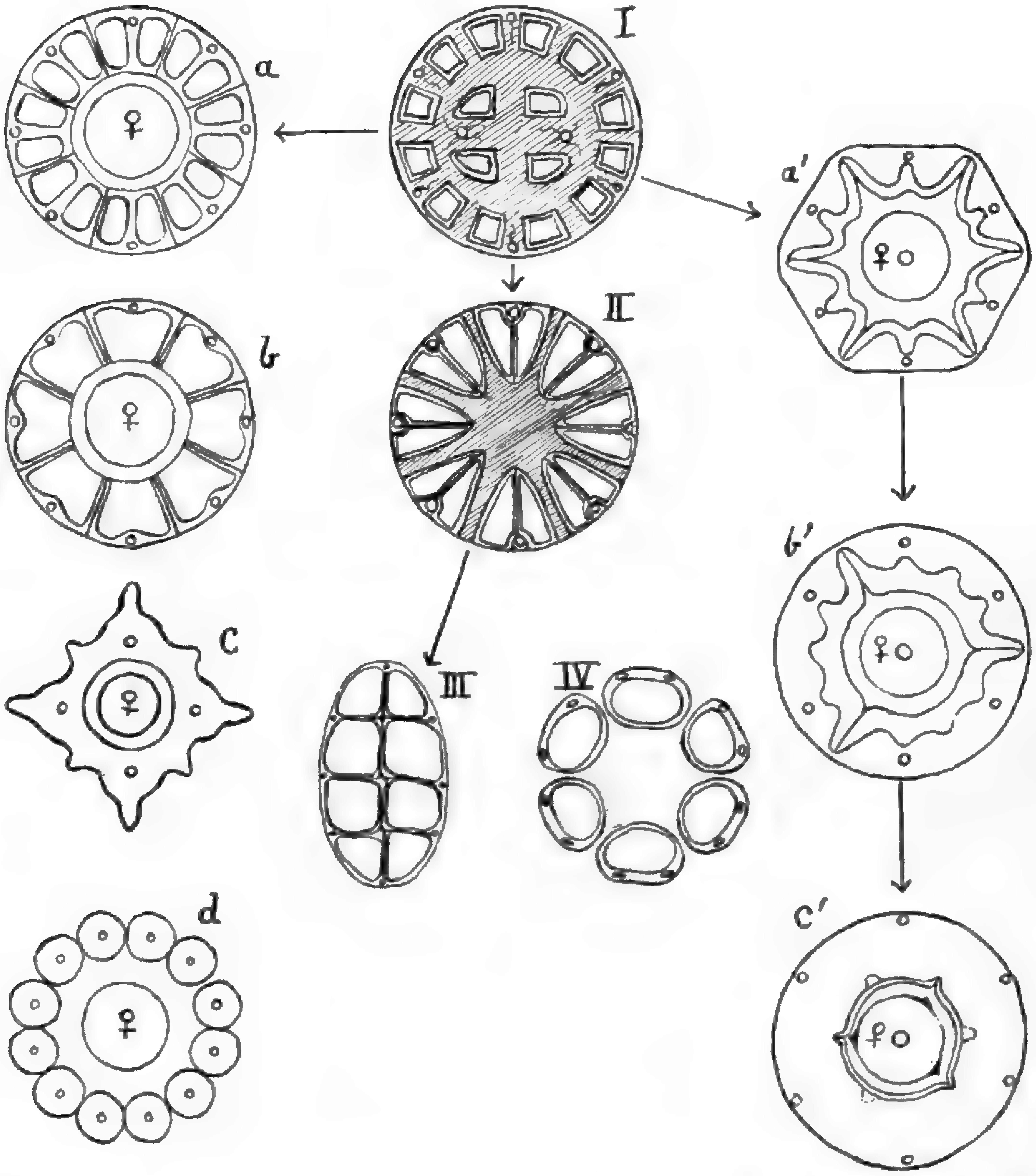

FIG. 8.—Diagrammatic transverse sections of various Pteridosperm ovules and pollen sacs to illustrate numerical relations they show to *Heterotheca* and one another: on left are *a*, *Sphaerostoma; b*, *Lagenostoma; c*, *Conostoma; d*, *Physostoma;* on right are a^1, *Polylophospermum;* b^1, *Trigonocarpus Parkinsoni;* c^1, *T. Shorensis;* in center are *I*, *Heterotheca Grievii; II*, *Crossotheca Hoeninghausi; III*, *Telangium Scottii; IV*, *Codonotheca caduca.* Vascular bundles indicated by small ring in each; arrows denote different phyletic lines; diagrams have been adapted from those of SCOTT and MASLEN, OLIVER, OLIVER and SALISBURY, SELLARDS, and three of writer's, references to which occur in text; relative size of bodies concerned has been neglected for convenience of comparison.

Lastly, a difficulty in the synangial theory of the seed has often occurred to the writer, but she has not heard it expressed by others. It is that in the compartments of the canopy of *Lagenostoma* a vascular bundle occupied a central position instead of, as one might have expected, a position in the plane of the lateral walls. As will be seen in text fig. 8, this difficulty is completely removed by the structure of *Heterotheca* and *Sphaerostoma*. Each compartment in the canopy of *Lagenostoma* is equivalent to a pair of loculi, which, although completely merged in *Lagenostoma*, can still be faintly traced in *Sphaerostoma*.

We owe much to Professor OLIVER for the open expression of his views published in 1909. The conclusions he finally arrived at were opposed to those which seem necessarily drawn from our present knowledge. Both theories as to the origin of the seed were mere hypotheses in 1908. Unfortunately, the view that the canopy of *Lagenostoma* was the product of a cupular or indusial upgrowth, led to the further hypothesis that the *Physostoma* segmented integument was a relatively primitive form, and even contributed to a suggestion that *Physostoma* was perhaps the most archaic type of seed known, a suggestion wholly contrary to what is known of its geological history. In the light of recent work, these latter hypotheses necessarily fall together, and it is to be regretted that a recent writer (14) should have referred to them as facts. OLIVER merely claimed that certain conditions should be proved to exist "in the ancestor," but evidently the necessary conditions have persisted in *Heterangium*, so that they coexist with the formation of an ovule for the megasporangial apparatus. Thus a link is provided between an ovule and a microsporangium which is stronger than was demanded, and there can be no longer any question as to the seed of a Pteridosperm, a seed that may well have been the homologue of all Pteridosperm seeds, having been produced in the course of evolution as a transformation product of a synangium. In this investigation we thus stand upon the threshold of the origin of at least one group of the Spermatophyta, and find more indications of it than were expected in the structure of a plant of the Lower Carboniferous.

The following is a more concise statement of the features of resemblance between *Heterotheca Grievii* and various ovules and microsynangia regarded as homologous with it. For the better elucidation of the subject, text fig. 8 has been constructed showing the possible numerical relations, etc., between the various sporangia of the Pteridosperms. We will commence with a comparison of *Sphaerostoma* and *Heterotheca*.

Heterotheca Grievii resembles *Sphaerostoma ovale* in the following particulars. It is approximately of the same form and dimensions (length 3.5–4 mm., width averaging about 2 mm.). It has probably six peripherally placed bundles in the buttresses between every two loculi as seen in transverse section. Besides these, there are two in the foci of the ellipse of the cross-sectional area, making eight in all, as in *Sphaerostoma*, although in the latter all eight are peripheral. The bundles are accompanied by enlarged water storage elements, and branch as in *Sphaerostoma* (5), fig. 3*a*. There is a central fertile region in each. In *Heterotheca* there is a large amount of vegetative tissue in the region between the loculi, forming buttresses vertical to the surface similar to those of the canopy of *Sphaerostoma*.

The differences can be explained by progressions of well known type. Thus the overarching of the central region by the peripheral at the apex to form the micropyle and sinus may have been partly in relation to the necessity of harboring the pollen grains, and partly a direct result of the freer vegetative development of the periphery. The later segmentation of synangia is a familiar phenomenon in Pteropsids, as may be seen by a comparison of *Angiopteris* and *Marattia*. The regular circumscissile dehiscence of the pollen chamber may be reminiscent of the time when there was a whorl of central loculi such as still exist in *Heterotheca*.

That there should be so few differences and so many resemblances between the microsporange and the ovule of the same species, makes it impossible to homologize merely the nucellus with the microsporange; as already stated, we must accept the synangial origin of the ovule as a whole, and regard the nucellus as derived from the central part of the common ancestor of both *Heterotheca* and *Sphaerostoma*. That the two structures, borne by the same species, should be homologous is undoubtedly the simplest explanation of their origin. They both separate from the frond, and in this respect differ from later forms, where, on the attainment of dry dehiscence in the microsporange, the pollen escaped before the fall of the sporange. The indusium or cupule which surrounds the ovule as long as it remains on the parent plant is one of the distinctive features of microsporange and ovule, for there is no evidence of an indusium surrounding *Heterotheca*. This difference, however, is really confirmatory of the synangial origin of the inner integument, as such an origin does not involve a double indusial formation in a relatively primitive ovule. On grounds such as these, it may be assumed that in the particular case of *Heterangium Grievii* its microsporangia and ovules are homologous, both being transformation products of a common ancestor.

Those who accept this thesis will undoubtedly be prepared to accept it for all the other Lagenostomales, such as *Lagenostoma*, *Conostoma*, and *Physostoma*. A necessary corollary as respects *Physostoma* is that there has been a meristic variation in the periphery. The bundles are approximately, or we might say, potentially twice as numerous as in the periphery of such a sporange as *Heterotheca*, and each dominates one radially symmetrical "tentacle" instead of a dual compartment as in *Spharostoma* (text fig. 8, *a*, *d*). Thus instead of regarding *Physostoma* as a *Lagenostoma* with unfused chambers (OLIVER **13**), we should, in the light of *Heterotheca*, regard it as a *Lagenostoma* with a lobed canopy.

Turning from the Lagenostomales to the Trigonocarpeae, which are regarded as the ovules of some at least of the Neuropterideae, we may ask if they bear any internal evidence of origin from such a type of synangium as *Heterotheca*. They show obvious series in the structure of the integument, series which in some respects run parallel to those in the Lagenostomales. Excellent details of the integument will be found in SALISBURY'S (**14**) work on *Trigonocarpus Shorensis*. In the Trigonocarpeae the nucellus is free from the inner integument except in the plane of its basal attachment, through which runs a vascular bundle which eventually forms a tracheal envelope round the nucellus. Six other bundles enter the integument and travel in its periphery. *Polylophosper-*

mum, *Trigonocarpus Parkinsonii*, and *T. Shorensis* show an obvious series in the reduction of the compartmental character of their integument (text fig. 8, a^1, b^1, c^1). In the two former the six bundles obviously alternate with compartments as in *Sphaerostoma* and *Heterotheca*, but in *T. Shorensis* all trace of the buttresses in the plane of the bundles has disappeared, as in *Lagenostoma*, and the bundles appear to occupy a central position.

The numerical relations of the bundles in *Heterotheca* and the Trigonocarpeae correspond, if we may assume in the latter a simple fusion of the two central bundles to supply the megasporange (nucellus), which in this series of seeds is free from the integument, and thus necessarily requires a central water supply. The tracheal mantle which becomes such a prominent organ in some of the Neuropterid seeds, for example, *Stephanospermum* (OLIVER 12), needs no explanation if the nucellus were derived from an ancestral sporange such as *Heterotheca*, with its large water storage equipment. Further, the succulent sarcotesta of the Trigonocarpeae is extraordinarily similar in structure to the succulent ground tissue of *Heterotheca*, so far as respects the macaroni-like tubules (text fig. 3). There is thus a considerable sum of evidence in support of the homology of the Pteridosperm seeds with a microsynangium such as *Heterotheca*.

Let us now turn to a consideration of the other microsporangia attributed with general assent to Pteridosperms. I shall only refer to *Crossotheca Hoeninghausi* (KIDSTON 9), *Telangium Scottii* (BENSON 4), and *Codonotheca caduca* (SELLARDS 15). Of these, *Telangium Scottii* is the only one in which the details of the anatomy have been published, but the preservation of the other sporangia as incrustation fossils is exceptionally good, and it has been possible to learn much of their organization. *Crossotheca Hoeninghausi* shows eight peripheral pairs of loculi, each pair showing a single vascular bundle running up in its dividing wall. In the young condition the eight pairs are seen to be in close lateral approximation, so that they form a body resembling a stout undehisced *Telangium* with a wide base. In specimens recently provided by MR. HEMINGWAY, there seems no reason to doubt that this younger condition (occurring nearer the tips of the fronds) represents an undehisced condition of a synangium. In KIDSTON's original description (9) the synangium was regarded as built up of eight discrete dual sporangia. In a transverse section kindly lent by Dr. KIDSTON, it was noted that the matrix within the ring of paired loculi was stained yellow, and this is suggestive of decayed ground tissue having been present originally where now only a boss of rock can be seen.

ZEILLER thought that the members of the whorl were sometimes coherent. If this be the true interpretation, *Crossotheca Hoeninghausi* occupies a position almost halfway between *Heterotheca* and *Telangium*. It resembles *Telangium* in the possession of a single peripheral series of loculi, in apical dehiscence, and its retention on the frond until the spores are shed. It resembles *Heterotheca* in the loculi being distributed one on either side of longitudinal discrete vascular bundles, in the possession of much sterile ground tissue, and in the number (sixteen) of its longitudinal loculi or loculi series. It differs from *Telangium Scottii* in the number of its loculi, and from *Heterotheca* in the absence of central loculi and in its apical dehiscence. I have no evidence as to the character of its ground tissue, but it is probable that it was non-cuticularized.

From these considerations I have included in text fig. 8, II an adaptation of KIDSTON's fig. 9, indicating by hatching the supposed ground tissue which had perished in the incrustation. It would seem best to retain the name *Crossotheca*, as the body is sufficiently distinct from any species of *Telangium* to retain its separate form genus rank.

Codonotheca caduca is regarded by SELLARDS as probably a Neuropterid sporange. It is fairly safe to regard it as belonging to some Pteridosperm, and a reference to it may be useful. Like the *Trigonocarpus* seeds, it is provided with six peripheral bundles, but in this case each becomes duplicated. Like the canopy of *Physostoma*, the peripheral series have become lobed. There are no central loculi, which are so far only recorded for *Heterotheca*. *Telangium Scottii* has but half the number of loculi recorded for *Crossotheca*, a mutation probably having occurred comparable with that which gave rise to the reduced number of bundles of *Conostoma*, which shows but four (text fig. 8, *c*). The three series shown in this figure, for example, the Lagenostomales (*a–d*), the Trigonocarpeae (a^1–c^1), and the microsporangia (I, II, III, IV) illustrate the fact that at least two lines of progression can be traced in Pteridosperm seeds, each with members suggesting an early descent from a sporange with paired loculi. These paired loculi occur in both *Heterotheca* and *Crossotheca*. A tendency to simplification is seen in all three groups, *Conostoma* among the Lagenostomales and *Telangium* among the microsporangia showing a halving of the number of parts. Again an example of lobing occurs in one seed of the Lagenostomales (*Physostoma*, text fig. 8, *d*), and in one type of microsporangium (*Codonotheca*, text fig. 8, IV).

Summary and conclusions

1. Among the remains of *Heterangium Grievii* (vegetative organs and seed) in the calcified deposits of plants occurring at Pettycur, Fifeshire, have been found large numbers of a new type of microsporange for which the form genus *Heterotheca* has been constructed.

2. Its mechanical structure is similar to that of the petioles of *Heterangium*, and, although so far found detached, it is attributed to *Heterangium* on the same grounds as are the vegetative organs, that is, the presence of vertical and horizontal sclerotic plates.

3. Its spore bearing tissue is distributed in sixteen series of loculi, of which twelve are peripheral and four central. The vascular bundles are similar to those of the seed, and near the apex each is nearly surrounded by a pair of loculi as in the canopy of the seed.

4. The structure throughout is strongly confirmatory of the homology of the seed and synangium, and is regarded as supplying ample proof of the synangial origin of the seed.

5. In this investigation we seem to stand upon the threshold of the origin of at least one group of the Spermatophyta, and the conclusions reached cannot fail to have their influence on the study of other groups.

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SURREY, ENGLAND

LITERATURE CITED

1. Arber, E. A. N., On a new pteridosperm possessing the *Sphenopteris* type of foliage. Ann. Botany **22**: 57–62. *pl. 7*. 1908.
2. ———. Devonian floras. Cambridge Univ. Press, p. 61. *fig. 34*. 1914.
3. Benson, Margaret, Contributions to the embryology of the Amentiferae. Part I. Trans. Linn. Soc. London **3**: 412. *figs. 7*. 1894.
4. ———, *Telangium Scottii*. Ann. Botany **18**: 161–177. 1904.
5. ———, *Sphaerostoma ovale*. Trans. Roy. Soc. Edinburgh **50**: 1–15. 1914.
6. ———, *Mazocarpon*. Ann. Botany **32**: 586. 1918.
7. Coulter, J. M., and Land, W. J. G., *Torreya*. Bot. Gaz. **39**: 163. 1905.
8. Gordon, W. T., On the nature and occurrence of the plant-bearing rocks at Pettycur, Fifeshire, Scotland. Trans. Geol. Soc. Edinburgh **9**: 1909.
9. Kidston, R., On the microsporangia of the Pteridospermeae. Trans. Roy. Soc. London, Series B. **198**: 428. 1906.
10. Kidston, R., and Lang, W. H., Old red sandstone plants. Part IV. Trans. Roy. Soc. Edinburgh **52**: 852. 1921.
11. Nathorst, A. G., Paleobotanische Mitteilungen. Handl. Kgl. Svensk. Vetensk.-Akad. **43**: 1–12. *figs. 3*. 1908.
12. Oliver, F. W., Structure and affinities of *Stephanospermum*. Trans. Linn. Soc. London **6**: 361–400. 1904.
13. ———, On *Physostoma elegans* Williamson, an archaic type of seed from the Paleozoic rocks. Ann. Botany **23**: 103. 1909.
14. Salisbury, E., *Trigonocarpus Shorensis*. Ann. Botany **28**: 74. 1914.
15. Sellards, E. H., Notes on the spore-bearing organ *Codonotheca*, and its relationship with the Cycadofilices. New Phytol. **6**: 175–178. 1907.
16. Starr, A. M., The microsporophylls of *Ginkgo*. Bot. Gaz. **49**: 53. 1910.
17. Treub, M., Sur les Casuarinées. Ann. Jard. Bot. Buitenzorg **10**: 172. 1891.

EXPLANATION OF PLATES IV AND V

The following abbreviations are used in the illustrations: *b*, buttress or interstitial tissue between peripheral loculi; *e*, epidermis; *g*, gap in stone section; *gt*, ground tissue; *l*, loculus; *cl*, central loculus, *ne*, nerve ending; *s*, spores; *sp*, sclerotic plate; s^1p^1, plate attached to loculus wall; *vsp*, vertical sclerotic plate; *vb*, vascular bundle.

PLATE IV

Figs. 1, 2.—Two successive cross-sections of immature synangium; epidermis is preserved, and in center of body occur small sclerotic plates; no central loculi can be distinguished, and content of peripherous ones is amorphous; matrix has been left around fig. 1 (CN.386.11, 12); ×33 diameters.

Figs. 3, 4.—Two successive cross-sections of synangium nearer base; central loculi well shown (CN.383.12, 13); ×29 diameters.

FIG. 5.—Transverse section of mature specimen, showing many of loculi with ripe spores, some of which, owing probably to solution of loculus wall, lie free in ground tissue; rough diagram of this section given in text fig. 4 (CN.411.31); ×37 diameters.

FIG. 6.—High power photograph of central ground tissue of obliquely longitudinal section, showing highly organized nerve ending (*ne*), various horizontal sclerotic plates, and succulent ground tissue like that of sarcotesta of Trigonocarpeae; in original, striated elements of transfusion tissue can be seen above nerve ending (CN.304.2); ×100 diameters.

FIG. 7.—Transverse section through upper part of synangium, showing central loculi and position of nerve ending (CN.411.12); ×18 diameters.

PLATE V

FIGS. 8, 9.—Two sections across another synangium, showing position of loculi in spite of some degree of maceration and subsequent shrinkage; in fig. 8 plane is nearer apex; surface is seen abutting on other tissues in matrix; in none of the four sections of this series are spores to be detected; fig. 8 (CN.391.23), ×25 diameters; fig. 9 (CN.391.24), ×40 diameters.

FIG. 10.—Nearly peripheral tangential section, showing in original the transversely oriented series of loculi, of which majority show tetrads of spores; cf. text fig. 3, constructed from two successive longitudinal sections; it is given to show transversely running strand (*vb*), for which reason the rest is slightly over exposed (CN.386.17); ×42 diameters.

FIGS. 11, 12.—Two successive transverse sections of class I synangium, in which spores are free from one another and all loculi are full of them; synangium had undergone some degree of maceration, as shown by sclerotic plates (*sp*), cells of which show partial solution of middle lamella; key provided for fig. 12 in text fig. 6, to indicate position of sclerotic plates, loculi, etc., which are clearer in the fossil, although many of the walls have given way; fig. 11 (CN.307.17), ×27 diameters; fig. 12 (CN.307.18), ×50 diameters.

FIG. 13.—Transverse section clearly showing vertical sclerotic plates (*vsp*); most of loculi have perished, but one is indicated (*l*); several others are easily detected in the slide (CN.393.6A); ×32 diameters.

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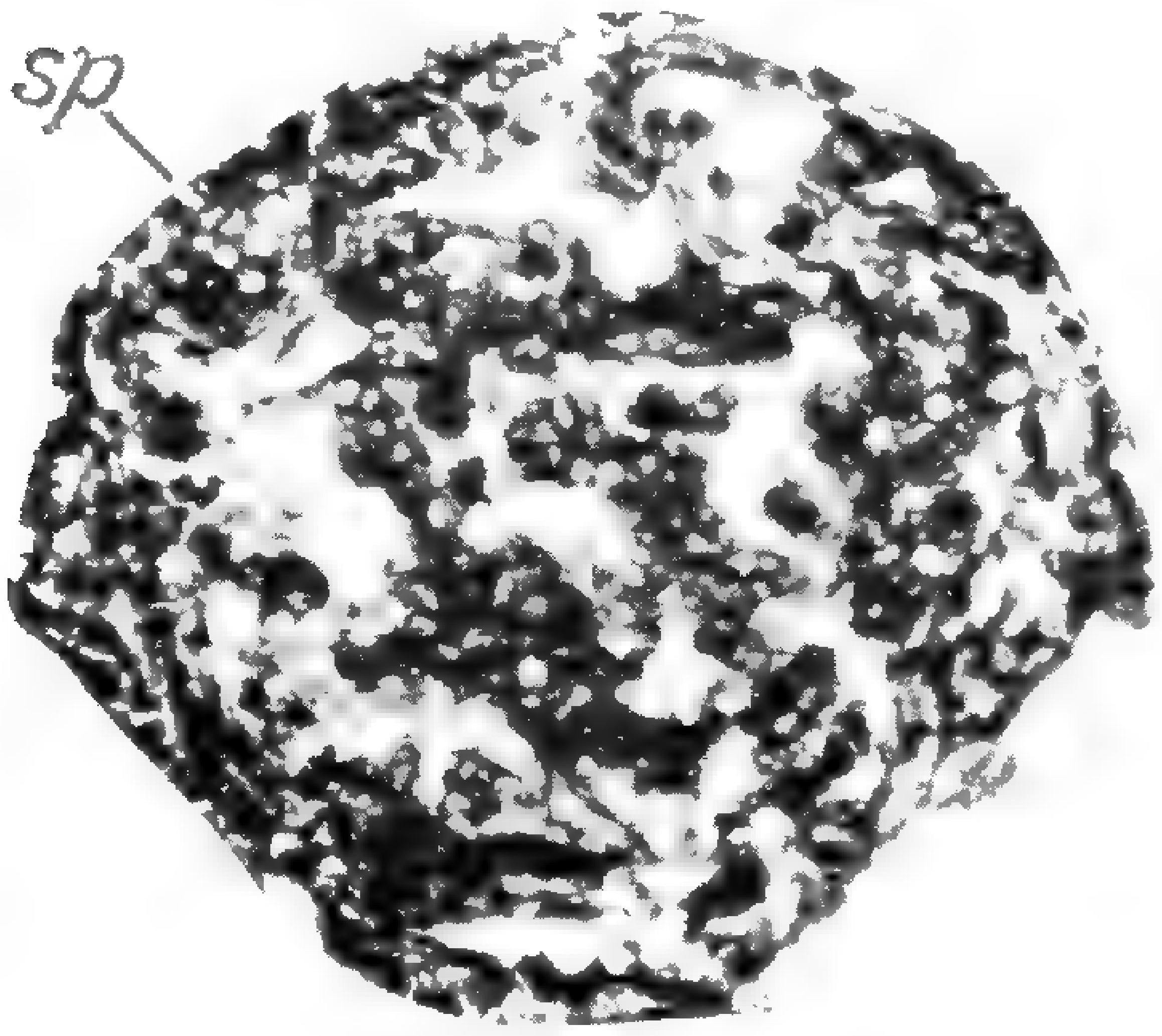

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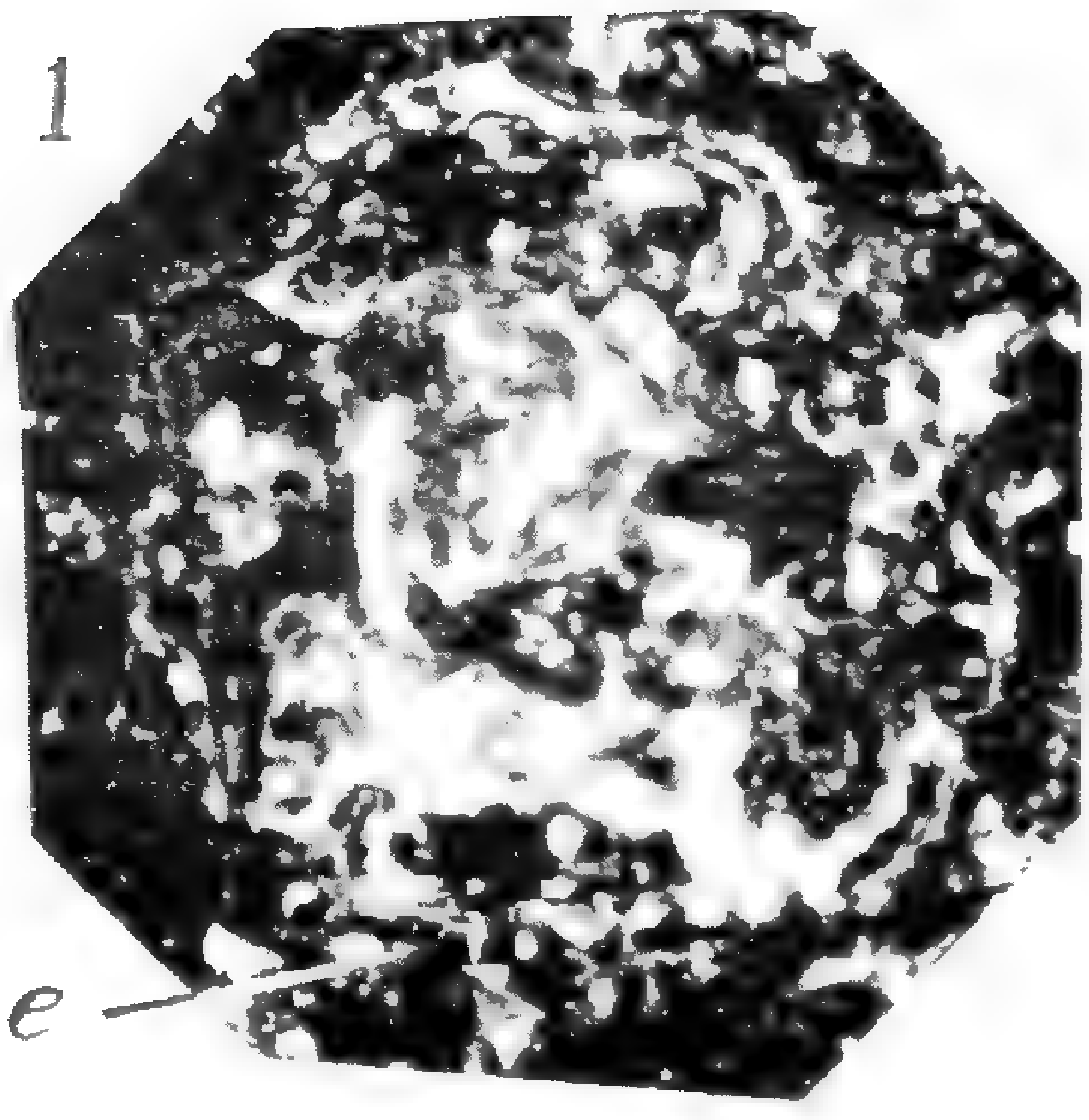

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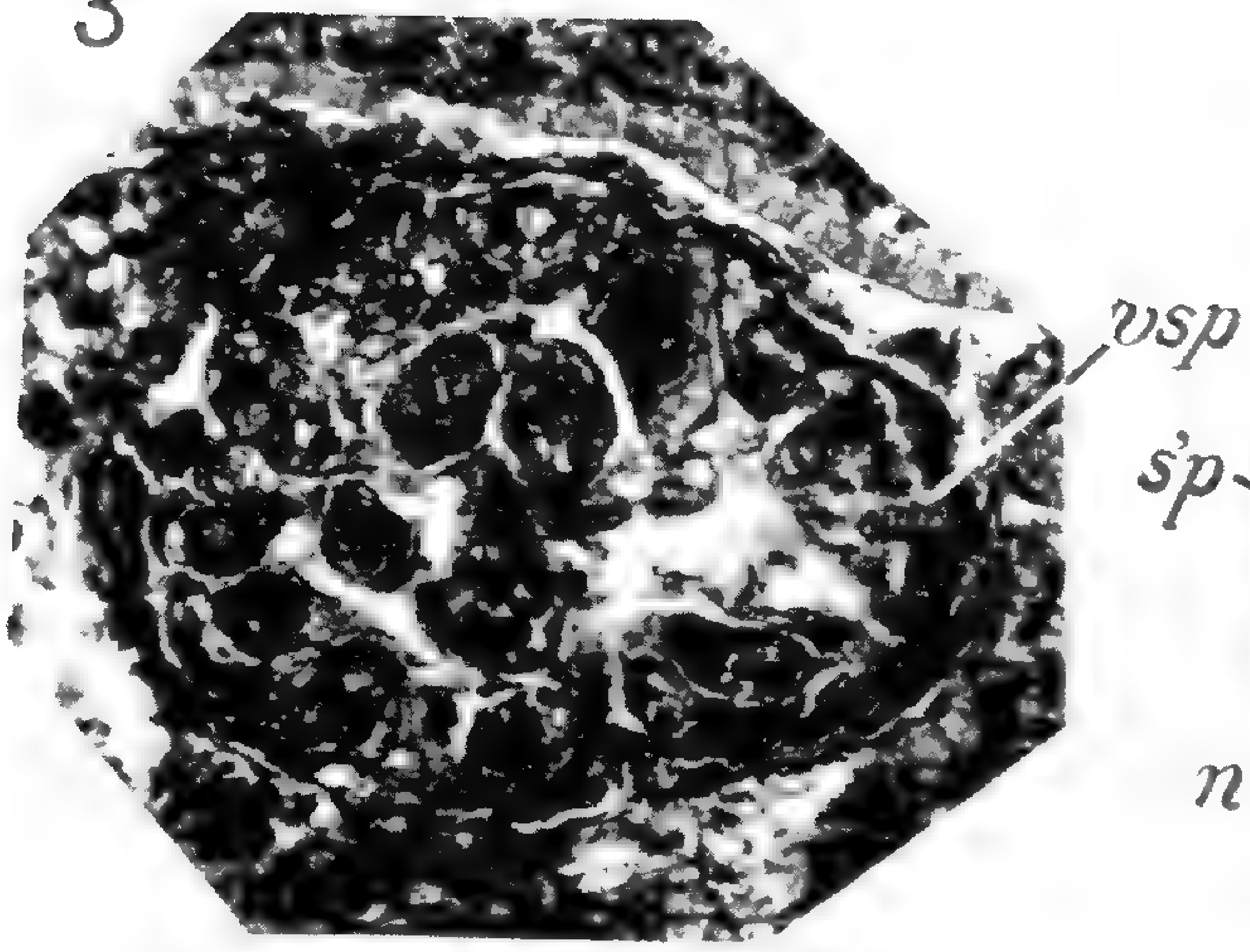

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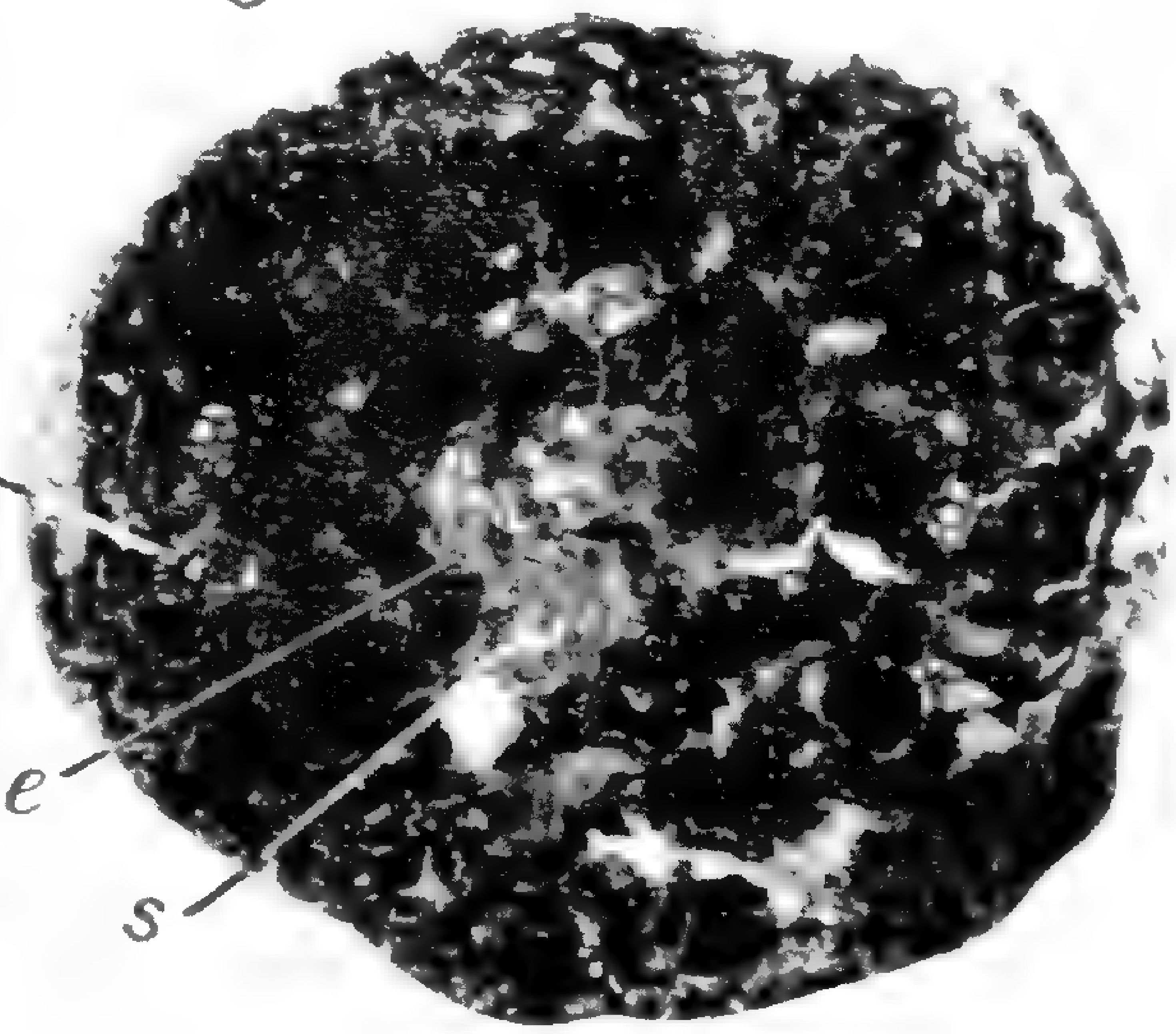

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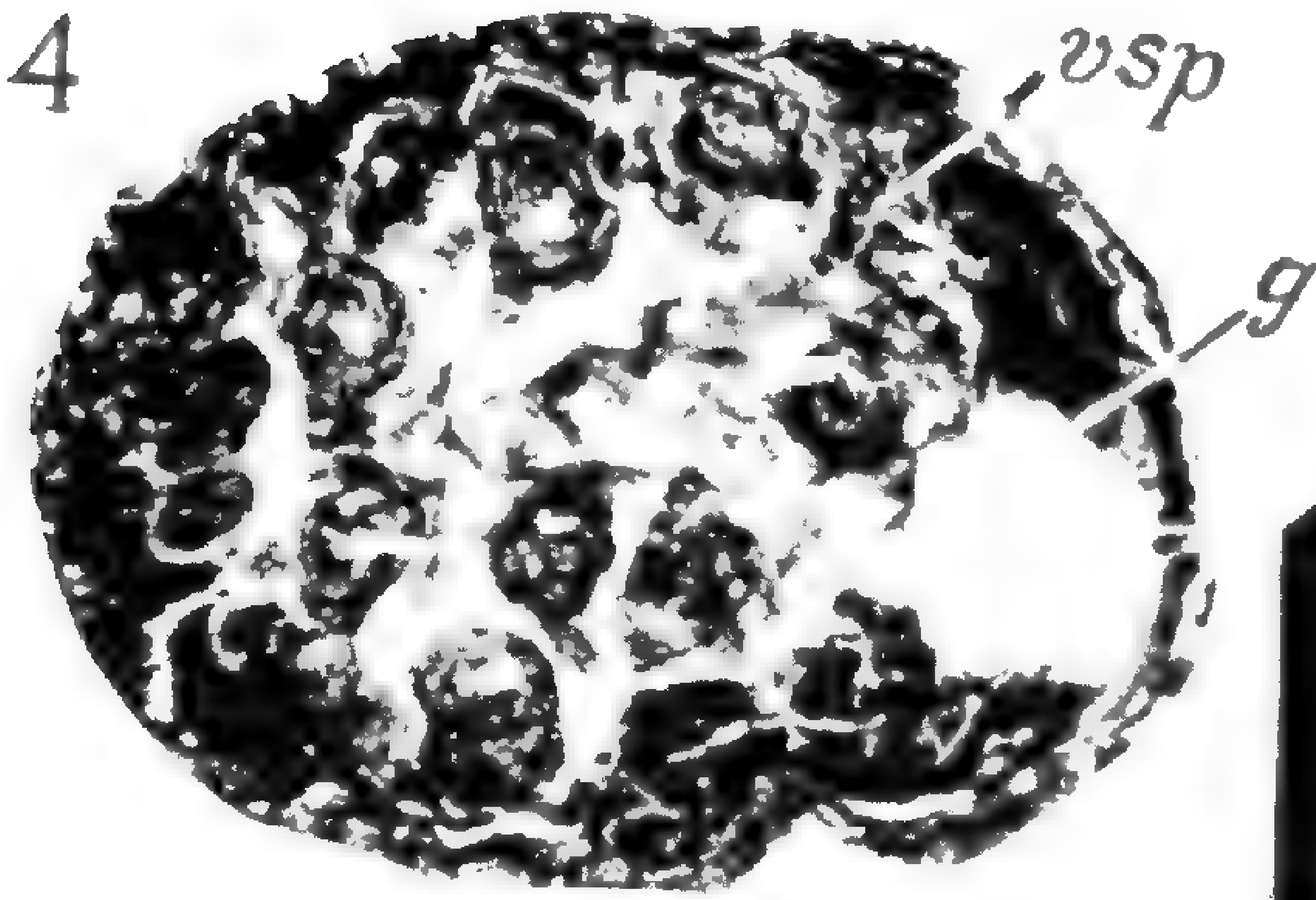

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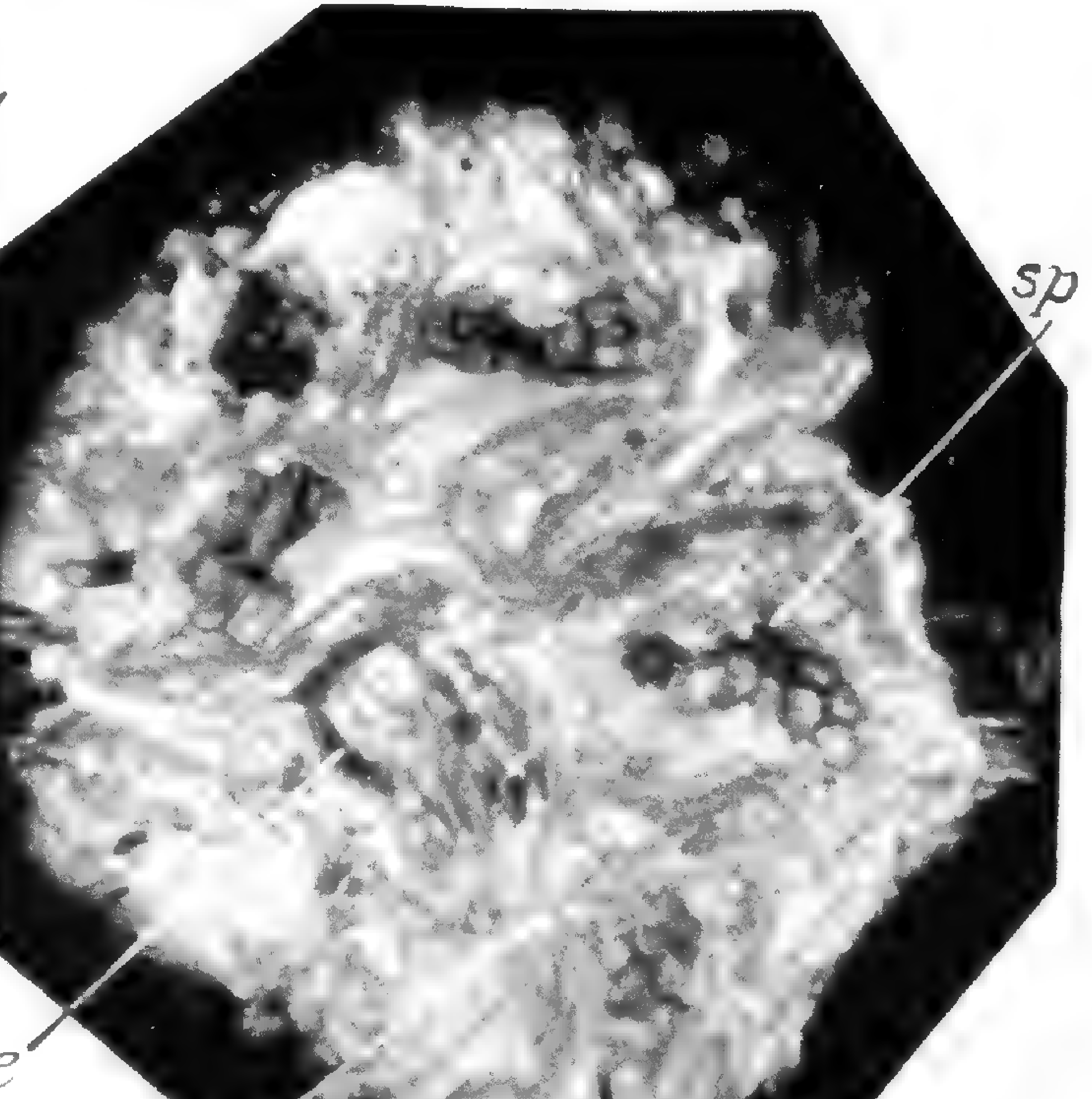

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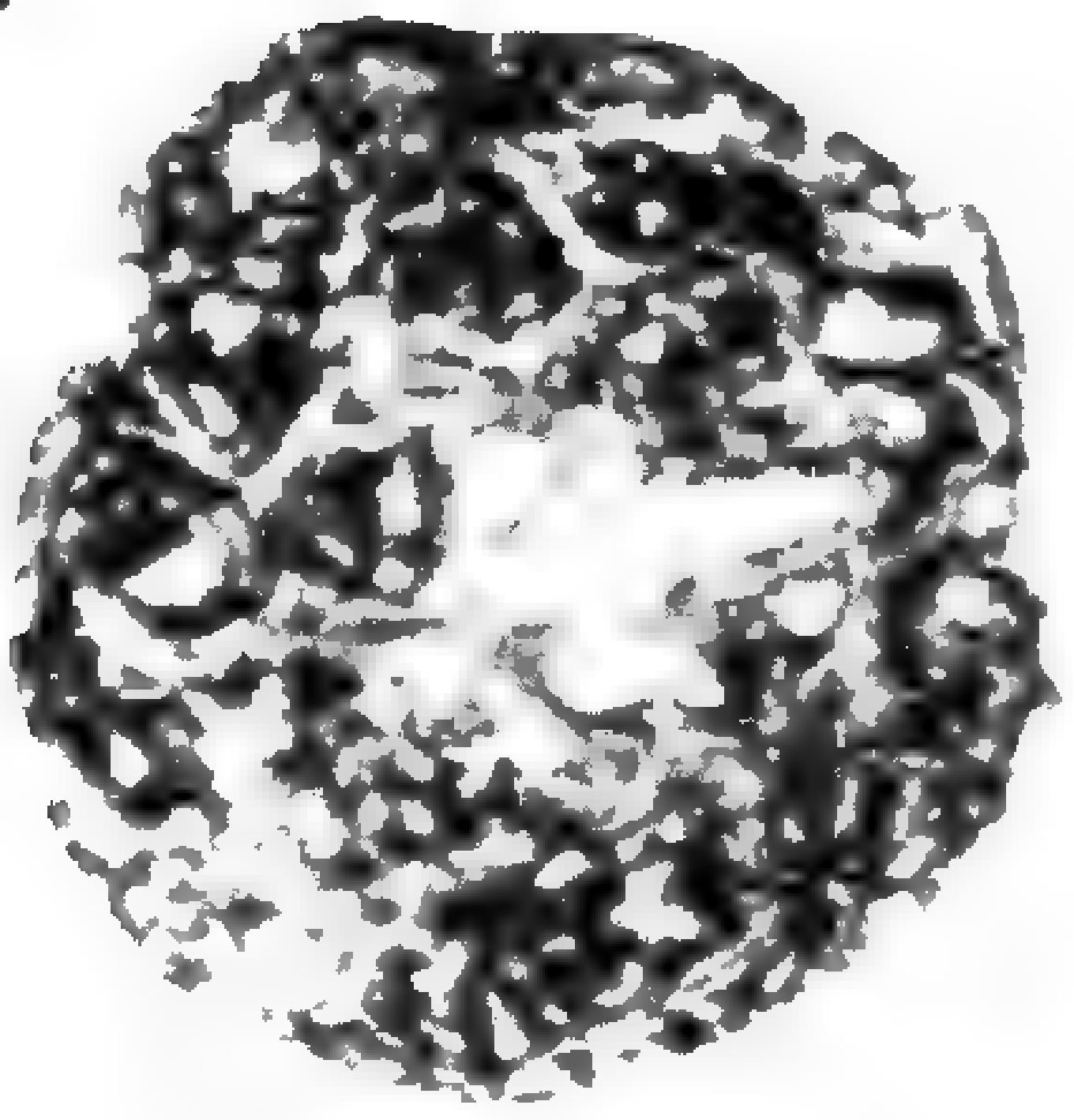

BENSON on HETEROTHECA

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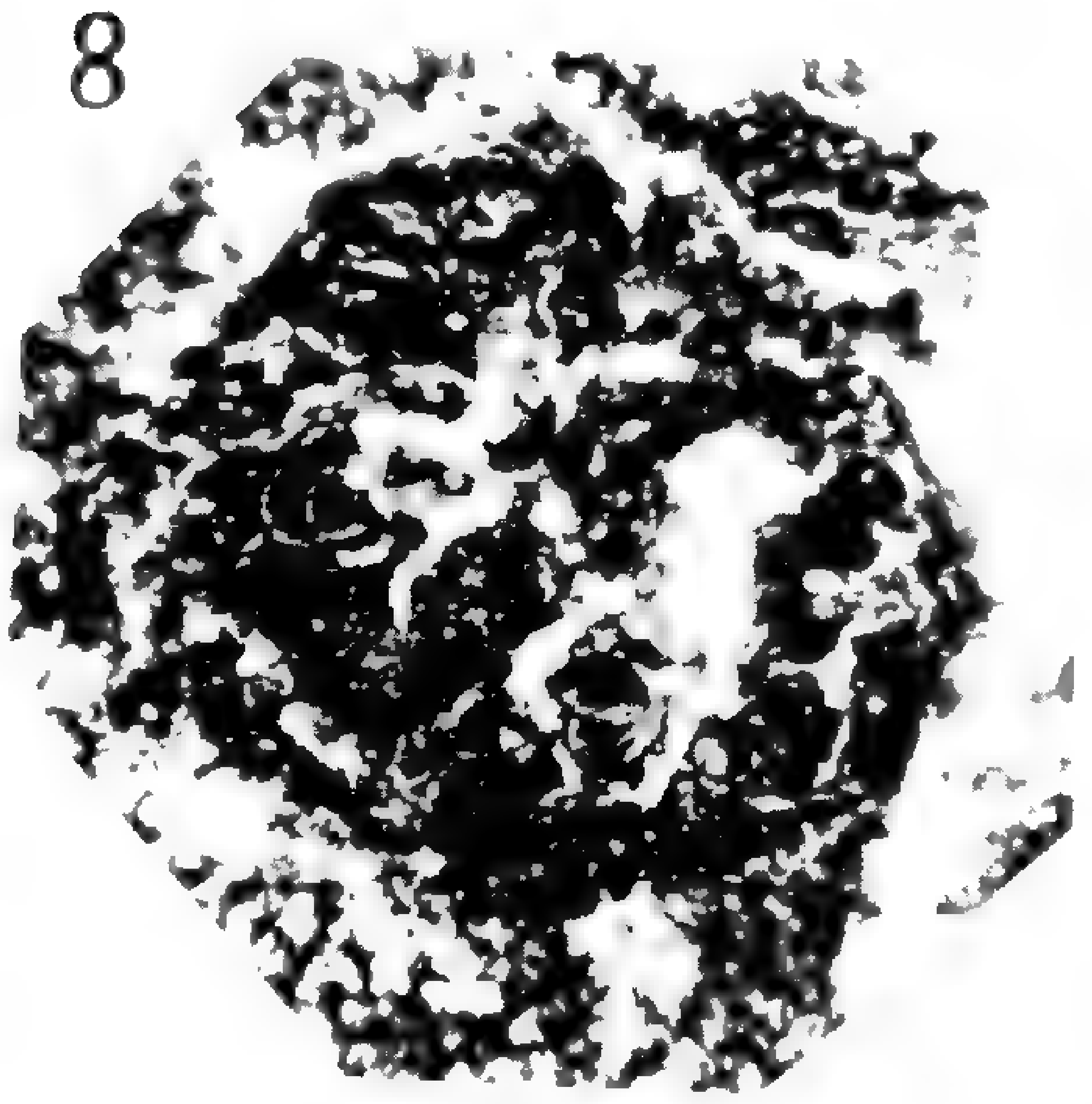

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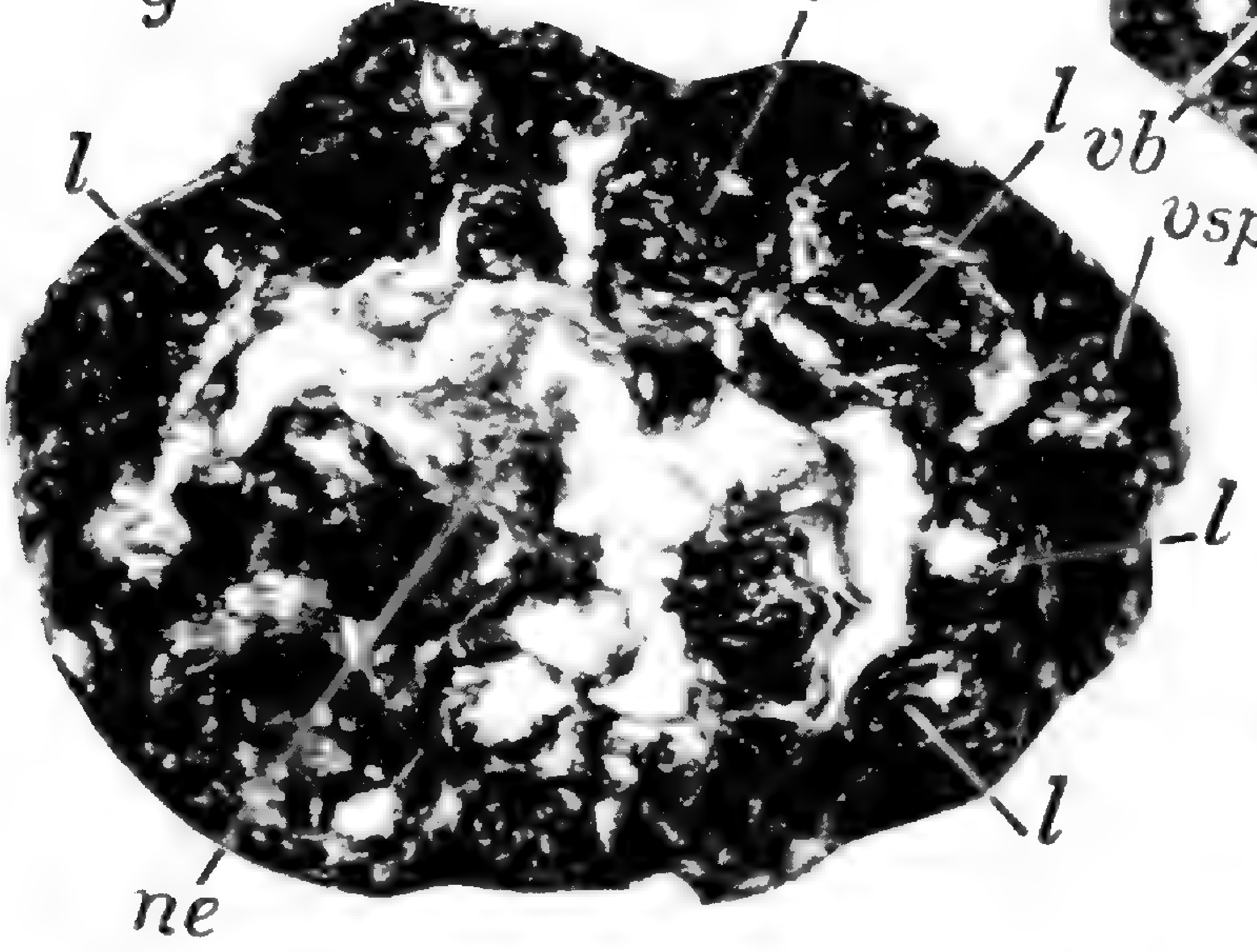

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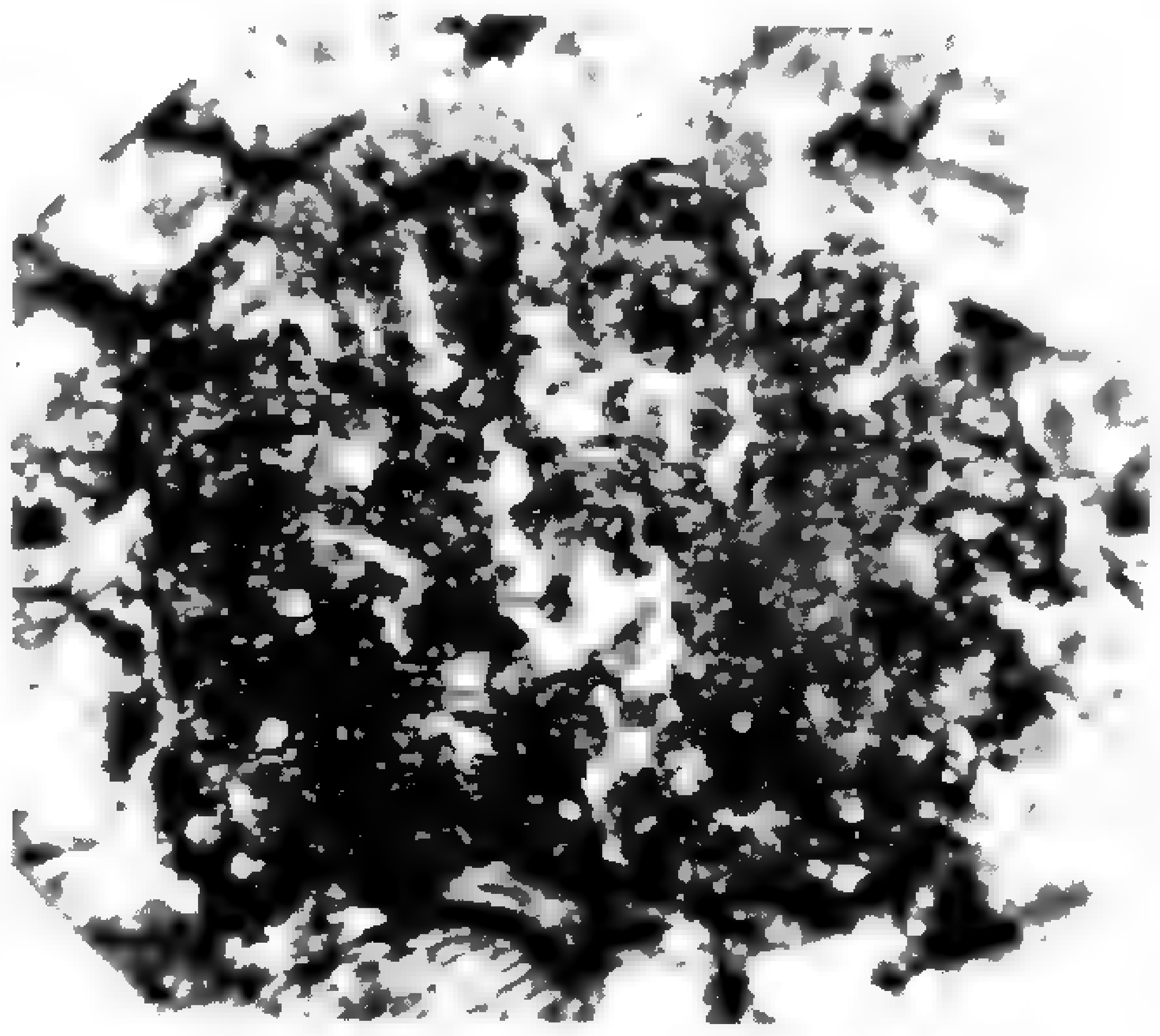

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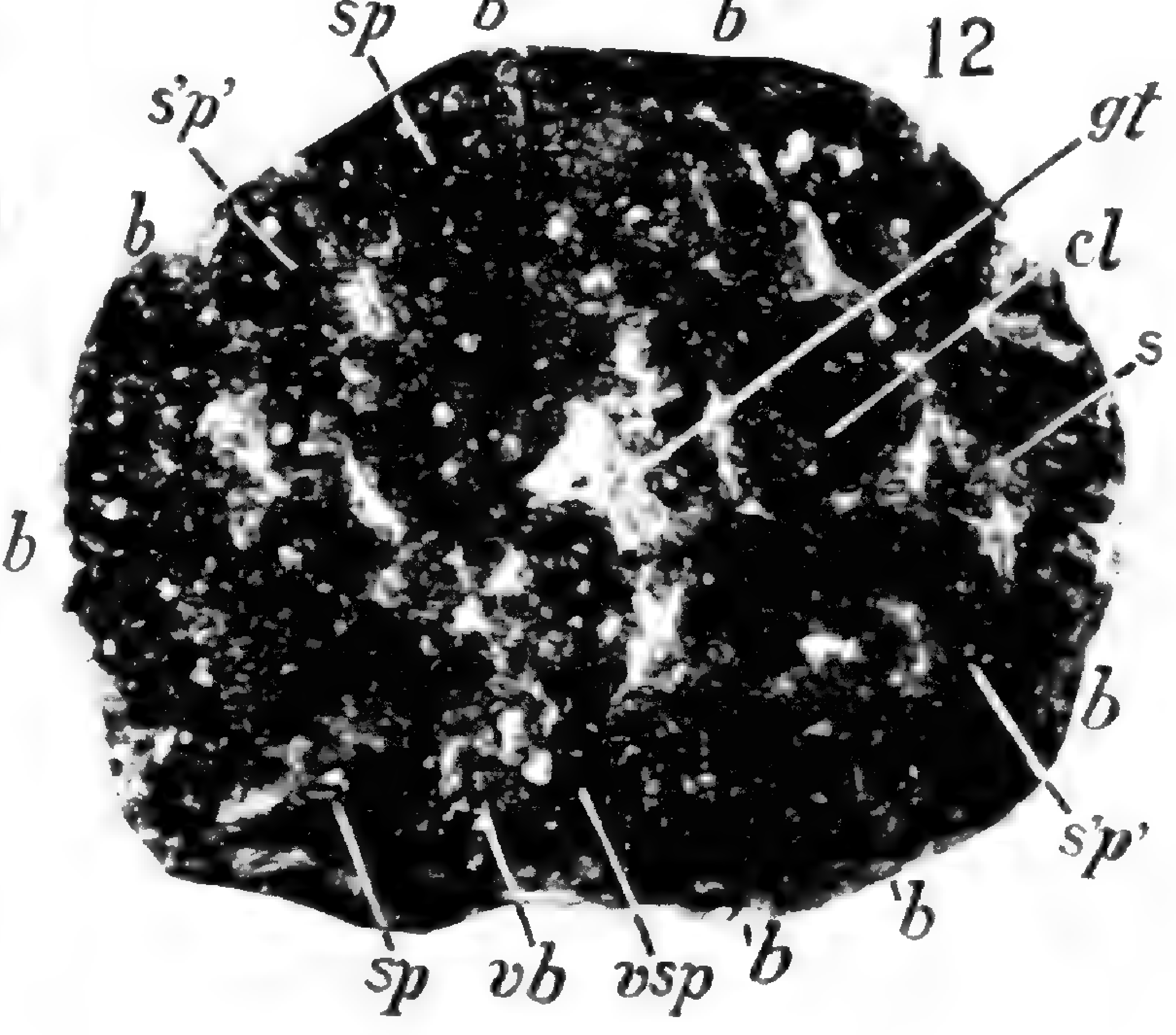


BENSON on HETEROTHECA

EARLY EMBRYOGENY OF REBOULIA HEMISPHAERICA

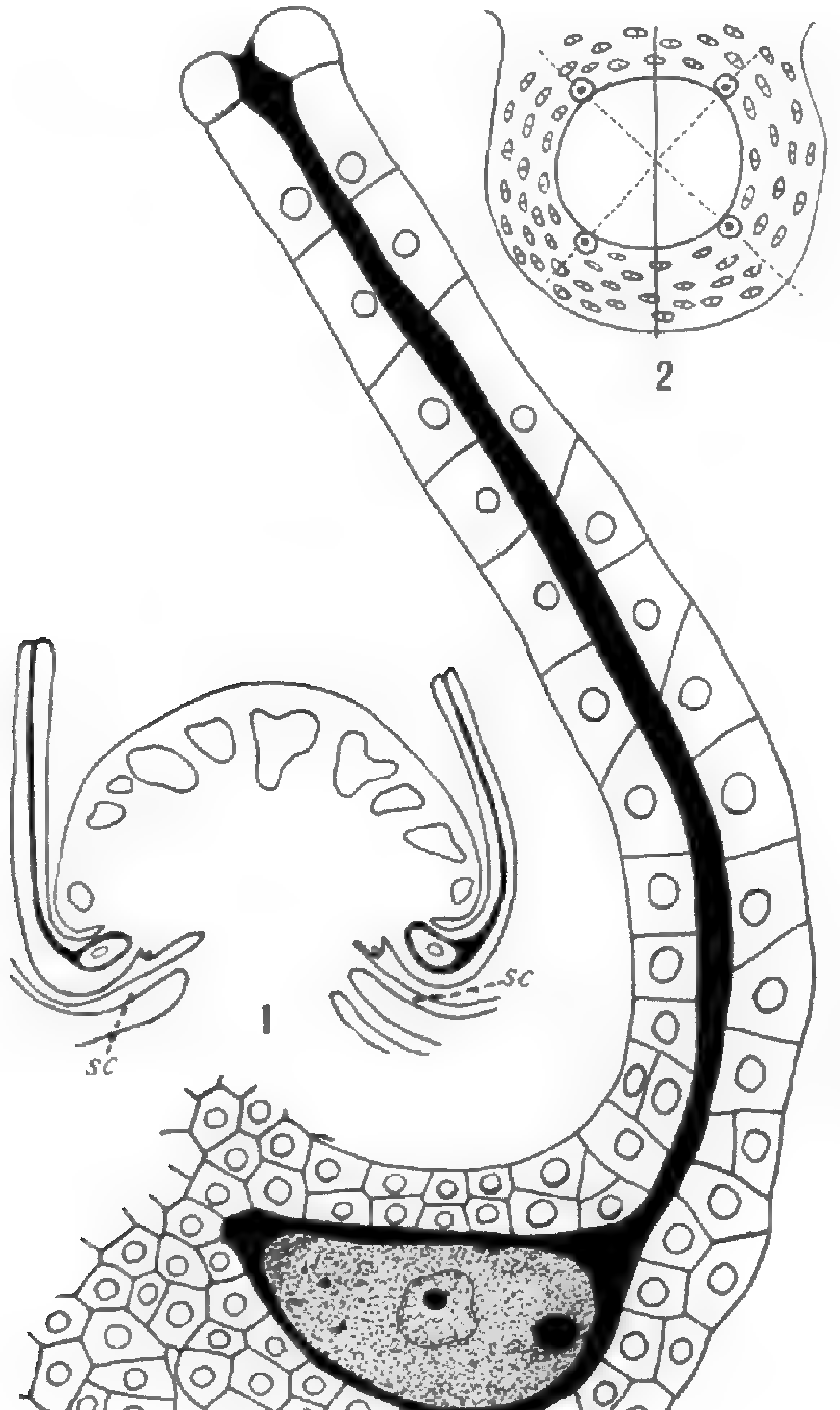
(WITH FORTY-SEVEN FIGURES)

A. W. DUPLER

Our knowledge of the development of the sporophyte of *Reboulia* dates back to HOFMEISTER (11), who described its early stages and its rapid growth as it approaches maturity. KIENITZ-GERLOFF (13), who studied the embryogeny of a number of forms, claimed the *Reboulia* embryo to be similar to that of *Grimaldia*, certain stages of which he described in some detail, but not including the earliest stages. LEITGEB'S (14) work on the Marchantiaceae included a study of *Reboulia*, of which he described the development of the sporophyte in a general way. CAVERS' (3) observations also included the early and late sporophyte of *Reboulia*. The more recent work on the embryo has been done by WOODBURN (18) and HAUPT (10), the former dealing with the very early stages, the latter describing the development from beginning to maturity. These two recent accounts differ somewhat from the earlier studies, and in certain features from one another. A study of the writer's collections of material has yielded certain results which may be of interest in comparison with these accounts, especially where they bear on their differences, and in the addition of certain facts not mentioned by them. Altogether it is clear that the embryo of *Reboulia* shows considerable variation in the early phases of its development.

Material

The material for this study was collected near Huntingdon, Pennsylvania, the greater bulk of the embryos figured having been secured from collections made from the early part of October to the latter part of November, 1919. None of the material collected in September shows embryos so far as it has been examined. The early winter condition (figs. 46, 47) was secured from a collection made December 23, 1920. The material was killed in 25 per cent chrome-acetic acid, and for the most part stained by the iron-alum haemotoxylin method. As DURAND (7) found in *Marchantia,* the embryo stains much lighter than the surrounding calyptra, and it was found that dipping the slides for a short time in an alcoholic solution of Lichtgrün served to bring out clearly the delicate cell walls. This clouds the cytoplasm of the cells to a slight extent, and is not to be recommended when the details of cell structure are to be studied.



FIGS. 1–3.—Fig. 1, vertical section of young female receptacle with mature archegonia; fig. 2, diagram showing relation of archegonia to receptacle and longitudinal axis of thallus (solid line); dotted line indicates median plane through bilateral archegonium and embryo; fig. 3, median longitudinal section of mature archegonium; dark area about embryo represents space between embryo and calyptra; fig. 3, ×400.

Archegonium

The writer has found nothing in the development of the archegonium differing from the account given by HAUPT (9). It first appears when the female receptacle is yet quite small, and when the sex organ is mature the receptacle is still a low conical structure (fig. 1), surrounded by a large number of sterile scales (fig. 2). The venter of the archegonium is inclined somewhat below the horizontal, the neck curving upward more or less to a perpendicular position among the scales. HAUPT (9) regards these scales as probably protective in function. They also probably serve in holding a film of water about the archegonia, functioning much as do the paraphyses about the sex organs of mosses, resulting in conditions which increase the probabilities of fertilization.

Usually there are four archegonia on each receptacle, one close behind the apical cell of each of the four growing points of the receptacle. Rarely three, occasionally five or six such growing points and archegonia may occur. In the typical condition the archegonia are so situated on the receptacle that a median section through the entire archegonium can be secured only by vertical sections cut on a plane at an angle of 45° to the long axis of the thallus (fig. 2). Owing to their curvature, both the archegonium and the early embryo are bilateral and not radial, and a strictly median section can pass through but one plane. With but few exceptions all the embryos figured in this account are from sections along this plane.

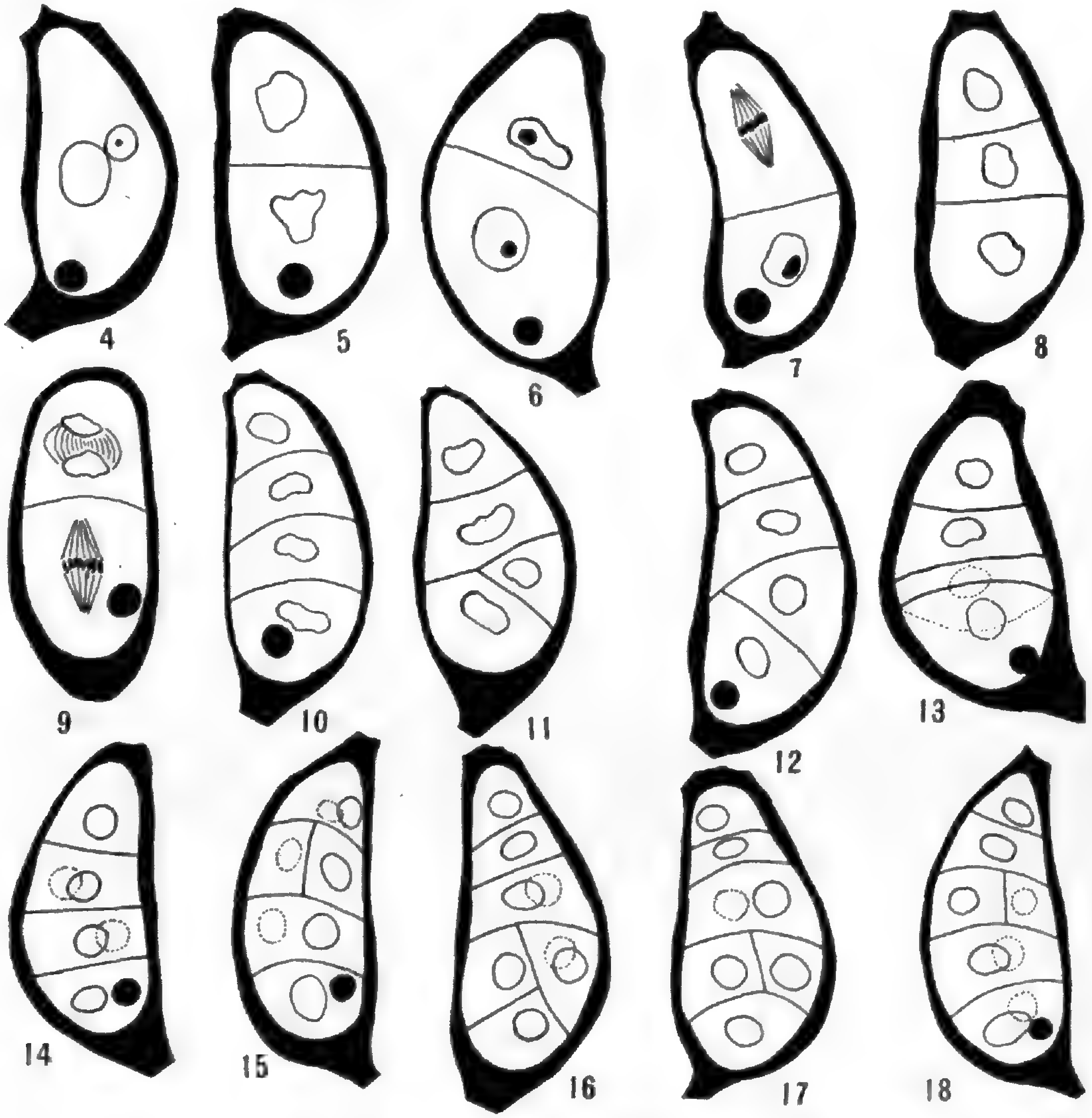
The egg at maturity is about twice as long as its transverse diameter, bluntly rounded at both ends, slightly more tapering at the hypobasal end, and with its long axis describing the arc of a circle (fig. 3). The nucleus is centrally placed, the egg cytoplasm uniformly distributed, and containing plastids and oil globules. There is usually a very conspicuous oil globule near the anterior end, which persists even in late stages of the embryo.

Fertilization

The close proximity of the male and female receptacles on the same branch of the thallus usually insures fertilization, although occasionally it fails to occur. WOODBURN calls attention to the change which the motile sperm undergoes from the time it leaves the antheridium until its nucleus is ready to fuse with the egg nucleus. The writer found a number of cases in which the sperm nucleus had penetrated the egg cytoplasm and lay close to the egg nucleus (fig. 4). At this time the egg nucleus has about twice the diameter of the sperm nucleus, whose more compacted chromatin results in a denser staining body. No attempt was made to study the nuclear changes involved in fertilization. Apparently the fusion nucleus passes into the resting stage before division of the egg takes place.

Embryo

FIRST DIVISION.—Without any considerable enlargement after fertilization, the egg divides by a transverse wall, usually perpendicular to the long axis, and giving nearly equal epibasal and hypobasal cells (fig. 5). This agrees with the accounts for *Reboulia* as given by Cavers (3), Woodburn (18), and Haupt (10), and with



Figs. 4–18.—Fig. 4, egg with male and female nuclei, black circle representing oil drop; fig. 5, first wall transverse; fig. 6, first wall oblique; fig. 7, mitosis in hypobasal cell; fig. 8, embryo of three cells; fig. 9, mitosis in both epibasal and hypobasal cells; fig. 10, typical filamentous embryo of four cells; figs. 11–13, epibasal cell divided by oblique wall; fig. 14, vertical division of two middle cells of row, basal and apical cells undivided as yet; fig. 15, apical cell not yet divided by vertical wall; fig. 16, epibasal cell divided by oblique wall, vertical division in middle of embryo, basal cell of row of four probably transversely divided; figs. 17, 18, two cells at base probably resulting from transverse division of basal cell; ×430.

such forms as *Targionia* (Campbell 1, O'Keefe 16), *Plagiochasma* (Starr 17), *Conocephalum* (Cavers 2), *Riccia* at times (Garber 8), and practically all the Jungermanniales which have been examined, among which are *Sphaerocarpus* and *Geothallus* (CAMPBELL 1), *Aneura* (LEITGEB 14, CLAPP 5), *Fossombronia* (HUMPHREY 12), *Pellia* (KIENITZ-GERLOFF 13), and *Symphyogyna* (MCCORMICK 15), and is in contrast with forms in which the first wall is more or less oblique, as occurs in *Riccia* (KIENITZ-GERLOFF 13, CAMPBELL 1, GARBER 8), *Marchantia* (DURAND 7), and *Preissia* (KIENITZ-GERLOFF 13).

HOFMEISTER thought the *Reboulia* egg divided first by a strongly inclined wall. LEITGEB claimed the first wall to be generally oblique, occasionally perpendicular, to the long axis. HAUPT (10) states that the first division is "always accompanied by a transverse wall." WOODBURN'S statement is not so positive, and one of his figures shows the first wall slightly inclined. The writer found several cases in which the first wall was more or less oblique, sometimes with the epibasal cell the larger of the two (fig. 6).

FILAMENTOUS EMBRYO.—The published accounts differ considerably as to the behavior following the first division. HOFMEISTER described the growth as due to an apical cell with two cutting faces, the epibasal cell dividing repeatedly by alternately inclined walls, resulting in a slender embryo of two rows of cells. KIENITZ-GERLOFF was not convinced by HOFMEISTER'S account, but concluded from analogy with *Grimaldia* that in *Reboulia* an octant is formed by vertical walls perpendicular to the first transverse wall. LEITGEB claimed quadrant formation by walls perpendicular to the first, the apical and basal cells of the quadrant being the larger, since the first wall is usually oblique, and both the epibasal and hypobasal cells are divided unequally. CAVERS (3) also claimed an octant by the formation of perpendicular walls, and regarded the epibasal half of the octant as giving rise to the capsule, the hypobasal to the foot and stalk. The studies by WOODBURN, HAUPT, and the writer do not agree with these earlier statements. In most cases the second and third divisions are parallel to the first, resulting in a filament of four cells, with the walls between them more or less parallel to one another.

WOODBURN and HAUPT both claim the second division to be in the epibasal cell, resulting in a row of three cells. WOODBURN says the third division may be in either the apical or the middle of these three cells; Haupt states that it is in the apical cell. According to both workers the first division of the hypobasal cell does not take place until after the formation of the row of four cells, and then usually by a vertical wall. Haupt says that occasionally this vertical wall formation takes place before the third transverse division, that is, when the embryo consists of but three cells. Neither Woodburn nor Haupt show mitotic figures definitely proving this sequence of division. The sequence of these early divisions may hold a definite relation to the later differentiation of the sporophyte into foot, stalk, and capsule regions. The writer's preparations show that transverse division may take place in both hypobasal and epibasal cells (fig. 9), and that the division of the hypobasal cell precedes that of the epibasal cell (fig. 7). The division of the hypobasal cell may be completed before the epibasal cell begins to divide, resulting in a three-celled filament (fig. 8). Such embryos are probably quite rare, however, the writer having found but a single case. It is more probable that the division of the epibasal cell is generally initiated before that of the hypobasal cell is complete (fig. 9), and the four-celled embryo results with the completion of the two division processes (fig. 10). The writer concludes, therefore, that at this stage the embryo consists typically of a row of four cells with parallel walls, as a result of the transverse division of both hypobasal and epibasal cells, the division of the former preceding slightly that of the latter. Not a single case was found suggestive of the quadrant, as claimed by Cavers, who probably based his interpretation on later stages without having observed these early ones, as he shows no figures of early embryo development.

Variations from the typical situation are of interest. For example, the transverse walls are often more or less curved, with the concave side toward the apex, the curvature often being especially pronounced in case of the apical cell (figs. 10, 17); or the division of the epibasal cell may be by an oblique wall (figs. 11–13) whose inclination to the perpendicular may show considerable variation. Such a division of the epibasal cell is more likely to occur when the first division has been an oblique one (figs. 11, 12), although the inclination of this wall may be independent of the first wall (figs. 13, 16). Woodburn figures several embryos showing oblique walls in the epibasal portion. HAUPT thinks oblique walls do not occur at this stage or later. It seems to the writer that the evidence for the occasional occurrence of oblique walls is conclusive.

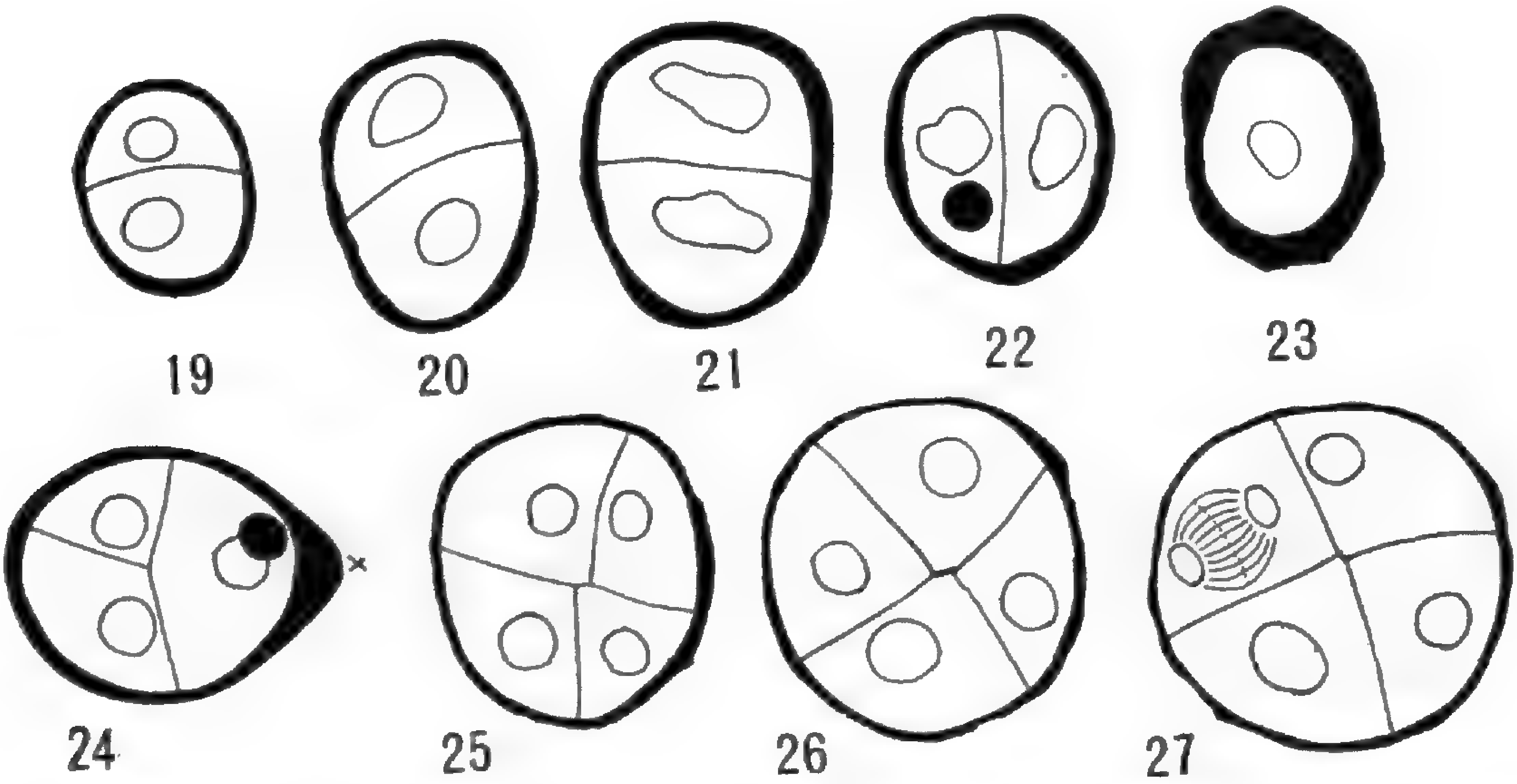
The occurrence of a filamentous embryo in *Reboulia* agrees with the embryo described for *Plagiochasma* (STARR **17**), *Targionia* (O'KEEFE **16**), *Geothallus*, and *Sphaerocarpus* (CAMPBELL), as well as practically all the Jungermanniales. Quadrant formation by walls vertical to the first wall occurs in *Riccia* (CAMPBELL **1**), in *Marchantia*, as given by a number of writers, DURAND's account being the most complete, *Conocephalum* (CAVERS **2**), and *Fimbriaria californica* (CAMPBELL). KIENITZ-GERLOFF made a similar claim for *Grimaldia* and *Preissia*, but did not have the early stages. GARBER found occasionally a row of three cells in *Riccia natans*, although the quadrant form was the rule. In the following account the innermost cell of the filament of four cells will be designated as the basal cell, the outermost as the apical cell.

VERTICAL WALL FORMATION.—Vertical walls now begin to form in the young embryo. According to HAUPT, "these vertical divisions begin at the lower end of the embryo, a feature which is also noted by WOODBURN's figures." This probably is the general rule, and is evidenced by HAUPT's figures, which show mitoses in the hypobasal portion before occurring in the epibasal portion, the basal cell evidently dividing first. It is quite common to find embryos of this stage with the apical cell yet undivided (figs. 14, 15). This cell also soon divides, either by a vertical wall or otherwise, as described later. A series of cross-sections of an embryo at this stage shows that the vertical walls do not usually lie in the same plane, but are inclined to one another at various angles (figs. 19–23). These vertical walls are usually perpendicular to the transverse ones, which, if obliquely inclined, usually result in oblique vertical walls, and the embryo may, in surface view of this and later stages, appear spiral. Occasionally some of the vertical walls are oblique to the transverse wall, even in the middle of the filament (figs. 17, 28).

The first vertical walls are soon followed by a second series, usually at right angles to the first, typically dividing each segment into four cells (figs. 25, 26). These divisions may be more or less simultaneous (fig. 36), although not ordinarily so even in the same segment.

Should the apical cell also divide by the two series of vertical walls, the apex of the embryo will consist of four octohedral cells (fig. 33). This is the situation as described by Haupt. Leitgeb describes a similar condition in *Blasia*. Owing to the curvature of the embryo two of these will be nearer the neck of the archegonium than the other two.

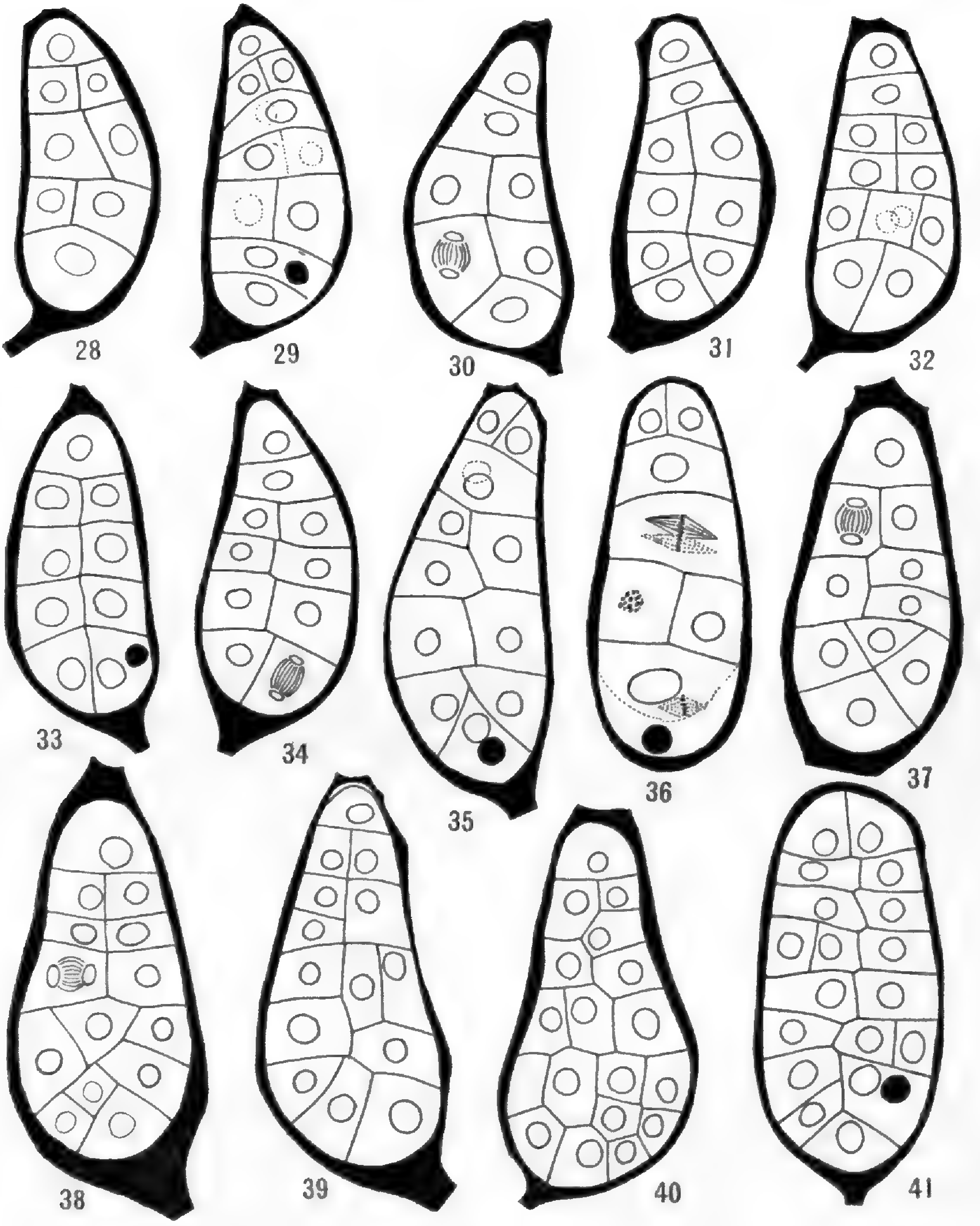
Apical cell.—The apical cell of the row of four may divide vertically, or, as certain embryos suggest, it may divide again transversely (fig. 29) before vertical division takes place, although in the absence of a mitosis one cannot be absolutely positive on this point. It may also divide by an oblique wall, whether its basal wall is oblique or transverse (figs. 31, 32, 34, 35). Woodburn shows a similar situation, to which Haupt takes exception, claiming that in his investigation "a truly median section has never revealed the presence of a triangular apical cell." Leitgeb found that in *Blasia* the apical cell may divide by oblique walls, and he figures several embryos with a triangular apical cell. The writer's observations of *Reboulia* confirm Woodburn's statement. In fact, owing to the curvature of the embryo, a truly median section is the one



Figs. 19–27.—Figs. 19–23, series of cross-sections through embryo, showing inclination of vertical walls to one another in successive segments; fig. 19 is basal segment; fig. 23, apex; fig. 24, transverse section of apical segment of embryo, *x* is neck of archegonium; figs. 25–27, transverse section of embryo with segments in quadrants; compare fig. 27 with fig. 42, noting position of walls; ×570.

most likely to show the oblique inclination of the wall and the consequent triangular apical cell, although one would not necessarily err even in the interpretation of an oblique section. It seems to the writer that there is no room for doubt as to the presence and functioning of a triangular apical cell (figs. 34–39, 41, 45). It is not probable that this cell functions as an apical cell more than a very few times, being soon "lost" in the growing embryo, where the apical function becomes distributed to a number of cells (figs. 40, 46).

BASAL CELL.—The basal cell of the row of four also does not perform uniformly in all cases. It may divide by a vertical wall into two approximately equal cells (fig. 36), or it may divide by an oblique wall (fig. 35). WOODBURN found "basal cells of triangular shape" to occur, probably arising as a result of oblique wall formation in the basal cell. HAUPT found no case of this, and in the writer's preparations it is not common. Should the first wall of the divided egg be an oblique one, it might be probable that the hypobasal would divide obliquely, resulting in a triangular cell at the base. While no mitosis was found as a direct proof, the appearance of a number of embryos (figs. 16–18, 30–32, 34) suggests that the basal cell may divide transversely instead of vertically, the basal of the two cells thus formed behaving as here described for the basal cell itself, while the other cell sooner or later becomes divided by vertical walls in the same way as its neighboring segment (figs. 28, 29). Vertical wall formation in this cell would probably be delayed for a time and the cell remain undivided, even after vertical walls have formed in the segments anterior to it (figs. 34, 36). It seems to the writer that very frequently the basal cell of the row of four undergoes no further division whatever, but very early becomes differentiated as a large conspicuous foot cell at the base of the embryo, retaining its hemispherical shape, very early showing denser contents than the other cells of the embryo, and becoming coated on its free margin by a heavy thickening (figs. 45–47). This cell often remains quite distinct, even in late embryos, and may clearly be recognized both in sections (fig. 47) and in surface views of dissected embryos. Should the basal cell have divided transversely (as already suggested) the basal of the two cells formed may remain undivided. WOODBURN



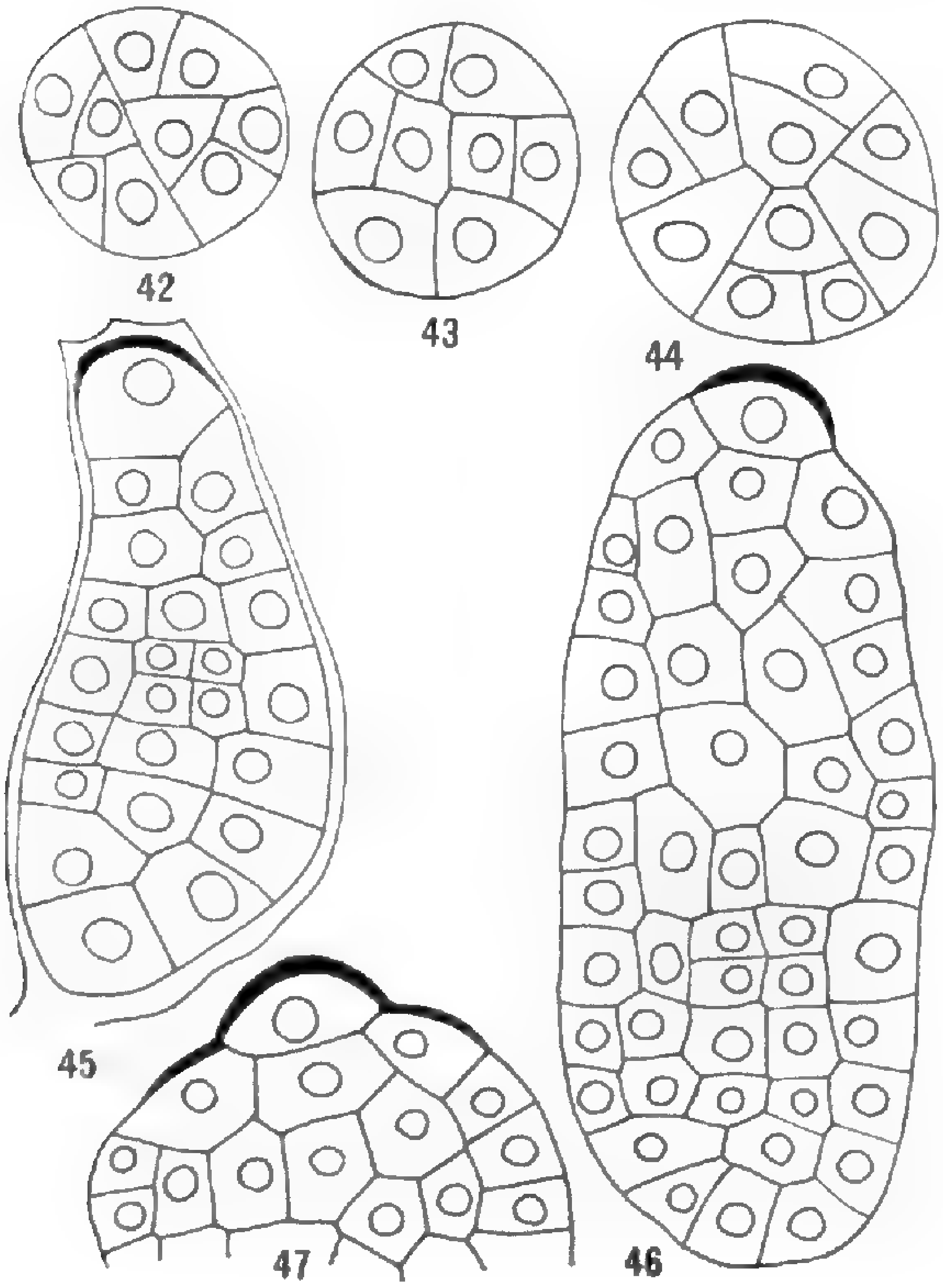
FIGS. 28–41.—Fig. 28, embryo with segment divided by oblique wall; fig. 29, apical cell (of row of four), probably divided transversely; figs. 30–41, embryos showing varying features, figs. 36, 37, and 41 cut at plane perpendicular to median longitudinal plane; in fig. 36 division is going on at several different regions of embryo; dotted line at apex represents oblique wall separating deeper cell (in process of division) from superficial cell with oil drop and undivided nucleus; figs. 34, 35, 37–39 show functional triangular apical cell; ×430.

cites a case where the basal cell has divided into a small group of irregular cells, probably also a very rare feature.

It is evident that the basal cell of the filamentous embryo undergoes but few, and in some cases no further divisions, and therefore makes a relatively small contribution to the tissue of the sporophyte, which therefore is built up almost entirely from the three anterior cells of the filament of four cells.

LATER GROWTH AND EMBRYO DIFFERENTIATION.—Along with or following the formation of vertical walls, transverse divisions in some or all of the segments result in additional tiers of cells, the divisions occurring in different planes without any definite sequence (figs. 36–41). At first the ventral side of the embryo will probably show the greater number of cells (figs. 35, 39, 40), but as growth continues the dorsal side also grows rapidly, and the embryo soon becomes a radial instead of a bilateral structure (fig. 46). Periclinal walls now form, especially in the epibasal portion of the embryo, without any definite sequence, forming inner and outer cells (figs. 38–44). These first periclinal walls are most likely to form in the capsule-forming region of the embryo, which becomes considerably broader than the more slender hypobasal portion (figs. 40–45). This portion, however, soon broadens out somewhat and reaches the winter condition (fig. 46). While it is impossible to trace back absolutely the origin of the different regions of the sporophyte, it seems most probable from the writer's study that the first division of the egg determines the capsule region as distinguished from the foot and stalk region, the epibasal cell giving rise to the capsule, therefore, the hypobasal to the stalk and foot, with the bulk of both foot and stalk derived from the anterior half or three-fourths of the original hypobasal cell. They are derived from the anterior half if the basal cell (of the row of four) does not undergo transverse division, and from the anterior three-fourths if the basal cell should divide transversely. Both WOODBURN and HAUPT regard the foot as derived from the hypobasal cell, the stalk and capsule from the epibasal cell; the capsule being formed from the two anterior cells of the three derived from the epibasal cell, according to HAUPT. The sequence of the early divisions and the behavior of the hypobasal portion of the embryo would seem to warrant the writer's interpretation.

While it may be probable that the future sporogenous tissue is cut off from the capsule wall by the first periclinal walls which form in this portion of the embryo, it does not show differential staining until later, when the physiological differentiation becomes evident, as shown in the more massive capsule (fig. 46) of the winter condition. CAVERS (4) holds to the view that "the capsule wall in Marchantiales is not differentiated until a relatively late stage; that is, the separation of the archesporium is not determined by the first periclinal divisions in the young capsule." Further development takes place the succeeding spring, with the sporophyte reaching maturity, in this latitude from the middle of May to the middle of June. The writer has not made a careful study of sporogenesis, HAUPT's paper giving an account of the features in detail.



FIGS. 42–47.—Figs. 42–44, transverse sections of embryos showing first periclinal walls; fig. 45, longitudinal section of young embryo late in November (note prominent basal cell); fig. 46, embryo in winter condition; ×350; fig. 47, base of embryo in winter condition (large basal cell quite conspicuous).

Calyptra and involucre

The calyptra grows apace as the embryo develops, becoming several layered and relatively somewhat massive. The longitudinal axis of both embryo and calyptra becomes more and more vertical, until finally it is practically perpendicular to the substratum, with the neck of the archegonium hanging downward. The calyptra incloses the sporophyte until spring, when the rapid growth of the latter breaks through the slower growing calyptra and becomes exposed, excepting where covered by the receptacle tissue, which has grown downward and formed an involucre about both calyptra and sporophyte, dorsally and laterally.

Discussion

The finding of filamentous embryos in an increasing number of Marchantiales makes it evident that the octant type of embryo is not necessarily the rule in this group, in contrast with the filamentous embryo of the Jungermanniales. This, together with the occurrence of oblique walls and even a triangular apical cell, tends to bring the Marchantiales and Jungermanniales closer together as regards their embryogeny, and in an occasional partial agreement with that characteristic of the Musci.

In a previous paper, the writer (6) referred to the plasticity of *Reboulia* as shown by the male reproductive structures. The variations found in the development of the embryo give additional support to that view.

In the differentiation of the capsule region from the foot and stalk, *Reboulia* is probably like that of most Marchantiales, in that the capsule is generally derived from the epibasal half of the egg. Even in *Reboulia* there is no absolute proof that the epibasal cell may not contribute in part to the stalk region, as is the case in the Jungermanniales. The behavior of the basal cell of the filament is suggestive of an approach to the situation in some of the Jungermanniales where the entire hypobasal cell is a mere appendage to the embryo.

Summary

1. The mature egg and early embryo are elongated, slightly curved, bilaterally symmetrical bodies.

2. Fertilization takes place in October, the development of the embryo beginning at once, the sporogenous tissue becoming differentiated by winter, the sporophyte maturing in May and June.

3. The early embryo shows considerable variation in its development, the chief features being: (1) the first division of the egg may be transverse or oblique; (2) transverse division of both hypobasal and epibasal cells results in a filamentous embryo of four cells;

(3) vertical wall formation occurs in these four cells, with the exception, commonly, of the basal cell; (4) oblique walls may occur in any part of the embryo, and are not uncommonly to be found in the apical region where they may form a triangular apical cell, functional in the cutting off of a few segments; (5) the foot and stalk are probably derived from the hypobasal cell, the epibasal cell giving rise to the capsule, although it may conceivably make some contribution to the stalk as well; (6) the basal cell of the row of four varies in its contribution to the tissue of the foot, at times apparently remaining undivided, in which case the remainder of the foot and the stalk is derived from its sister cell.

4. The variations in the early embryo support the view that *Reboulia* is a plastic form, and as such may occupy a genetic position among the Hepaticae.

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LITERATURE CITED

1. CAMPBELL, D. H., Mosses and ferns, 3d ed. New York. 1918.
2. CAVERS, F., On the structure and biology of *Fegatella conica*. Ann. Botany **18**:87–120. *pls. 6, 7. figs. 28–31.* 1904.
3. ———, Notes on Yorkshire Bryophytes, III. *Reboulia hemisphaerica* (L.) Raddi. Naturalist 1904:242–250. *pl. 8. figs. 2–6.*
4. ———, The interrelationships of the Bryophytes. New Phytol. Reprint no. 4. Cambridge. 1911.
5. CLAPP, GRACE L., The life history of *Aneura pinguis*. BOT. GAZ. **54**:177–193. *pls. 9–12.* 1912.
6. DUPLER, A. W., The antheridium and male receptacle of *Reboulia hemisphaerica*. Amer. Jour. Bot. **9**:285–295. *pl. 14. figs. 24.* 1922.
7. DURAND, E. J., The development of the sex organs and sporogonium of *Marchantia polymorpha*. Bull. Torr. Bot. Club **35**:321–335. *pls. 21–25.* 1908.
8. GARBER, JOHN F., The life history of *Ricciocarpus natans*. BOT. GAZ. **37**:161–177. *pls. 9, 10. figs. 4.* 1904.
9. HAUPT, A. W., The gametophyte and sex organs of *Reboulia hemisphaerica*. BOT. GAZ. **71**:61–74. *figs. 21.* 1921.
10. ———, Embryogeny and sporogenesis in *Reboulia hemisphaerica*. BOT. GAZ. **71**:446–453. *pl. 33. figs. 11.* 1921.
11. HOFMEISTER, W., On the germination, development, and fructification of the higher Cryptogamia. Eng. trans. by F. Curry. London. 1862.

12. Humphrey, H. B., The development of *Fossombronia longiseta*. Ann. Botany **20**:83–108. *pls. 5–7. figs. 8.* 1906.

13. Kienitz-Gerloff, F., Verglichende Untersuchungen über die Entwicklungsgeschichte des Lebermoossporogons. Bot. Zeit. **32**:161–172; **33**: 777–782. 1875.

14. Leitgeb, H., Untersuchungen über die Lebermoose. I. *Blasia pusilla*. 1874; III. Die frondosen Jungermannieen, 1877; VI. Die Marchantiaceen. 1881.

15. McCormick, Florence A., A study of *Symphyogyna aspera*. Bot. Gaz. **58**:401–418. *pls. 30–32.* 1914.

16. O'Keefe, L., Structure and development of *Targionia hypophylla*. New Phytol. **14**:105–116. *figs. 2.* 1915.

17. Starr, A. M., A Mexican *Aytonia*. Bot. Gaz. **61**:48–58. *pls. 1–4. figs. 33.* 1916.

18. Woodburn, W. L., Preliminary notes on the embryology of *Reboulia hemisphaerica*. Bull. Torr. Bot. Club. **46**:461–464. *pl. 19.* 1920.

SPECIFIC ACIDITY OF WATER EXTRACT AND OXALATE CONTENT OF FOLIAGE OF AFRICAN SORREL[1]

GEORGE PELHAM WALTON

(WITH ONE FIGURE)

Foreword

This report is submitted, not so much as a contribution to the accumulated data on the composition of the leaves of *Rumex abyssinicus* Jacq., as for the purpose of directing the attention of food and drug analysts to a comparatively simple procedure by which much of value may be learned about the source of the acidity in certain acid materials, with the minimum expenditure of time and effort. The scheme outlined in this paper has already been applied by the writer to a study of dried apple pomace and pectin pulp, with gratifying results, and it is believed that it should prove of value in the study of other feeding stuffs of an acid character.

Material examined

Among the plants brought to the United States for further study by the Office of Foreign Seed and Plant Introduction of the United States Department of Agriculture in 1919 was a native African sorrel, *Rumex abyssinicus* Jacquin.[2] The stock was brought from Angola, Portuguese West Africa, where the foliage is reported to be used as greens for human food. Dr. DAVID FAIRCHILD states as follows:

> Preliminary trials at various points in this country have shown this species to possess distinct promise as a summer vegetable. By sufficient parboiling or boiling in a large amount of water, its acidity can be reduced to a point where it is distinctly agreeable, and since it is devoid of all stringiness it deserves to be widely known in America. The plant grows to a height of eight feet and produces an amazing amount of greens throughout the summer. It is as resistant to heat as New Zealand spinach and Swiss chard.

Dr. ARNO VIEHOEVER suggests caution, however, in the use of this material for food. The following statement by him[3] was written after the completion of the chemical work reported in this paper.

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[1] From the Cattle Food and Grain Investigation Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington.

[2] S.P.I. no. 48023.

[3] Amer. Food Jour., January 1922.

The presence of considerable amounts of oxalic acid, as found in the Bureau of Chemistry, in the form of soluble oxalates, as well as calcium oxalate, suggests caution. It is a well known fact that some people are especially susceptible to poisoning from oxalic acid, and the poisoning cases reported after eating rhubarb leaves are by no means rare. It appears possible, however, that by the addition of calcium carbonate the soluble oxalates may be precipitated, and thus the major portion of the objectionable ingredient may be eliminated. Another means suggested, and possibly equally effective to make the product available for general consumption, would be the removal of the water in which the material has been soaked and boiled. At any rate, we have here a product which may be placed on the market, and which may be used as a substitute for spinach and other greens, but the identity and characteristics of which should be known to the consuming public and especially the food officials concerned with the welfare of the people.

Several plants started in the vicinity of Washington in 1920 made vigorous, healthy growth (fig. 1). Because of the promising character of the plant as a source of summer greens, it was decided to submit a sample to the Bureau of Chemistry for a determination of its oxalic acid content. The suggestion was made, however, that in order to decide the question of its wholesomeness it was essential that both the total (titrable) acidity and specific acidity (H-ion concentration) of a water extract of the material be determined, as well as the total oxalate content. These determinations were undertaken in conjunction with the colorimetric determination of specific acidity in certain feeding stuffs.

TABLE I

DIMENSIONS OF LEAVES OF SAMPLES IN MM.

DIMENSION	SAMPLE NO. 38339			SAMPLE NO. 38340		
	Largest	Smallest	Average of 20	Largest	Smallest	Average of 11
Length of blade	195	75	149.0	260	230	249
Extreme breadth of blade	150	45	118.0	250	200	220
Length of petiole	120	40	86.5	140	100	125

On August 5, the plants in Washington being at a suitable stage of growth, a sample of about one pound of fresh foliage was analyzed. Practically all of the material obtained consisted of sound, crisp leaves with petioles. As there were two distinct sizes of leaves, the material was divided into two samples. The dimensions of the leaves constituting these samples are given in table I.

FIG. 1.—Row of plants of Abyssinian *Rumex*, about 6 feet high, grown at Yarrow Plant Introduction Garden near Rockville, Md. Photograph furnished by Office of Foreign Seed and Plant Introduction, Bureau of Plant Industry, U.S. Department of Agriculture.

Procedure

MOISTURE

The bulk of the material of each sample was used for the determination of moisture, but only entire leaves, with their petioles, were taken. After recording the total green weights, the midribs, larger veins, and petioles were split by a sharp knife to facilitate evaporation, and the material was rapidly dried at 65°–70° C. in a well ventilated oven, until friable. It was then crushed, care being taken to avoid loss of substance, and the samples finally dried to constant weight at 65°–70° C. in a vacuum oven. The loss in weight, about 90 per cent of the green weight, was taken to be total moisture. The dried material was ground and reserved for further study.

TOTAL ACIDITY OF WATER EXTRACT

Several sound leaves with petioles, representative of the fresh green material, were selected from each sample, and the weights of the two charges recorded. In the case of the larger foliage two leaves, weighing 43 gm., constituted the charge, while several leaves, weighing in the aggregate 25 gm., represented the sample of smaller foliage. Each charge was thoroughly macerated in a glass mortar, and the resulting pulp transferred to a four-sided glass 8 oz. sample jar, with exactly 200 cc. of distilled water, previously boiled and cooled to room temperature. After violently stirring the mixture for 30 minutes by means of an electric mixer,[4] it was thrown on dry filter paper. The first (cloudy) portion of the filtrate was rejected, and the total acidity in an aliquot of the clear extract was determined by titrating with N/10 sodium hydroxide solution, using phenolphthalein as indicator. The presence in the water extract of a natural indicator,[5] the strongly darkening color of which tended to obscure the end-point of the titration, made it necessary to carry through concurrently a blank with a similar aliquot of extract with-

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[4] Described in Circular 68, Office of the Secretary, U.S. Department of Agriculture.

[5] This natural dye in the leaves of *R. abyssinicus* Jacq. appears pink in dilute aqueous extracts of natural acidity. As titration with a fixed base progresses, the color changes through yellow to brown, at about the neutral point, and the solution becomes inky when made distinctly alkaline.

out phenolphthalein. In this blank titration, the addition of N/10 sodium hydroxide solution kept pace with the quantity added in the true titration, and the end-point was determined by contrasting the colors of the titrated extracts. The extract containing phenolphthalein developed a noticeably redder brown color than the other.

In the computations allowance was made for the water present in the green material. The total titrable acidity is expressed as cubic centimeters of normal acid per kilogram of leaf material, termed the "degree of acidity."

SPECIFIC ACIDITY OF WATER EXTRACT

The specific acidity was estimated on a portion of the clear extract by a slight modification of the colorimetric method described by GILLESPIE (3) after BARNETT and CHAPMAN (1), in which use is made of the principle introduced by CLARK and LUBS (2), following SALM (7), of "superimposing the two extreme colors of an indicator in determining its half-transformation point." Instead of using a system of nine pairs of tubes having drop ratios 1:9, 2:8, etc., MEDALIA'S (4) system of seven pairs, having P_H exponent intervals of 0.2 between each pair for the indicators used, was adopted. For convenience the procedure is briefly sketched here.

The color comparisons are made in the small "block" comparator described by GILLESPIE. Seven pairs of test-tubes, selected to fit the comparator and for their uniformity in bore, are calibrated for 5 cc. capacity and arranged in a double row test-tube rack. A total of eight drops of the suitable indicator solution is delivered into each pair of tubes, 1 to 7 drops in the front seven tubes and 7 to 1 drops in the back row, care being taken to hold the delivery pipette in an upright position. Sufficient alkali[6] (dilute acid in the case of the indicator thymol blue, acid range) is then added to the tubes in the front row to produce the full alkaline color, and sufficient acid to develop the full acid color is added to those in the rear row. The tubes are then carefully filled to the 5 cc. mark with distilled water, previously boiled and cooled. Similar tubes are used for the solutions under examination (the water extracts of the sorrel). Eight drops of the indicator solution are required, of course, and the

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[6] Quantity of alkali or acid varies somewhat for the different indicators.

5 cc. volume is completed with the "unknown" solution. The contents of all tubes are well mixed before making the color comparisons. (Mixing may be accomplished by rolling the tube back and forth between the palms of the hands.)

In making the color comparisons, the tubes, held vertically in the comparator, are arranged in two files of three tubes each, one file being made up of the tube containing the "unknown," *with* indicator solution and two tubes of distilled water, and the other file consisting of a pair of the standard tubes and a tube containing the "unknown" solution, *without* indicator. This arrangement is necessary to obviate optical differences caused by thickness of liquid viewed on the one hand, and on the other to offset the natural color and any turbidity of the extract under examination. Different pairs of standards are tried until the color of light passing horizontally through that file of tubes matches the color from the file containing the tube of "unknown," *with* indicator.

As stated by GILLESPIE, the tubes are viewed best against the sky. Occasionally, in the case of certain indicators, such as bromphenol blue, trouble is experienced in matching the colors because of a dichroic effect, especially noticeable in turbid solutions. In such cases the tubes may be viewed by the yellow light of a carbon electric lamp, screened as advised by CLARK and LUBS. Only two indicator solutions were needed in estimating the specific acidity of the sorrel extracts, an 0.05 per cent aqueous solution of bromphenol blue,[7] and an 0.02 per cent solution of thymol blue (thymolsulphonphthalein) in 80 per cent alcohol.

To develop the full acid and "alkaline" colors respectively, in the standard paired tubes, the following quantities of reagents were used for the two indicators:

Bromphenol blue.—To produce the acid color, 0.5 cc. of N/10 hydrochloric acid solution; to produce the alkaline color, 1 drop of N/20 sodium hydroxide solution.

Thymol blue (acid range).—To produce the full acid color, 2 cc. of 1.25 per cent hydrochloric acid solution; to produce the color of

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[7] Tetrabromophenolsulphonphthalein. The 0.05 per cent solution was prepared by diluting one volume of the indicator solution, furnished in the LaMotte field set, to twenty volumes, with freshly boiled and cooled distilled water.

the "alkaline" end of the range, 1 cc. of 0.005 per cent (N/700) hydrochloric acid solution.

The volume in all the tubes should be made up at once to 5 cc. The specific acidity values accepted for the several drop ratios are given in table II. This specific acidity is based on the H-ion concentration of pure neutral water as unity, as defined by WHERRY (10). The articles by WHERRY and ADAMS (11) and CLARK (11) discuss this system of stating H-ion concentration.

TABLE II

SPECIFIC ACIDITY VALUES IN ROUND NUMBERS

DROP RATIO	BROMPHENOL BLUE		THYMOL BLUE, ACID RANGE	
	Specific acidity	P_H	Specific acidity	P_H
1:7	6300	3.2	400,000	1.4
2:6	4000	3.4	250,000	1.6
3:5	2500	3.6	160,000	1.8
4:4	1600	3.8	100,000	2.0
5:3	1000	4.0	63,000	2.2
6:2	630	4.2	40,000	2.4
7:1	400	4.4	25,000	2.6

These specific acidity (and P_H) values, while not absolutely exact, are close enough for the purposes of this investigation, particularly as it was found that the specific acidity of the sorrel extracts fell at the extreme acid end of the bromphenol blue series, or between that and the "alkaline" end of the thymol blue, acid range, where a close estimation is impossible.[8] The sorrel extracts, however, were checked up by comparison with the straight acid color of the first indicator and the straight "alkaline" end color of the thymol blue, acid range.

The color standards are quite permanent, and if the tubes are stoppered and kept in the dark they may be used over a long period (4). The literature citations, particularly 3 and 4, contain details on the use of indicators covering P_H values from 1.2 to 9.8. Both titrable and specific acidity were determined also on water

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[8] As stated by GILLESPIE, measurements cannot be accepted at the point where the drop ratio is 9:1 or 1:9 (7:1 or 1:7), as the percentage transformation of the indicator is so nearly 100 or zero that the H^+ exponent may be far from that represented by the ratio, and this would not be disclosed by a difference in color.

extracts of dried and ground material of both samples. All determinations were made at room temperature, 25°–30° C. The higher temperature was usually reached in the afternoon during August.

Total oxalate

The estimation of total oxalate was undertaken for the purpose of verifying the figures obtained in the acidity work, and a critical study of oxalate methods was not attempted. After the completion of this work, the writer's attention was directed to a method, perfected by W. F. Kunke, Bureau of Chemistry, for the determination, with a high degree of accuracy, of the total oxalate content of plant material. Using this method on another sample of the sorrel foliage, Kunke obtained materially lower figures than those reported in this paper for total oxalate. Because of this and the relative crudity of the usual method, the figures for total oxalate herein reported are probably somewhat high. This, however, in no way invalidates the data dependent on the acidity determinations (the figures for potassium binoxalate), as whatever error there may have been appears in the figures for calcium oxalate.

The total oxalate content was estimated only on the dried and ground material. Two gm. was weighed into a 150 cc. volumetric flask, about 100 cc. of 2 per cent hydrochloric acid solution added, and the mixture, after being heated to boiling, was digested for thirty minutes on the steam bath. After cooling and completing the volume to 150 cc. with distilled water and mixing, the extract was filtered through dry filter paper. The total oxalate in 100 cc. of the hydrochloric acid extract was estimated by precipitation with calcium chloride and titration of the oxalate with standard solution of potassium permanganate, in the usual way. Contamination of the calcium oxalate precipitate with organic matter necessitated double precipitation, and the final precipitate was washed with 1 per cent acetic acid solution in the cold, for further purification.[9] Two control determinations on pure sodium oxalate were conducted under the same conditions as those for the sorrel samples. In the titration, which was carried through rapidly, there appeared to be a

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[9] Final precipitation of calcium oxalate in acetic acid solution would be preferable for a material of this nature.

definite end-point, at which the pink color of the permanganate persisted for an appreciable interval of time, although organic matter, small amounts of which undoubtedly were present, continued slowly to reduce additional permanganate.

Results

The data obtained in the chemical examination of the fresh and dried sorrel leaves are given in tables III, IV, and V.

TABLE III

ANALYSIS OF LEAVES OF *Rumex abyssinicus*

SAMPLE	PERCENTAGE MOISTURE AT 65°–70° C.	DEGREE OF ACIDITY (cc. normal acid per kg.)	PERCENTAGE TOTAL OXALATE AS (C_2O_4)*
Misc. Div. No. 38339, small leaves with petioles (fresh)	90.77	152.7	1.69
Dried at 65°–70° C. and ground	2.99	1490.0†	17.8
Misc. Div. No. 38340, large leaves with petioles (fresh)	89.59	185.4	1.73
Dried at 65°–70° C. and ground	2.50	1500.0†	16.2

* As stated previously, according to KUNKE these figures may be somewhat high.

† Evidently there was a decrease in acidity during drying, for the degrees of acidity as determined on the dried material, computed to the original moisture bases, would be 141.8 and 160.2 respectively.

TABLE IV

WATER EXTRACT OF LEAVES

SAMPLE	QUANTITY OF SAMPLE PER 100 CC. OF EXTRACT (gm.)	TOTAL ACIDITY IN 100 CC. OF EXTRACT EXPRESSED AS NORMAL ACID (cc.)	SPECIFIC ACIDITY, H-ION CONCENTRATION			
			Of water extract of *Rumex* leaves (observed)		Of aqueous solution of HKC_2O_4 having same total acidity as water extract of *Rumex* leaves (computed)*	
			Specific acidity	P_H	Specific acidity	P_H
No. 38339, small leaves with petioles (fresh)	11.26	1.72	6300–10,000	3.2–3.0	9000	3.05
Dried	1.00	1.49	6300	3.2	8400	3.08
No. 38340, large leaves with petioles (fresh)	18.07	3.35	10,000–16,000	3.0–2.8	12600	2.90
Dried	1.00	1.50	6300	3.2	8400	3.08

* The method of computation is discussed later under Discussion. Attention is directed to the close agreement between the observed values and those computed for a pure potassium binoxalate solution.

Table IV gives the figures relating to the acidity of water extracts of both the fresh and dried material, and specific acidity values computed for pure solutions of potassium binoxalate of the same respective normalities (titrable acidities) as the water extracts.

TABLE V

PERCENTAGE OF OXALATES AND EQUIVALENTS IN LEAVES

Sample	HKC_2O_4 in leaves (based on acidity of water extract of dried material)	(C_2O_4) equivalent of HKC_2O_4 in preceding column	Total (C_2O_4) in leaves from table III	Total (C_2O_4) minus acid (C_2O_4), column 4 minus column 3	Calcium oxalate (CaC_2O_4) in leaves equivalent of (C_2O_4) in preceding column
No. 38339, small leaves (fresh)	1.82	1.25	1.69	0.44	0.64
Dried	19.09	13.10	17.80	4.70	6.80
No. 38340, large leaves (fresh)	2.05	1.41	1.73	0.32	0.47
Dried	19.22	13.20	16.20	3.00	4.40

The data in table V are derived entirely from determinations made on the dried material. In the second and last columns the data from tables III and IV are correlated to show the percentage amounts of salts of oxalic acid presumably present in both the fresh and dried leaves. The percentages given for calcium oxalate are for the anhydrous salt, for convenience in comparing with data in pharmacological literature.

The assumption that most of the oxalate is present as potassium binoxalate and calcium oxalate (as the monohydrate) is substantiated by additional information obtained through the kindness of DR. WHERRY. The work of MR. DEUEL on total soluble oxalate also checks in a striking manner the figures for binoxalate.

CRYSTALLOGRAPHIC-OPTICAL EXAMINATION

WHERRY, who examined some of the dried and ground material by polarized light, under a petrographic microscope, identified numerous crystals of potassium binoxalate, and a smaller number of crystals of calcium oxalate monohydrate in groups. The potassium binoxalate crystals were readily identified, because of their characteristic of having a relatively low alpha index. WHERRY states that four substances might conceivably be present in the sample of plant tissue, and first determined their optical properties as follows:

	Alpha	Beta	Gamma	2 E.	Sign
Oxalic acid dihydrate	1.445	1.505	1.540	120°	−
Potassium binoxalate	1.415	1.545	1.565	65°	−
Potassium oxalate monohydrate	1.440	1.485	1.550	160°	+
Calcium oxalate monohydrate	1.490	1.555	1.650	Over 180°	+

Examination of the sample showed two distinctly different crystalline substances to be present, one in rosettes of acute crystals, the other in nearly equant grains. The first proved to have the refractive indices characteristic of calcium oxalate as indicated, the second to have those of potassium binoxalate. The calcium salt occurs as aggregates of crystals, and therefore looks more prominent, but considering the large number of small grains of the potassium salt which are scattered around, it is evident that the potassium salt is present in the greater amount. Immersion liquids 1.490 and 1.565 are most suitable for distinguishing the two, the potassium salt having one index much lower than the first, the calcium salt one much greater than the second. As no crystals were found to be present with gamma less than 1.560, or beta less than 1.540, it is to be inferred that neither oxalic acid nor neutral potassium oxalate is present, at least in significant amounts.

TOTAL DISSOLVED OXALATES

DEUEL has kindly consented to the inclusion in this paper of figures obtained by him for the soluble oxalate in a sample of foliage from the same group of plants from which these samples were obtained. His procedure was to boil the finely minced leaves in water for 1.5 hours, filter, make up the extract to definite volume, and in an aliquot determine the total soluble oxalate gravimetrically by precipitation as calcium oxalate, and after ignition, weighing as calcium oxid. In the two samples of foliage examined he found oxalate equivalent to 2.06 and 1.96 per cent potassium binoxalate. The percentage of moisture on the samples was not reported, but the figures are for the fresh leaves.

Discussion

Much may be learned about acid material of the same general type as the foliage of *Rumex* sp., merely from a determination of the total (or titrable) and the specific acidity of a water extract of it. By "same general type" is meant here acid material in which, as in the sorrel, the "acidity" is due almost entirely to forms of a single acid. If, in addition to total and specific acidity, the total amount of the acid radical present in the water extract and in the material itself be known, it is possible to draw fairly accurate conclusions as to the quantities of the several salts of that acid actually present, without making elaborate determinations of the several basic elements. Auxiliary use of the petrographic microscope may afford valuable qualitative substantiation of the conclusions.

In this investigation the mere determination of "degree of acidity" (total titrable acidity of the water extract) of the sorrel foliage meant little; the acidity might have been caused by the presence of free oxalic acid. Determination of the specific acidity (H-ion concentration), however, proved conclusively that the acidity of the water solution could not have been caused by the presence of the free acid; as for the normality involved, the specific acidity value of oxalic acid would have been approximately ten times[10] the values actually found. On the other hand, the agreement between the "specific acidity" values determined experimentally for the sorrel extracts and those computed for pure solutions of potassium binoxalate of the same normality is striking. These values for potassium binoxalate solutions were computed by the help of the formula: Percentage ionization $=100\left(\sqrt{KV}-\frac{KV}{2}\right)$,[11]

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[10] Computed from data reported by THOMAS (9) after OSTWALD (5). Oxalic acid of a dilution comparable with the water extract of sample No. 38339 ($N\times 0.0172$) is highly dissociated, the percentage ionized being 88.4 at 25° C., according to THOMAS' table.

[11] By an evident typographical error this formula in THOMAS' article was incorrectly stated: "Per cent ionization $=100\sqrt{KV-\frac{KV}{2}}$." The method of calculating the P_H value is here appended in more detail, using as an example the data for the potassium binoxalate solution corresponding to the extract of the larger leaves: Normal-

derived from OSTWALD'S dilution law, as explained in detail by THOMAS (9). In this formula V = the volume in liters, in which one gram molecular weight of the substance is dissolved, and K = the dissociation constant. The values used for K are those given in SCUDDER'S (8) tables, and are for 25° C.

The data from the acidity determinations, therefore, point to potassium binoxalate as the sole source of the acidity of the sorrel extracts, at least of those of the dried material. Furthermore, a calculation of the percentages of this salt present in the samples based on the titrable "acid" in the extracts of the dried leaves yields figures agreeing very closely with those obtained by DEUEL for the oxalate (as potassium binoxalate) dissolved by boiling water.

These data, per se, do not preclude the possibility of the presence in the foliage of approximately equivalent quantities of free oxalic acid and normal potassium oxalate, which would simulate the acid salt, and, in fact, in aqueous solution would be identical with it. WHERRY'S observations on the dried material decides the point beyond a doubt. The acid nature of the leaves is unquestionably due to the presence of potassium binoxalate.

On recalculating the figures obtained for titrable acidity in the dried material (on which are based the figures for potassium binoxalate in the leaves) to the original (green) moisture bases, it becomes apparent that there is a loss of titrable acid during the drying. These figures (footnote, table III) become 141.8 and 160.2 for "degrees of acidity" on the original bases, respectively equivalent to 1.82 and 2.05 per cent potassium binoxalate, while the acidity actually titrated in the fresh material yielded the figures 152.7 and 185.4 (in terms of potassium binoxalate corresponding to 1.96 and 2.38 per cent); therefore 10.9 and 25.2 cc. respectively of normal acid per kilogram of fresh leaves disappeared during the drying of the two samples. This lost "acid" may have been carbon dioxide or other weak volatile acids, or may be accounted for in part by changes in colloidal, acid-reacting protein. The discussion by

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ity of solution $= 0.0335\ N$; hence $V = 29.85$, $K = 4.9 \times 10^{-5}$. Percentage ionization $= 3.75+$. H per liter $= 0.0335 \times 1.008$ gm. H^+ per liter $= 0.0375 \times 0.0335 \times 1.008 = 0.001267 = 1.267 \times 10^{-3}$ $\therefore$ P_H = algebraic sum of -3, and log. $1.267 = -2.897$. Omitting the negative sign, $P_H = 2.9$ (specific acidity $= 12670$).

PFEFFER (6) of decrease in acidity in plant tissues (in life) and in sap, due to a rise in temperature (from 15° to 45°) and to exposure to sunlight, fully explains a loss in acidity of this magnitude, on drying fresh material in which the cells are still functioning.

From the figures obtained for total oxalate it becomes apparent that there is more oxalate present in the leaves than is accounted for by the potassium binoxalate. Further, this excess oxalate must be either insoluble or neutral in reaction if soluble. Again the crystallographic-optical examination made by WHERRY decides the point. The only normal oxalate found was the insoluble calcium salt. A recapitulation of analytical results is presented in table VI.

TABLE VI

ANALYSIS OF LEAVES OF *Rumex abyssinicus*

Leaves	Percentage moisture at 65°–70° C.	Percentage potassium binoxalate (HKC_2O_4)	Percentage calcium oxalate monohydrate ($CaC_2O_4 . H_2O$)	Degree of acidity (cc. normal acid per kilo)
Smaller leaves (fresh).......	90.77	1.82	0.73	152.7
Larger leaves (fresh).......	89.59	2.05	0.53	185.4
Smaller leaves (after drying).	2.99	19.09	7.80	1490.0
Larger leaves (after drying).	2.50	19.22	4.98	1500.0

A discussion of the influence of such quantities of oxalate on the edibility of the sorrel foliage, or of the physiological effects following its use as food, is outside the scope of this paper.

Summary

1. The study here reported of the acidity and oxalate content of the leaves of *Rumex abyssinicus* (an African sorrel) demonstrates the advantages of determining the specific acidity (H^+ concentration), as well as the total (titrable) acidity of a water extract of acid material of this nature.

2. This paper brings together descriptions of relatively simple procedures, worked out by the investigators cited, for (1) colorimetrically determining, without the use of buffer solutions, the specific acidity of such water extracts, and (2) computing, for purposes of comparison, the specific acidity and P_H value of pure solutions of the acid substance suspected of being the source of the

acidity. Through a comparison of these values, matching the specific acidity actually determined against that computed for those substances causing the acidity, a means of identifying, or at least indicating, the principal source of the acidity is described.

3. The data indicate that only two compounds of oxalic acid, potassium binoxalate and calcium oxalate monohydrate, occur in the *Rumex* leaves examined. The percentages in which these salts occur are computed from the data for acidity and total oxalate in the dried material.

4. Attention is directed to the value of a crystallographic examination in corroborating the results of the chemical work. It is believed that the scheme of investigation described should prove of value to analysts in examining drugs, foods, or feeding stuffs of an acid character.

5. The presence of a natural indicator in the leaves of *R. abyssinicus*, the aqueous solutions of which are pink in the natural acid solution, is noted. On adding a fixed alkali the solution changes its color through yellow to brown, becoming nearly black when distinctly alkaline.

The writer wishes to thank Mr. Paul G. Russell of the Office of Foreign Seed and Plant Introduction for making examination of this material possible, for furnishing information as to the history of the plant and its culture in this locality, and for providing the fresh foliage for analysis. Also acknowledgment is due Messrs. Deuel and Kunke for their kindness in permitting the inclusion of notes of their work, and Dr. Wherry for his helpful suggestions and for the crystallographic examination of the leaf material.

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LITERATURE CITED

1. Barnett, G. D., and Chapman, H. S., Colorimetric determination of reaction of bacteriologic mediums and other fluids. Jour. Amer. Med. Assoc. 70:1062. 1918.
2. Clark, W. M., and Lubs, H. A., The colorimetric determination of hydrogen-ion concentration and its application in bacteriology. Jour. Bact. 2:1–34, 109–136, 191–236. 1917.

3. Gillespie, L. J., Colorimetric determination of hydrogen-ion concentration without buffer mixtures, with especial reference to soils. Soil Science **9**:115. 1920.
4. Medalia, L. S., "Color standards" for the colorimetric measurement of hydrogen-ion concentration P_H 1.2 to P_H 9.8. Jour. Bact. **5**:441. 1920.
5. Ostwald, W., Über die Affinitätsgrössen organischer Säuren und ihre Beziehungen zur zusammensetzung und Konstitution derselben. Zeit. Physik. Chem. **3**:281. 1889.
6. Pfeffer, W., The physiology of plants. Vol. 1. pp. 328, 329. trans. by Ewart, A. J., 2d ed. Oxford. 1900.
7. Salm, E., Studie über Indikatoren. Zeit. Physik. Chem. **57**:471. 1906.
8. Scudder, H., Conductivity and ionization constants of organic compounds. New York: Van Nostrand Co. 1914.
9. Thomas, A. W., Tabulation of hydrogen and hydroxyl ion concentrations of some acids and bases. Jour. Amer. Leather Chem. Assoc. **15**:133–146. 1920.
10. Wherry, E. T., Soil acidity and a field method for its measurement. Ecology **1**:160. 1920.
11. Wherry, E. T., and Adams, E. Q. (and reply by Clark, W. M.), Methods of stating acidity. Jour. Wash. Acad. Sci. **11**:197. 1921.

MICROBIOLOGY OF FLAX RETTING

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Linum usitatissimum has been cultivated for thousands of years as a textile fiber producing plant. The Egyptians must have raised it, since their mummies are found today wrapped in fine linen. Frequent allusions to flax and linen in the Bible indicate that the ancients were acquainted with the usefulness of the bast fibers in flax and had methods of separating them from the rest of the plant. They were also familiar with other types of fibers, since these are found in their papyri today. The United States cannot be regarded as a great flax or linen producing country; it has had to depend mainly on importation to supply the increasing demands for linen. In the spinning of flax the United States was at the bottom of the list of the larger countries in 1915, with slightly over 8000 spindles against Great Britain's 1,161,000.[1] Most of these were in Ireland, although they were not kept busy on fiber produced in Ireland. Russia was once the largest flax fiber producing country, contributing 80 per cent of the flax fiber used in making linen. Since the world war, however, this has changed on account of the industrial disorganization in that country. Statements in the press, said to come from the Office of Fiber Investigation of the United States Department of Agriculture, indicate that the spinning mills in this country have used about 10,000 pounds of flax fiber per annum. For the production of this amount of fiber about 60,000 acres of land would be required. In 1920 only 6000 acres of flax were grown in this country, while the low price paid for it will restrict the acreage to about 3000 in the future.

Flax is raised in this country mostly for seed which is pressed for linseed oil; a smaller amount is raised for the fiber. Flax raised for seed is of a different quality from that usually required for fiber. Fiber flax is taller and produces less seed. It requires greater care in cultivation, and especially careful handling at the

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[1] MILES, C. F., Fiber flax. U.S. Dept. Agric. Farmer's Bull. no. 669. 1915.

harvest. Some claim that it must be pulled, not cut, and tied up carefully in bundles. This may be one reason why it has been difficult to utilize the flax from seed flax for spinning. It might be possible in the future to combine profitably the seed and fiber crop. This would tend to reduce the value of each crop taken by itself perhaps, but the value of the combined crops of seed and fiber might compensate for any decrease in the value of the single crop.

The bast fibers, which are those used in making linen, are cemented to the other parts of the stalk and to each other by means of materials, for convenience, called pectins. Undoubtedly this term is used only in a general way to cover a number of compounds closely related chemically. The aim of the retting process is to remove these "binders" without harming the cellulose fiber. The fermentation must be checked when these fibers have been freed by the hydrolysis of the pectose or salts of pectic acid. These binding materials which hold the stalk together are undoubtedly carbohydrate in nature, and thus susceptible to the action of microoganisms.

Preparation of flax fiber

The fiber is prepared from the flax straw by a special process which seems to have been built up after a long period of time without much assistance from the sciences. Proper harvesting is very important. Fiber flax should be pulled either by hand or by machinery and tied into bundles which are shocked for curing. Cutting the flax is claimed by some to leave the ends of the stalk exposed for undesirable decompositions. When the heads are shocked for curing, this cut end becomes susceptible to the attacks of undesirable bacteria. The fibers become badly stained also. This may not be entirely true, however, under actual practice.

After curing, the stalks are retted. This is really a rotting process, which indicates the origin of our present term. Three general methods may be used to dissolve the binder which holds the cellulose fibers to the woody materials: dew retting, water retting, and chemical retting. The first two only are of bacteriological interest, and were studied in the present investigation.

Dew retting was used by our forefathers in this country for preparing flax fiber for spinning. It represents the earliest method of preparing flax fiber. No special apparatus is needed, since the flax straw is merely spread on the ground in the fall and allowed to remain throughout the winter. Dew retting has been used for the preparation of most Russian flax fiber. Its greatest objection is the time required, but this may be reduced greatly by carrying the process out under conditions where the retting organisms may be made to work harder.

Water retting was introduced undoubtedly to get away from certain of the distinct disadvantages of dew retting. It is carried out either in slow flowing rivers or in ponds and other inclosed bodies of water. The bundles of flax straw are packed into these basins and weighted down. The retting process starts with a gaseous fermentation of the carbohydrate materials in the flax straw. If conditions are favorable, a little over ten days is necessary for the completion of the fermentation. The flax should be removed when all the pectic materials are dissolved, or over-retting will result. The bundles are removed, dried in sun and air, and are then ready for scutching. The river Lys in Belgium is famous for its flax retting. River retting has certain economic features which limit its wide application. As KUHNERT[2] has shown, the stream becomes putrescible, which is detrimental to fish life. It carries amounts of organic materials in the reduced conditions which may give off objectionable odors. Water retting has not had wide application in this country.

Several attempts have been made to improve on the water retting. One of the earliest of these was proposed by SCHENCK in 1846. The flax straw was packed tightly into a tank and the water kept at a temperature of 75°–95° F. This warm environment was more favorable to the development of the bacteria concerned in this process, and a vigorous fermentation quickly established itself. The vats had all of the characteristics of a fermentation mixture. Others have proposed similar methods with a higher temperature.

Scutching is the process by which the woody material is broken away from the cellulose fibers after they have been retted and

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[2] KUHNERT, Landw. Wochenbl. Schleswig-Holstein 70:540–543. 1920.

dried. Different methods have been used, all of which rest on breaking the woody particles and mechanically removing them from the stalk. The fibers are finally combed to separate the "tow" from the fibers which are not long enough to remain in line. The latter may be used in paper, coarse linen, etc. The fiber from flax may be 30–40 inches in length, thus yielding a product which is valuable for spinning.

Microbiology of retting

Retting is indeed a natural process, and may be regarded as merely a step in the cycles of the elements. The various factors involved have been separated in an attempt to intensify certain ones in order to make the process shorter, and also to produce a better fiber. In retting flax man has simply made use of and intensified a reaction which is always going on.

One of the first investigations on the microbiology of retting was carried out by VAN TIEGHEM[3] in 1879, in his study of the process of water retting. An anaerobic organism named *Bacillus amylobacter* was reported as the organism which quickly decomposed the pectic materials of the flax stalk. In the same year VAN TIEGHEM stated that his *Bacillus amylobacter* was probably identical with the *Vibrion butyrique.* An aerobic spore-forming organism was also found by WINOGRADSKY.[4] FRIBES, working in this laboratory, tried various disinfectants for sterilizing the flax, but finally used the method of heating in water at 100° C. for three successive days, or at 115° C. for fifteen minutes. Various aerobes and anaerobes were isolated, none of which seemed to have any effects on the flax. Finally a specific anaerobe was isolated. It was a spore-former, the young cells of which were 10μ to 15μ by 0.8μ. Glucose, sucrose, starch, and lactose were fermented if some nitrogenous matter was present. A quite similar microorganism was also isolated by BEHRENS[5] from the water retting of hemp. The organism was a *Clostridium* form fermenting the binding materials of hemp straw.

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[3] VAN TIEGHEM, Sur la fermentation de la cellulose. Bull. Soc. Bot. France **26**:25. 1879.

[4] WINOGRADSKY, S., Sur le rouissage du lin et son agent Microbien. Comp. Rend. Acad. Sci. **121**:742. 1895.

[5] BEHRENS, J., Cent. Bakt. **10**:524. 1903.

It fermented glucose, sucrose, fructose, lactose, galactose, and starch with abundant gas formation, but could not attack arabinose, cellulose, gum arabic, or calcium lactate. Similar to WINOGRADSKY'S organism, it required some source of nitrogen, as peptones or proteins. It was an obligate anaerobe with large spores which had a greater diameter than the vegetative rod.

In 1902 HAUMANN[6] published an interesting paper which gave an entirely new aspect to the subject. He stated that many common microorganisms could ret flax. He first studied the flora on the stalks of retted flax and isolated a number of organisms, among which were *Bacterium coli-communis*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Streptothrix Forsteri*, *Penicillum glaucum*, *Cladosporium herbarum*, *Bacillus mesentericus fuscus*, *B. mycoides*, *B. termo*, *Micrococcus roseus*, and *Mucor mucedo*. The mere presence of these organisms would not indicate that they functioned in retting. The preponderance of certain species, however, might indicate some relation to the retting process. *Cladosporium herbarum*, *Bacillus mesentericus*, *B. subtilis*, and colonies of *Streptothrix* were common. To determine whether these organisms were important, HAUMANN inoculated sterile flax with pure cultures. The flax stalks were put into long culture tubes plugged with cotton. The tubes containing flax were heated to temperatures below 110° C. in the dry condition. He stated that three heatings under such conditions did not alter the flax. Retting was accomplished by using many of the common species of microorganisms. There was a difference in action, *Pseudomonas fluorescens* giving good results, while *Micrococcus roseus* was least satisfactory. HAUMANN concluded from this that all of the common bacteria were able to ret flax. Some of these bacteria were also able to split pectin. His results are unique in that they contradict those secured by many others and also those obtained in this investigation. In the light of some of the recent work on thermal resistance of the spores of anaerobic bacilli, HAUMANN'S method of sterilizing the flax is open to criticism. One would not expect these spores to be destroyed by a temperature of 110°, especially in the dry conditions. It is

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[6] HAUMANN, M. L., Étude microbiologique et chimique du rouissage aerobic du lin. Annals Past. Inst. **16**:378–385. 1902.

possible that some of the spores of anaerobic bacteria survived and produced the characteristic change in the flax fiber which was attributed to the pure culture of aerobes used.

An anaerobic organism was also found by BEIJERINCK and VAN DELDEN,[7] to which they gave the name *Granulobacter pectinovorum.* This organism was an obligate anaerobe and a vigorous spore-former. It required protein or its split products as sources of nitrogen, liquefied gelatin, and actively attacked carbohydrates. In general it had the characteristics of the anaerobes described by others, especially the one found by WINOGRADSKY.

A more recent extensive investigation has been reported by STORMER,[8] who found an anaerobic spore-former which he called *Plectridium pectinovorum.* The granular structure of the cells makes one believe that he had the organism described by earlier writers. STORMER'S bacillus seemed to differ from these, however, in being a facultative anaerobic organism.

In sharp contrast with these papers are a number of others indicating that an aerobic organism is involved. MARMIER[9] mentioned such an organism. BEIJERINCK and VAN DELDEN also reported that *Bacillus subtilis* and *B. mesentericus* would ret flax, although they found another organism, which they called *Granulobacter pectinovorum*, which seemed to ret flax more completely and quickly.

ROSSI[10] studied the retting of vegetable fibers and stated that the microbiological retting process has certain advantages over chemical retting. Whether this is true or not is probably determined by the uses to which the fiber will be put. He devised an aerobic method in which *Bacillus comesii* was allowed to act on material which had been steeped in water at from 28° to 35° C. The pure culture was added and the vat was aerated, the tempera-

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[7] BEIJERINCK, M. W., and VAN DELDEN, A., Over de bacterien welke bij roten van vlas werksdam zign. Kon. Ak. Wetensch. Amsterdam (Verstag. van de Gewone Vergadering der Wis en Natunkk) **12**:673. 1903.

[8] STORMER, K., Über die Wasserroste des Flachses. Chem. Zent. **76**:41. 1905.

[9] MARMIER, Le rouissage du lin. Bot. Centrabl. **83**:90. 1900.

[10] ROSSI, G., Industrial retting of textile plants by microbiological action. Bull. Agric. Intell. **8**:1067–1074. 1916.

ture being maintained between the extremes just mentioned, since the fermentation goes on most vigorously at that range. ROSSI's work was concerned with hemp, but the organisms may be similar to those used with flax. An anonymous article in the *Bulletin Imperial Institute* (**17**:605–607. 1919) confirms the work of ROSSI. Flax straw is immersed in water at 82° to 86° F. in vats, and after the addition of a special aerobic organism, *B. comesii*, it is aerated during the retting process. The pectinous materials are consumed and the retting process is completed in 36–40 hours. Another aerobic organism called *Bacillus felsineus* is reported which will also ret hemp, flax, ramie, nettle, and other plants. It is said to produce a rapid retting and furnish a fine, white, well separated fiber. LOESER,[11] in carrying out ROSSI's process, boiled flax for forty minutes and then treated it with pure cultures of *Bacillus comesii*. The vats were aerated and maintained at a temperature of 30°–32° C. These methods are more expensive than those which do not require boiling. Furthermore, the anaerobic organisms are so resistant to heat that they might pass through these preheating procedures and function in retting the flax, although the credit be given to certain aerobic bacteria.

CORRIGAN[12] has more recently discussed the relation of fungi to retting. His statements apparently are based on the researches which have been reviewed herein. Mrs. WYANT[13] has recently reported briefly some results on this problem. She isolated about forty cultures of both aerobes and anaerobes. Each of these cultures was tested for its retting ability. This narrowed the work down to one pure culture which received more intensive study.

Experimental work

The work which has been carried out at this laboratory has been done with pure cultures, and on materials from a large rettery

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[11] LOESER, R., Retting of flax by means of bacteria. Jour. Soc. Chem. Ind. **38**:407. 1919.

[12] CORRIGAN, J. FREDERICK, Bacteria and molds: their biological nature and their influence on vegetable fibers. Jour. Soc. Dyers Colourists **36**:198–201. 1920.

[13] WYANT, Z. N., Some bacteriological problems involved in the retting of flax. Abstracts Bact. **5**:208, 209. 1921.

in eastern Michigan.[14] Most of the flax used in these experiments was grown in Michigan, and was thoroughly cured before it was received at the laboratory. It was tied in bundles or "heads" and was in fine condition, since it had been cultivated for fiber. The other was the flax which had been raised for seed, and consequently had not been kept tied in bundles, but had become badly broken and bent during thrashing. While just as good retting was secured on this as with the stalks which were raised for the fiber and tied in bundles, the resulting fiber was not of good quality. Perhaps such a raw material could be better retted by the chemical process. The fibers were much shorter, but probably just as satisfactory for paper making.

The samples from the retting vats were taken in sterile bottles and subjected to the usual bacteriological examination. Both aerobic and anaerobic plates were made, from which pure cultures were picked and transplanted into various culture media. The flora from the vats was varied, but spore-forming bacteria of both aerobic and anaerobic types were common. The aerobic types were similar to the members of the *subtilis-mesentericus* group; they formed large spreading colonies on solid media and liquefied gelatin very rapidly. Spores were easily formed in large numbers.

The pure culture experiments were carried out with many of the common bacteria and several cultures of yeasts. The bacteria and yeasts used were:

Pseudomonas pyocyaneus
Proteus vulgaris
Erythrobacterium prodigiosus
Pseudomonas fluorescens
Zopfii Zenkerii
Bacterium enteritidis
Bacillus gasoformans
Bacterium colon
Bacillus butyricus
Bacterium aerogenes
Saccharomyces cerevisiae
Torula monosa
Saccharomyces ellipsoideus
Saccharomyces marxianus
Myoderma vini
Bacterium cloacae
Bacillus cereus
Bacterium capsulatum
Erythrobacillus arborescens
Bacillus subtilis

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[14] The author is indebted to Mr. B. S. SUMMERS of the Summers Linen Company of Port Huron, Michigan, for material upon which some of the results here reported were secured, and for his constant interest in the progress of the work. Dr. R. E. FINDFUSZ of the American Writing Paper Company kindly furnished some flax straw and exhibited a kindly interest.

Contrary to the statements of HAUMANN, successful retting could not be accomplished with these common bacteria. None of the organisms such as *Bacillus subtilis* was found to possess any activity. In order to test the retting ability of all these cultures and those isolated from vat liquors, flax straw was cut into pieces about 4.5 inches long. These were put into long test tubes and covered with water. Sterilization was accomplished by heating in the autoclave at 115° C. for fifteen minutes. The sterility of these flax straw culture tubes was determined by both aerobic and anaerobic cultures in sterile litmus milk and other media. Heating in the autoclave at 115° C. seemed to be sufficient for their sterilization, and did not seem to injure the straw or make the retting more quickly accomplished when the bacteria were applied. Experiments were carried out later in 3 gallon earthenware jars in order to test the retting activity of the organism which was finally isolated. Attempts were also made to find an aerobic organism such as was used by ROSSI. Flax straw was put into a large glass vat covered with distilled water and aerated for a week, with frequent examinations for aerobic pectin fermenting organisms.[15] No success was obtained, even after specimens of soil and decaying organic matter were added. Several workers have mentioned the use of aerobic strains. One who is familiar with bacterial metabolism would expect to find an anaerobic organism. Members of this group decompose more materials than aerobes which leave so much energy in their products. The anaerobes partially hydrolyze large amounts of material for a certain amount of energy, while the aerobes hydrolyze a smaller amount of material completely for the same amount of energy. Perhaps for the same reason the anaerobes are used in most of the fermentations which yield certain organic chemicals.

The study of the vat liquors from a large commercial rettery in eastern Michigan, and from the specimens of flax and retting liquors in small tubs in the laboratory, narrowed down to an anaerobic organism as the most specific. This was secured in pure cultures by anaerobic plating in plain agar. Transfers made into other media allowed the following characterization:

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[15] Industrial retting of textile plants by microbiological action. Bull. Agric. Intell. 8:1067–1074.

Vegetative cells: The vegetative cells grown on common media were large rods with a dense protoplasm. Many of the cells presented a granular structure. Iodine staining indicated the presence of starchy materials.

Spores.—Spores are formed which are larger than the vegetative cells, giving the cells the shape of a *Clostridium*. The spores were found to resist heating for thirty minutes at 80° C. Further studies on their thermal resistance seemed uncalled for in a study of this nature.

FERMENTATION REACTIONS.—Large amounts of gas were formed in lactose, glucose, saccharose, and glycerol. In most of these fermentation tubes there was a pronounced odor of butyric acid.

LITMUS MILK.—Litmus milk was quickly decomposed; the curd was peptonized with large amounts of organic acids, principally butyric.

GELATIN.—Gelatin was quickly liquefied at 20° C.

PLAIN BROTH.—The broth was rendered cloudy with a precipitate only after a long period of growth.

NITRATES.—Nitrates were reduced with the formation of nitrites and ammonia.

That this anaerobic organism is common in nature and on the stalks of the flax plant was shown in several ways. It was found to be present repeatedly on the stalk of flax by simply soaking it in distilled water. After about thirty hours a vigorous evolution of gas would start, which ceased in about forty-eight hours at room temperature. This could be reproduced at will. That the organism is present in soil was shown by adding garden soil to tubes of sterile flax in distilled water. All evidences of a rapid retting started in twenty-five hours, and was completed in forty-eight hours. Pure cultures of this anaerobe removed the carbohydrate binders in the flax stalk in forty-eight hours at room temperature (26°–32° C.). The fermentation is accompanied by a vigorous evolution of gas, which is forced out of the stalk, clinging to the side until the bubble is large or some jar removes it. The liquid becomes turbid and has a strong characteristic odor.

Whether pure cultures of this anaerobe would be valuable in retting is probably doubtful, since the organism is so widely distributed in nature. Several experiments were carried out to determine whether the organisms were on the flax stalk itself. There was no difficulty in the majority of attempts to demonstrate its presence. In a large sense the change brought about in the flax stalk is a natural one, which is continually going on in nature. It is an attempt on the part of nature to bring about the transformation of organic compounds, and to keep the elements moving through their cycles. In retting it is the desire to carry this to the point when the binding materials in the fiber are dissolved, thus releasing the bast fibers, and to check it just before the cellulose of the fibers is attacked. It is reasonable to expect that this could be done more quickly in a rettery, where favorable conditions are maintained and where the flora of microorganisms may easily be established.

The quality of the water seems to have great influence on the quality of fiber. During a few experiments in the beginning of this work tap water containing about 1 p.p.m. of iron was used. This yielded a fiber which was dark and discolored in appearance. The use of pure distilled water corrected this and yielded a silken glossy fiber nearly white in color. This supports the experience in water retting that a better fiber is secured where a softer water is available, and confirms the statements of workers that the quality of fiber produced in the Courtrai region in Belgium, where flax is retted in the waters of the river Lys, is superior to that retted elsewhere.

One of the earlier investigators stated that the presence of aerobic bacteria tended to produce more favorable conditions in which the anaerobic could act. To determine whether there was foundation for this, several experiments were carried out with mixtures of the anaerobe isolated in this investigation and certain common aerobes. *Bacillus subtilis* and *Bacterium coli* were used, but it could not be seen that they were of any value. Neither did they seem to lengthen or shorten the time required for completion of the retting process. Their presence seemed to have little effect. Under natural conditions they might favor the retting in that they would help to remove the products formed from the pectic binding materials. In favor of this assumption is the fact that nature does most of her work with mixed cultures, and many significant changes are brought about by symbioses.

Summary and conclusions

1. Retting of flax in the preparation of linen fiber is a natural process, and an attempt on nature's part to keep the elements moving through their cycles.

2. The organism isolated as the specific one in retting flax was *Clostridium amylobacter*. It is an anaerobic spore-forming bacterium which quickly hydrolyzes the carbohydrate "binders" in the flax stalk. It was found to be commonly present on flax stalks and widely distributed in nature.

3. Symbiosis of this organism with common aerobic bacteria did not seem to decrease the time required for retting or produce conditions under which the anaerobic *Clostridium amylobacter* could work better.

4. Temperature is an important factor in that it retards or increases the activity of the fermentation involved in retting. The best temperature seemed to be 30° C.

5. The retting process can be shortened and a better quality of fiber produced by carrying it out under controlled conditions where the optimum environment may be maintained.

6. Previous sterilization of the flax did not seem to affect the retting process. The flax retted as quickly when put into the water without previous treatment as when it was boiled or heated in the autoclave.

7. No real success was secured by the use of fifteen common aerobic bacteria and five yeastlike fungi.

8. Flax raised for seed was quickly retted, although the fiber was not in as good condition as that prepared from flax raised for fiber.

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ANATOMY OF A GALL ON POPULUS TRICHOCARPA

KARL C. HYDE

(WITH PLATE VI)

Early in the fall of 1919 the writer collected some galls upon the twigs of *Populus trichocarpa* T. and G., caused by *Macrophoma tumefaciens* Shear,[1] in Greenough Park, Missoula, Montana. These galls were relatively large woody growths, and appeared on the trees in great numbers. Within an area of about one acre, at the north edge of the park, there were as many as twenty-five trees of various sizes bearing galls, while the trees throughout the rest of the park were practically free from knots. A close examination of the infected trees showed that nearly every twig bore from one to many galls, and that above these galls in many cases the twigs were gradually dying. The disease, although apparently occurring only in isolated localities, appeared to the writer to merit further investigation, and accordingly the problem was attacked at such an angle as to show, if possible, what effect the fungus had upon the normal twig to bring about the hypertrophy resulting in the gall.

Introduction

Populus trichocarpa is the largest tree of the genus, sometimes attaining a height of 65 m. and a diameter of 2.6 m. It is a rapid grower, and is usually found in the lowlands. It is very common throughout the Pacific coast region along the banks of streams, from southern Alaska to northern California, extending as far east as the ontinental divide in Montana. In Washington, Oregon, and eastern Montana it is the largest of the broad-leaved trees, tending to break somewhat the monotony of the vast stretches of coniferous forests of the region in its range. The tree is widely used for shade and ornament for parkings and lawns of the cities of the northwest. In Missoula, for example, there are many trees of this species within the limits of the city. It is especially adaptable for regions of light

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[1] HUBERT, E. E., A new *Macrophoma* on galls of *Populus trichocarpa*. Phytopath. 5:182. *figs. 3.* 1915.

rainfall because of its long roots and ease of propagation. The roots were formerly used by the Indians of California and Oregon in the manufacture of hats, baskets, mats, and other ornaments, being well adapted for this purpose because of their toughness, fineness, and length. The wood is dull brown, with nearly white sapwood, soft, light, and weak, with a specific gravity of 0.38. It shrinks moderately, warps considerably, is easily worked, but is not durable. It has a dull silky lustre, and is used extensively for cooperage, boxes, tubs, bowls, canoes, wooden legs, and paper pulp.

The only published work that treats of this gall is that of HUBERT, who deals with the characteristics of the fungus which is thought to be the cause of the gall. He originally found the galls on twigs of *Populus trichocarpa* in 1909, and at first thought they were caused by an insect, *Saperde populnea* L. In March 1910 another collection was made, and he identified the causative organism as a species of *Macrophoma*, but was unable to determine its specific identity. In November 1910 he sent samples of the galls to C. L. SHEAR, United States Bureau of Plant Industry, who described and named the fungus *Macrophoma tumefaciens*.

According to HUBERT, the fungus and galls are widely distributed throughout Montana on the twigs of *Populus trichocarpa*. The several cities mentioned as localities in which he has observed the galls show that they occur at least throughout the western part of Montana and the eastern part of Idaho.

Method

As the xylem of the normal wood is comparatively soft, no difficulty was experienced in obtaining satisfactory sections. Sections of the galls were made with a sliding microtome because they were more uniform in thickness, and good photomicrographs could be secured more readily. First the knots were cut into cubes of suitable size for clamping into the microtome. These cubes were then placed in a mixture of equal parts of glycerine, alcohol (95 per cent), and water, and heated for one hour at a temperature of 100° C. This treatment served the double purpose of softening the wood and of removing a considerable amount of air. All of the air, however, was not eliminated by this process, and it was necessary to use the airpump to remove entirely the air from the blocks. Sections 10 μ in thickness were easily obtained by these methods. In staining sections it was found that Delafield's haematoxylin in combination with safranin gave satisfactory results.

Anatomy of healthy stem

XYLEM.—The normal wood is of the diffuse, porous type (fig. 1), corresponding in this respect to other species of *Populus*. The vessels are numerous, visible to the naked eye, and are slightly larger in the spring wood than in the summer wood. They form a conspicuous ring of large pores within the earlier wood where it joins with the late wood of the previous season. In most growth rings an irregular, oblique, tangential-radial arrangement of pores can be seen, which, however, do not cross the junction of the spring wood and summer wood of the preceding season's growth. The vessels are of the bordered pitted type in the xylem of secondary growth. In the xylem that arises from the growing point, however, a great many vessels with spirally thickened walls are formed.

MEDULLARY RAY.—The rays of this species, as in all the species of *Populus* which the writer has examined, are scarcely visible with the ordinary hand lens. Only uniseriate rays have been found to occur in normal wood, the cells of which are richly supplied with simple pits (fig. 3). The ray is made up of uniform parenchyma cells elongated horizontally. Viewed radially, a ray appears as a muriform structure composed of several rows of tabular cells. The walls of the cells of the medullary ray are pitted, and the walls therefore have a lattice-like appearance when seen in radial section. The ray cells in most cases contain a considerable amount of starch.

In tangential section, the cross-sections of the rays are shown to good advantage. The uniseriate character is very evident. The rays vary from three to seventeen cells in height, and are distinctly spindle-shaped, the end cells tapering to a decided point. The cells between the end cells vary in shape from cubical to prismatic (fig. 3).

In the radial aspect one is best able to study the wood fibers that compose the bulk of the xylem. These wood fibers are slender, nonseptate, spindle-shaped, sharp-pointed cells with narrow cavities. They extend nearly parallel to one another, and diverge from their course only in weaving around the medullary rays. The wood fibers are perforated with numerous, almost circular bordered pits.

In cross-section the fibers are nearly square in outline, and have relatively thin walls. The walls are somewhat thicker in the summer wood than in the spring wood. The fibers, according to RECORD,[2] have a maximum length of 1.90 mm., a minimum length of 0.50 mm., and an average length of 1.15 mm.

BARK.—The bark as herewith considered includes all that portion of the stem outside the cambium layer, and is composed of epidermis, cortex, and phloem.

The epidermis of young twigs is smooth and colorless. Beneath this is found a layer of parenchyma, from four to seven cells thick, encircling the stem. As the stem becomes older the outer tissues disappear and are replaced by suberized tissue produced through the activity of the phellogen. In the case of normal twigs this cork tissue never becomes very thick. The fibers are thick-walled elements, with sharp pointed, unbranched ends. They are arranged in groups which are in turn arranged in bands that extend around the stem, concentric with the cambium ring.

The phloem is composed of sieve tubes, companion cells, and phloem parenchyma. In many of the cells comparatively large spherical crystals are found.

The pith rays extend into the phloem. They are uniseriate, but broaden considerably as they progress into the cortex. This broadening is due to the fact that the cells are thickened tangentially, and not to any increase in number of rows of cells.

PITH.—The central pith of the stem is composed of cylindrical cells with thin walls. The pith area is about 0.8 mm. in diameter, and varies in color from a light gray to a reddish brown. Many of the cells contain starch and crystals. The crystals are spherical in shape, and never more than one is found within a cell. The outline of the pith area in cross-section is usually distinctly five-angled, but all gradations between this and a circular outline occur.

CAMBIUM.—The cambium of this tree is similar to that of other dicotyledons. It consists of a layer of thin-walled, delicate, tabular

[2] RECORD, S. J., Economic woods of the United States. New York: Wiley and Sons. 1919.

cells. These cells have their long axes extending vertically, and are wider tangentially than radially.

Anatomy of gall

Gross anatomy.—Many galls were examined and were found to vary considerably in size. The largest one measured was 3.5 cm. in diameter, and the smallest one had a diameter of 0.5 cm. They vary in shape from ovoid to globular, and usually encircle the stem. Ordinarily the galls appear singly, but it is not unusual to find them very close together or even confluent on the stem, and characteristically at the nodes. The outer surface of the gall is roughened much more than the outer portion of the normal bark, either above or below the hypertrophy. There has been such a development of suberized tissue on the periphery of the gall, due to the presence of the disease, that it becomes broken into deep, irregular, longitudinal fissures (fig. 5).

Pycnidia of *Macrophoma* are scattered irregularly over the surface of the gall. These pycnidia are flask-shaped, and are imbedded in the parenchymatous tissue of the gall. They have a pseudoparenchymatous wall and open to the exterior through an ostiolum. The pycnidia are barely visible to the naked eye, and are more abundant in the region of the fissures. Considerable sloughing of the bark from the surface occurs, especially at the time of the increased activity of the cambium region in the spring.

It is interesting to note the enormous increase of the hypertrophied part of the stem in comparison with the apparently normal stem above and below the gall. No less interesting is the macroscopic comparison of the normal wood and bark of the region below with the region through the gall (fig. 8). It shows to advantage the relative amount of increase of the tissue in question, as a result of the stimulation of the pathogene. The averages, obtained from the measurements of twenty-five galls of various sizes, are as follows:

Radius of galls	10.5 mm.
Radius of wood of galls	7.0
Thickness of bark of galls	3.5
Radius of stem immediately below gall	5.0
Radius of wood immediately below gall	4.5
Thickness of bark immediately below gall	0.5

It is apparent from these figures that the diameter of the normal stem is composed of 10 per cent bark and 90 per cent wood, while in the diseased twig 25 per cent is bark and only 75 per cent is wood. The section through the gall has increased 110 per cent, the wood 55 per cent, and the bark 600 per cent over the same tissues in the stem just below the gall.

ORIGIN.—The hypertrophy first makes its appearance on the twig in the form of a slight swelling, at or about the time of renewed cambial activity in the spring, which for the region of western Montana is about the first of May. This original swelling is brought about by the increase in number and also the increase in size of the cells of the recent phloem and xylem as secondary growth takes place. It would appear evident, therefore, that this increase in size and number of the cells is brought about by the stimulating effect produced on the cambium by the presence of the fungus. To say that this is due to enzymatic action is only a conjecture, but this is the most plausible explanation.

In the majority of knots sectioned the distortion reached to the pith, at least in some portion of the stem, which indicates that infection took place in the infancy of the twig, as xylem once formed in the region surrounding the pith ordinarily is not subsequently distorted. This, coupled with the fact that the hypertrophy is almost always formed where the twig branches, indicates that the pathogene gains entrance to the host in the region of, and during the formative period of the lateral buds.

The galls evidently arise as the result of the original infection. Frequently when a young lateral twig becomes infected its growth is stunted distad to the infection, and as a result numerous short lateral twigs are present that simulate spurs. Although these spurs may be only a few millimeters in length, they usually show several years growth, as evidenced by the number of terminal bud scars present. These spurs usually protrude from the branch at a distinct right angle, while the normal twig protrudes at an angle of approximately 30° (figs. 5–7). This increased angle is brought about mechanically, the gall forming in the axil exerting a pressure that forces the spur downward.

XYLEM.—In looking at a transverse section of diseased wood such as is shown in fig. 2, one is impressed by the enormous broadening of the rays. In this view the rays are clearly seen to be multiseriate. Occasionally the rays are seen to join together, giving rise to still wider ones. In a great many cross-sections the rays do not take a direct course through the xylem, but are often broken and their course considerably interrupted. In many cases this ray parenchyma is scattered among the wood fiber and wood parenchyma elements.

The vessels have become greatly distorted throughout (fig. 2). This is due to the flattening of the tubes in a radial direction. In most cross-sections the pores are few in number, and in sections of some galls they are entirely absent (figs. 2, 9). Considerable increase in wood parenchyma cells is seen in the diseased wood. In a great many cases the wood fibers are bent at right angles, the bend always being toward the periphery. Due to the bending, a transverse section often shows these fibers in a longitudinal view (fig. 2). This distortion is brought about largely as a result of crowding, due to the great increase in the number and size of the cells of the medullary rays.

In fig. 4 is shown a tangential section of diseased wood through a gall which illustrates to good advantage the characteristics of the medullary rays, which vary from one to several cells wide tangentially. This broadening is due to the increase in number, as well as to the increase in size of the individual cells making up the rays. That these ray cells are larger in the diseased wood than in the normal wood is apparent by comparison of fig. 3 and fig. 4, the magnification in both cases being the same. It is also evident that there is considerable increase in the size and number of the wood fiber and wood parenchyma elements. The medullary rays sometimes become so broad that their tangential diameter equals, or is even greater than their vertical diameter (fig. 4).

The average of one hundred measurements of the diameter of the medullary ray cells, in the tangential sections of normal and diseased wood of the same age, gave for the former 13.2 μ and for the latter 27.9 μ. This shows an increase of slightly over 100 per cent in these cells as a result of the diseased condition. Measurements of the diameters of the wood fibers in the same sections gave an average in the normal wood of 12 μ and in the diseased wood an average of 16 μ.

Here and there in the xylem of the diseased area uniseriate rays occur, but these are far outnumbered by the multiseriate rays. The ray tissue in the diseased xylem makes up approximately 30 per cent of the wood, while it is evident, by comparing the figures, that in the normal wood the ray tissue makes up a much smaller proportion.

BARK.—The distortion of the elements of the bark of the gall are not so pronounced as in the xylem. In the bark the principal effect is found to be a decided increase in size and number of the cells of the several tissues. As a result of the normal reaction of the host, in an attempt to overcome the injurious effects of the pathogene, the amount of suberized tissue is increased manyfold, so that now there are as many as fifty rows of cork cells. The parenchyma cells of the primary cortex are considerably larger and more than doubled in number. The average of numerous counts made of the number of rows of parenchyma cells between the cambium and the periderm in normal and diseased bark shows for the former 28, and for the latter 65. The phloem rays are multiseriate, often comprising six or seven rows of cells. These rays are sometimes bent tangentially, as seen in cross-section.

The phloem tissue is greatly increased in the diseased twig. This increase is largely due to the multiplication of the phloem parenchyma cells and the subsequent growth of the cells to a size slightly beyond the normal. Physiologically the phloem does not appear to be greatly interfered with during the younger stages of the gall. As the gall becomes larger, considerable pressure is exerted upon the sieve tubes, as is indicated by the fact that they are flattened radially.

The functional disturbance seems to be more closely connected with the xylem than with the phloem, as it is here that the distortion of elements is the greatest. This disturbance is manifested in the gradual dying of the twig above the hypertrophy (fig. 7), which, however, does not usually occur unless there are several knots upon the twig. In cases where only one knot is found on the twig there usually is no noticeable disturbance distad to the knot.

The writer believes the death of the twig to be due to the fact that sufficient water and minerals cannot get through the vessels of the xylem to the leaves beyond the galls. The supply of water and minerals is shut off because the xylem elements are so twisted and distorted that the vessels as vertical tubes and efficient water carriers have largely disappeared. The water and mineral nutrients are able to pass slowly one or two galls by diffusion, even though the vessels are greatly distorted, but when several galls are present in the path the movement is so interfered with that growth is retarded and sooner or later the tip part of the twig dies above the knot.

PITH.—No striking effects on the central pith as a result of the gall formation are noticeable, but in some larger galls the pith area is somewhat compressed, and the individual pith cells in these cases have lost their characteristic cylindrical form and have taken on an angular appearance.

CAMBIUM.—The cambium appears to be very active in the younger galls, as evidenced by the comparatively larger cells and nuclei. As the gall grows older this activity gradually grows less, until it is brought to an end by the death of the twig. In cases where death of the twig distad to the gall does not occur, the activity of the cambium eventually becomes almost negligible. The cambium ring as a whole becomes greatly distorted and interrupted in many places. The wood fibers that are bent toward the periphery are responsible for this interruption of the cambium. The cambium never entirely loses its identity (fig. 9). Many nascent cells are isolated from the cambium that give rise by continual division to isolated groups of phloem in the xylem region. These groups are more often formed between the spring wood and the summer wood of the preceding years' growth. They are usually crescent-shaped, and suggest the pith flecks often found in other woods. There are no isolated xylem groups formed in the phloem, but a great many wedgelike xylem elements extend through the cambium into this region (fig. 9).

Summary

1. The normal wood of *Populus trichocarpa* conforms closely to a typical dicotyledonous, diffuse, porous wood.

2. In western Montana and eastern Idaho a gall disease threatens to interfere with the commercial uses of this tree.

3. Only uniseriate rays are found in the normal wood of this tree, while in the diseased wood the rays are considerably broadened, often being several cells wide tangentially.

4. The average increase in the diameter of the stem, due to gall formation of several galls measured is 110 per cent, of xylem 55 per cent, and of phloem 600 per cent.

5. The xylem elements are greatly distorted, the vessels are flattened radially, and the wood fibers are often bent at right angles, due to crowding as a result of the great increase in number and size of the cells of the medullary ray.

6. In the bark the greatest effect noticeable is the increase in size and number of the parenchyma cells.

7. The phellogen is stimulated to unusual activity, and consequently the suberized tissue is considerably increased.

8. The distortion of the vascular elements, because of the interference with the transpiration stream, often results in the twigs dying above the galls.

9. The central pith is not greatly altered in the diseased stem.

10. The cambium is sometimes altered by distortion, but never completely loses its identity.

11. In addition to the evidence of constant association of *Macrophoma tumefaciens* with the lesions, the histological examination supports the idea that this fungus is the cause of the disease.

12. Infection experiments on pathogenicity are as yet lacking.

Acknowledgment is made to Professor W. W. Rowlee, under whose direction this study was made, and to Professor D. Reddick for many suggestions in the preparation of the manuscript.

Cornell University

EXPLANATION OF PLATE VI

All photomicrographs show magnification of 85 diameters.

Fig. 1.—Transverse section of normal wood, showing one annual ring complete.

Fig. 2.—Transverse section of diseased wood, showing distorted multiseriate medullary ray and radially flattened vessels.

Fig. 3.—Tangential section of normal wood, showing uniseriate medullary rays and normal fibers.

FIG. 4.—Tangential section of diseased wood, showing broadened medullary rays and enlarged and distorted fibers.

FIG. 5.—Twigs bearing characteristic galls; reduced $\frac{1}{3}$.

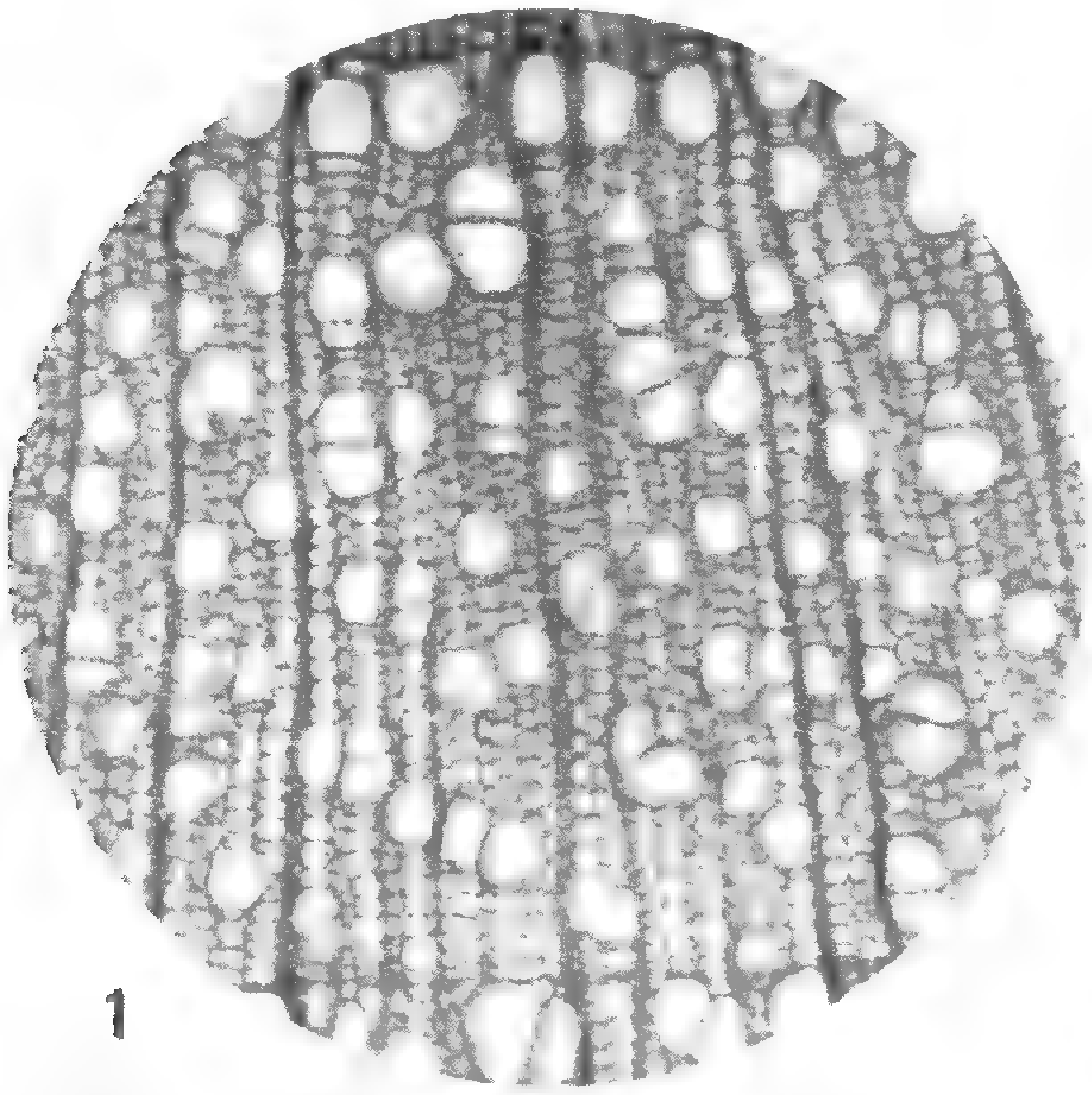
FIG. 6.—Normal twig, showing method of branching; reduced $\frac{1}{3}$.

FIG. 7.—Cluster of branches, showing characteristic habit of galls; many of these twigs were dead at tips; much reduced.

FIG. 8.—Transverse section of gall on five-year old twig; to right of this is shown transverse section of twig just below gall; reduced $\frac{1}{3}$.

FIG. 9.—Characteristic irregularity of cambium ring in diseased twig.

FIG. 10.—Normal one-year old twig and twig of same age bearing gall; reduced $\frac{1}{3}$.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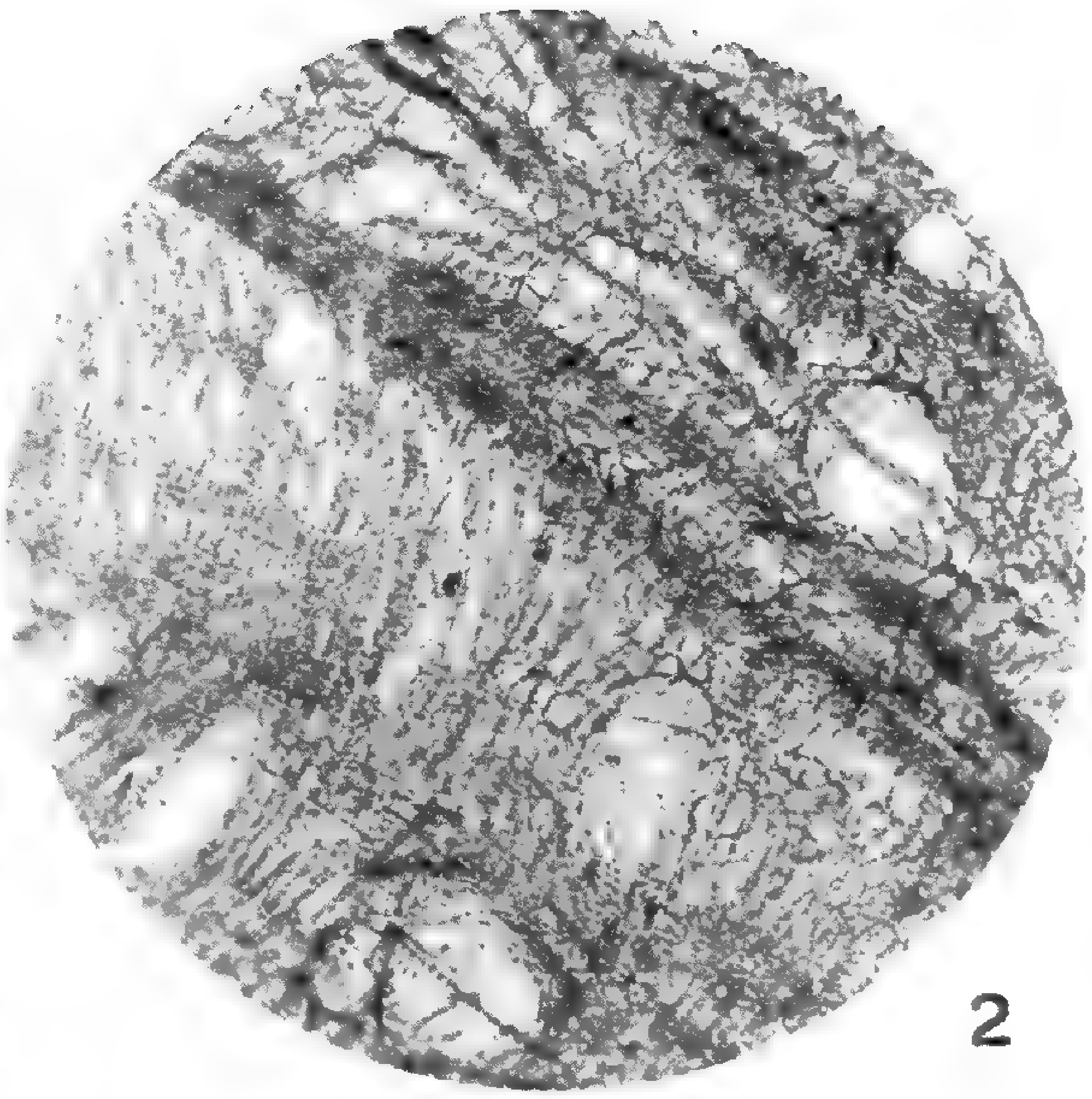
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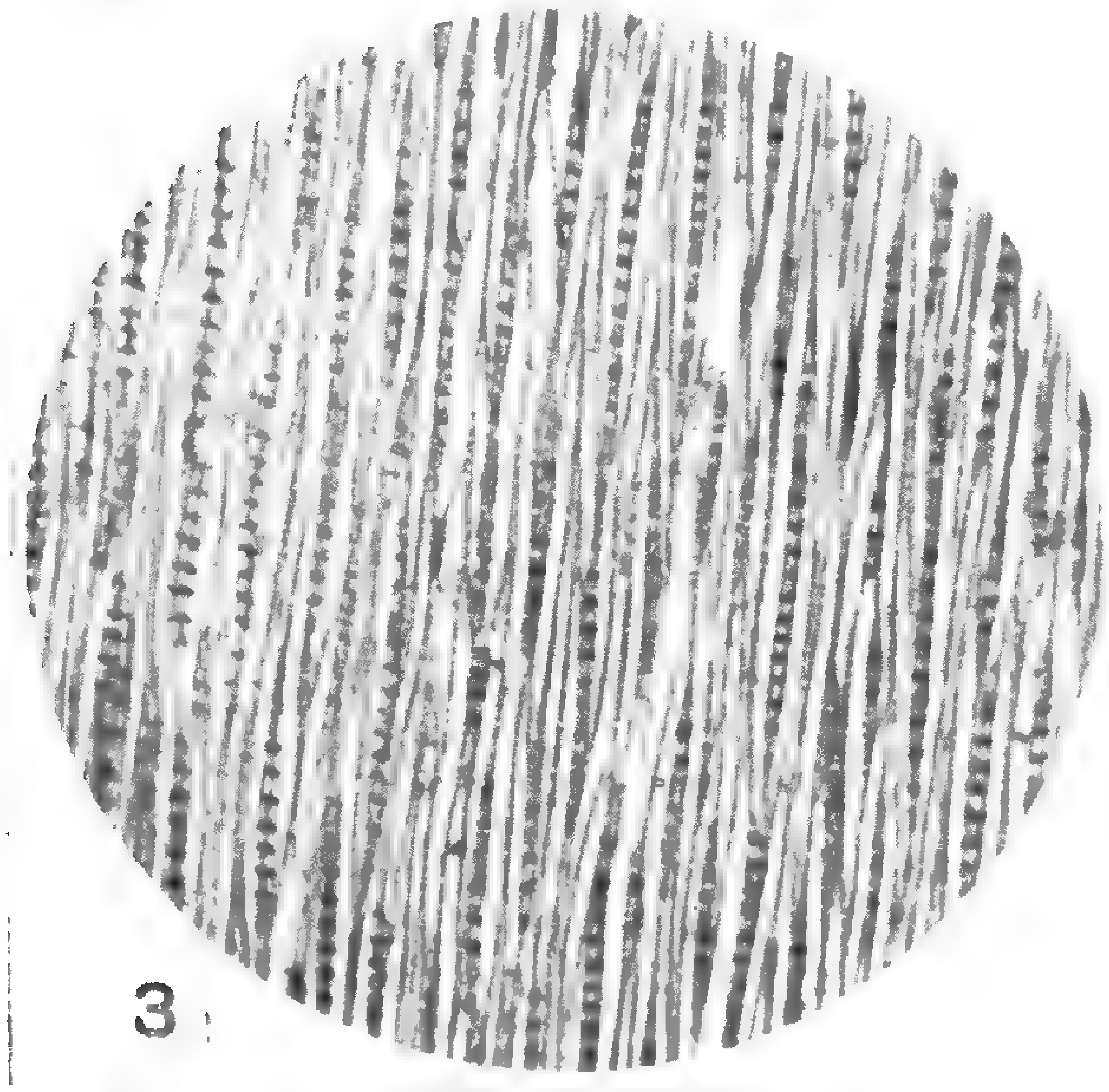
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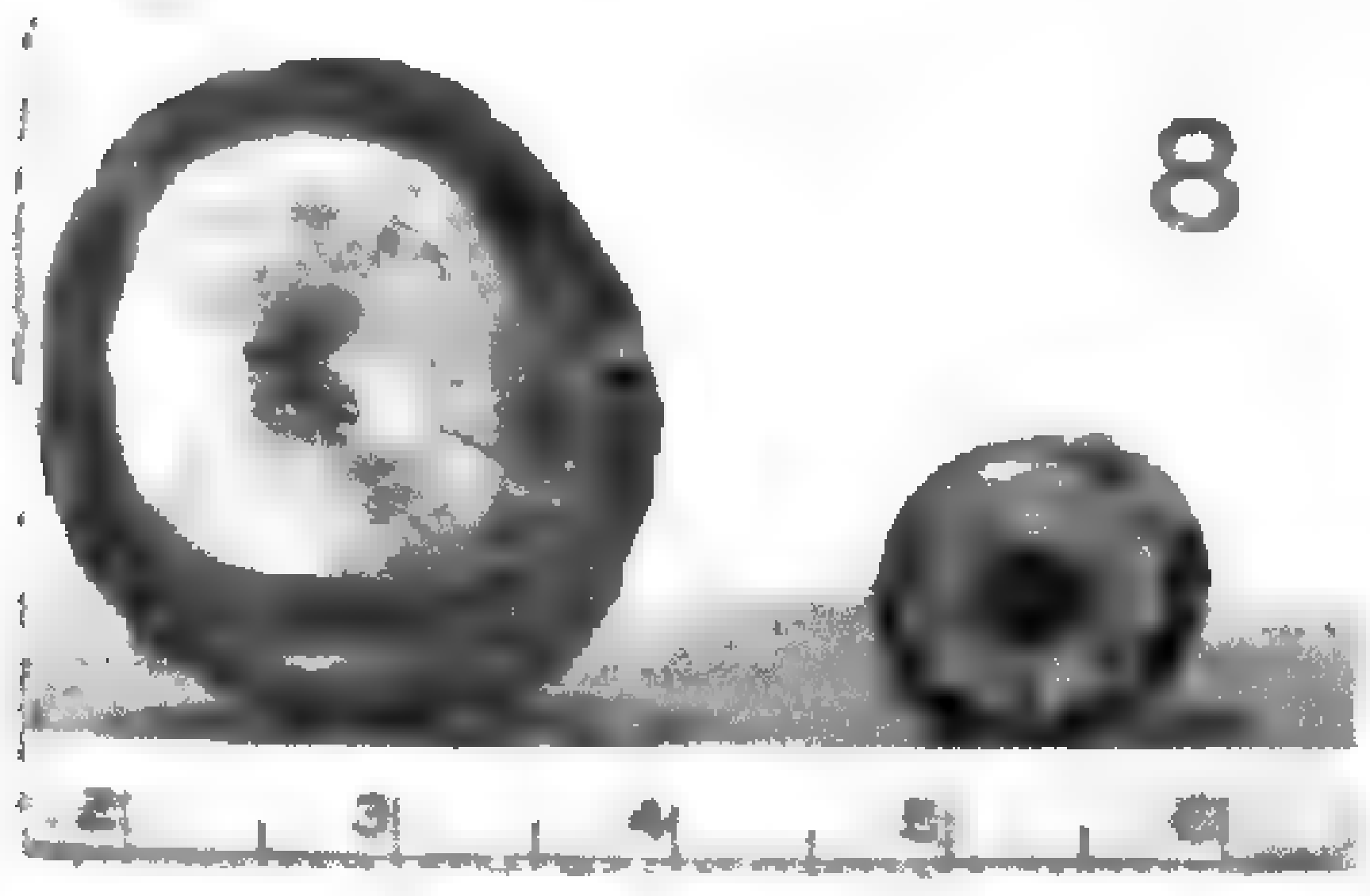

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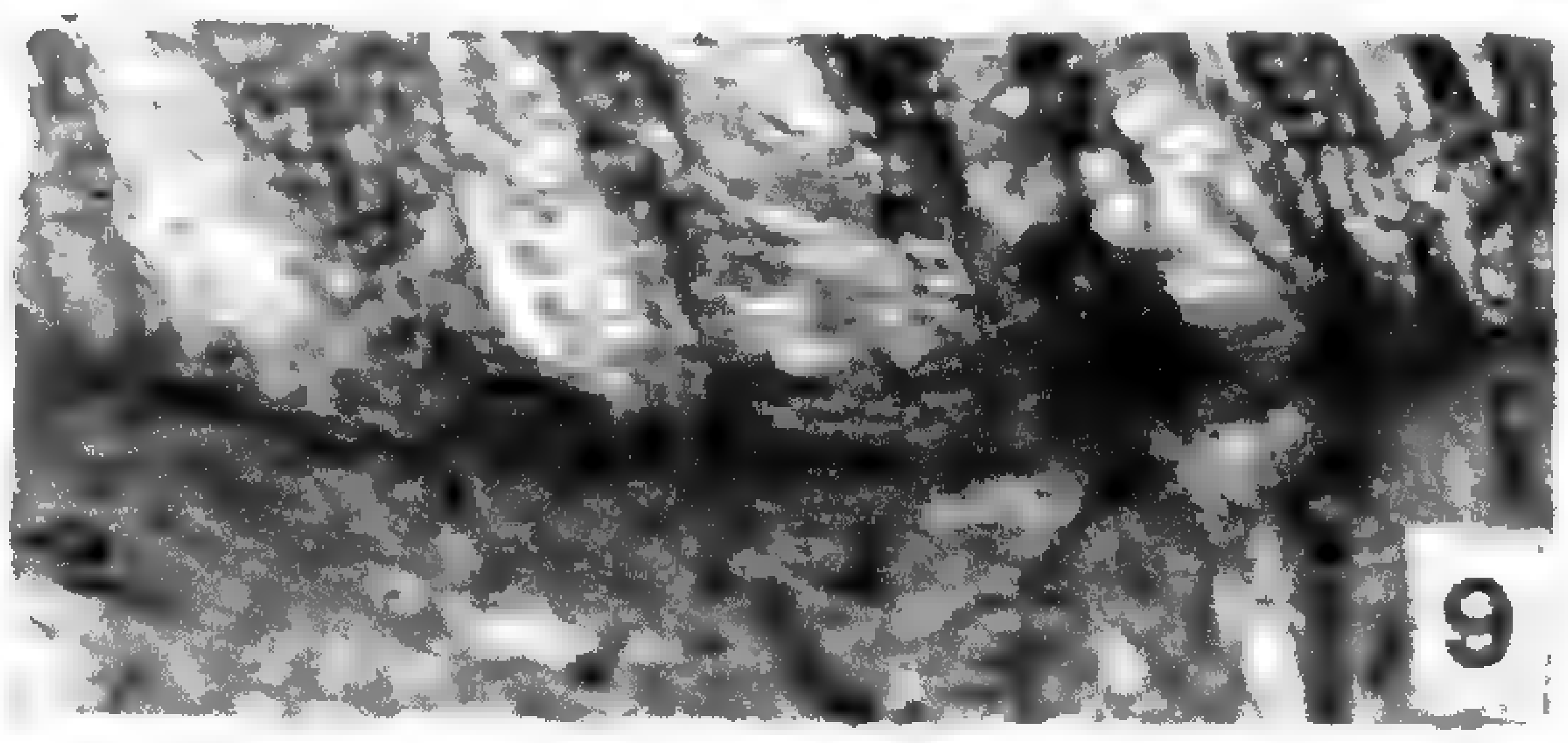
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HYDE on POPULUS GALL

POLLINATION IN ALFALFA

F. A. Coffman

(WITH FIVE FIGURES)

The problem of fertilization in alfalfa has been a matter of considerable controversy for several years. Many experiments have been performed in endeavoring to determine what factor is most largely responsible for pollination in this plant. In the review of investigations published by Piper, Evans, McKee, and Morse[1] it is stated that the amount of self-pollination varies with the season, and that its real importance in seed production is doubtful. Hildebrand[2] is cited as having believed as early as 1866 that fertilization may take place in untripped flowers, and Urban[3] (1873) is referred to as thinking that in some cases untripped flowers form pods. Most of the work of recent investigators has consisted of attempts to determine the most important agents in tripping the alfalfa blossom. Although a considerable number of experiments have been carried on, no definite conclusions seem to have been reached. Roberts[4] found anthers dehiscing and stigmas pollinated in the early bud stages of the alfalfa.

An investigation which was begun by the writer during the latter part of September 1916, and which was continued until killing frosts in October, had for its object the determination of the stage at which the stamens of the majority of alfalfa flowers really shed their pollen. It was found that light frosts do not seem to have any effect upon pollination, the percentage of pollinated to unpollinated flowers not being affected so far as could be noticed in any of the classes of buds examined.

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[1] Piper, C. V., Evans, M. W., McKee, R., and Morse, W. J., Alfalfa seed production; pollination studies. Bull. 75. U.S. Dept. Agric. 1914.

[2] Hildebrand, F., Über die Vorrichtungen an einigen Bluthen zur Befruchtung durch Insektenhulfe. Bot. Zeit. **24**: 75. 1866.

[3] Urban, I., Prodromus einer Monographie der Gattung *Medicago*. Verhandl. Bot. Ver. Provinz Brandenburg **15**: 13. 1873.

[4] Roberts, H. F., Alfalfa varieties, breeding, seed, and inoculation. Quarterly Rept. Kans. State Board Agric. **35**: 180. 1916.

The flowers were divided arbitrarily into four classes, according to the stages of their development, as follows: straight bud, fig. 1; pointed bud, fig. 2; hooded bud, fig. 3; and erect standard, fig. 4. The lengths of the flower in millimeters were determined before they were examined, and these measurements serve to some extent as checks on their stages of development. In the fourth class of blossoms, the flowers did not measure longer than in the hooded bud stage. This is because all measurements were taken from the base of the calyx to the tip of the flower in the first stages. After the standard began to arise, the measurements continued to be taken to the tip of the interlocking envelope of the wings and keel, which did not further elongate after the erection of the standard. After being measured, each flower was dissected by means of needles and forceps at a magnification of 23 diameters, under a Zeiss binocular microscope, using F-55 objectives and no. 5 oculars. The dissec-

FIG. 1.—Alfalfa bud representing "straight bud" stage; corolla in this stage has not grown out much beyond calyx; only standard petal can be seen; it is folded around all of the others.

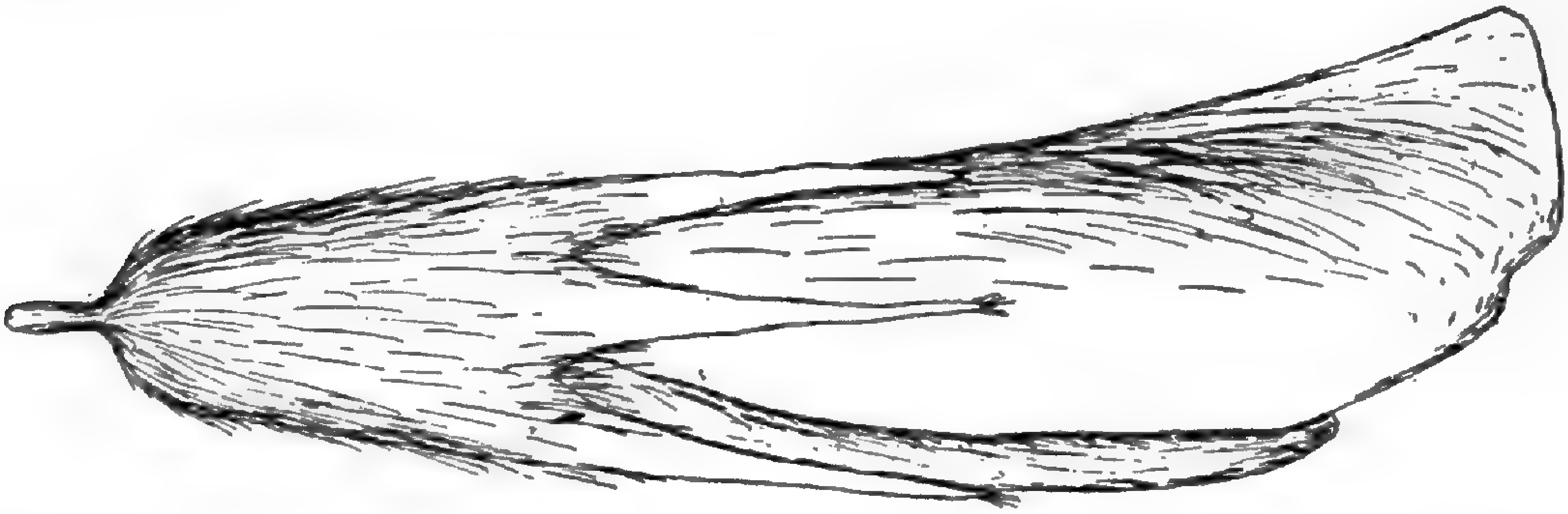


FIG. 2.—Alfalfa bud representing "pointed bud" stage; standard petal is seen to be distinctly curving upward in its growth; lower edges of wing petals, which together fold down over keel, are visible.

tions were made by removing first the standard, and then one of the wing petals, thus exposing the interior of the flower to view. Care was taken not to set off the tripping mechanism, nor to disturb the stamens or otherwise injure the flowers. Flowers believed to have been injured in the dissecting process were discarded and not recorded.

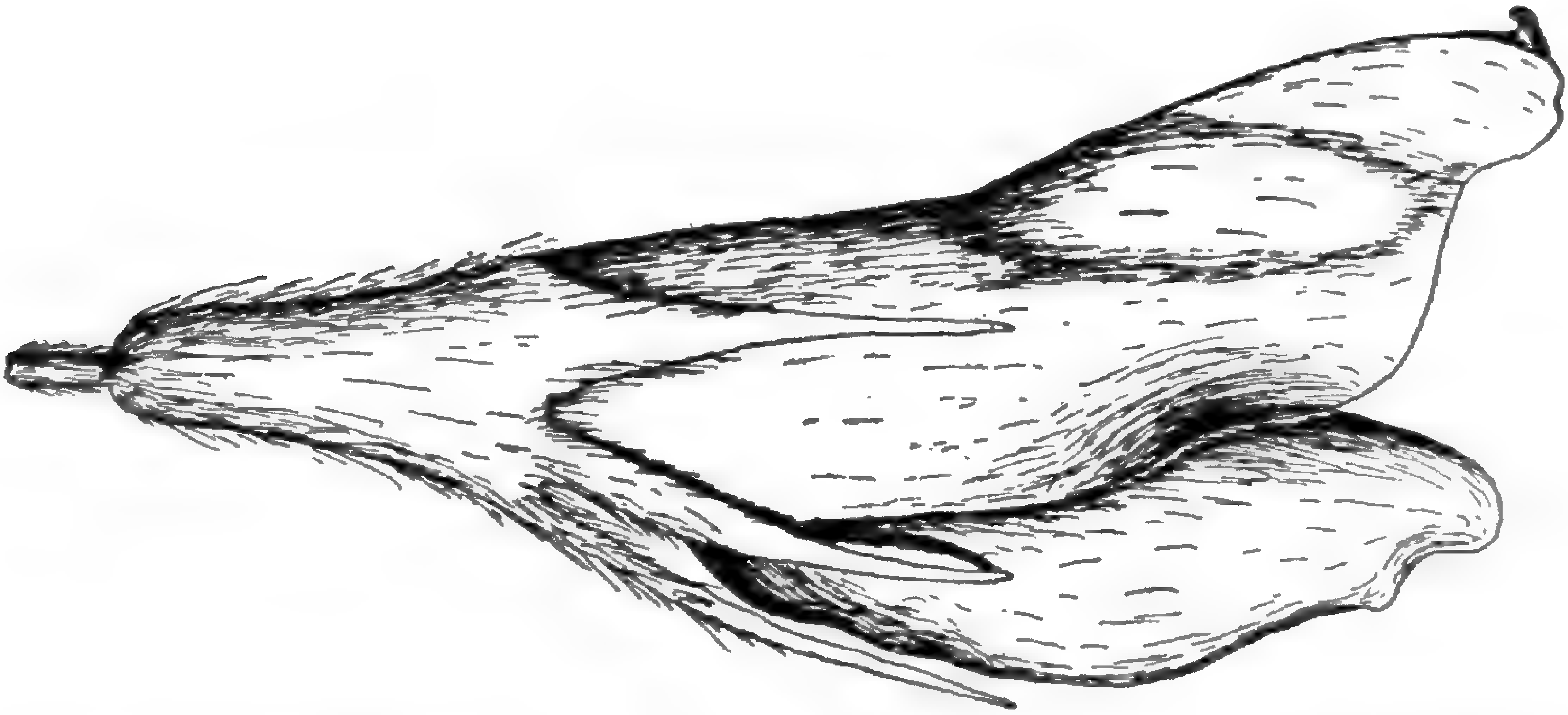


FIG. 3.—Young alfalfa flower called "hooded bud" stage; standard petal has risen nearly to full height, and is beginning to spread; wing petals distinctly seen protruding, folded over keel, which is not yet visible.

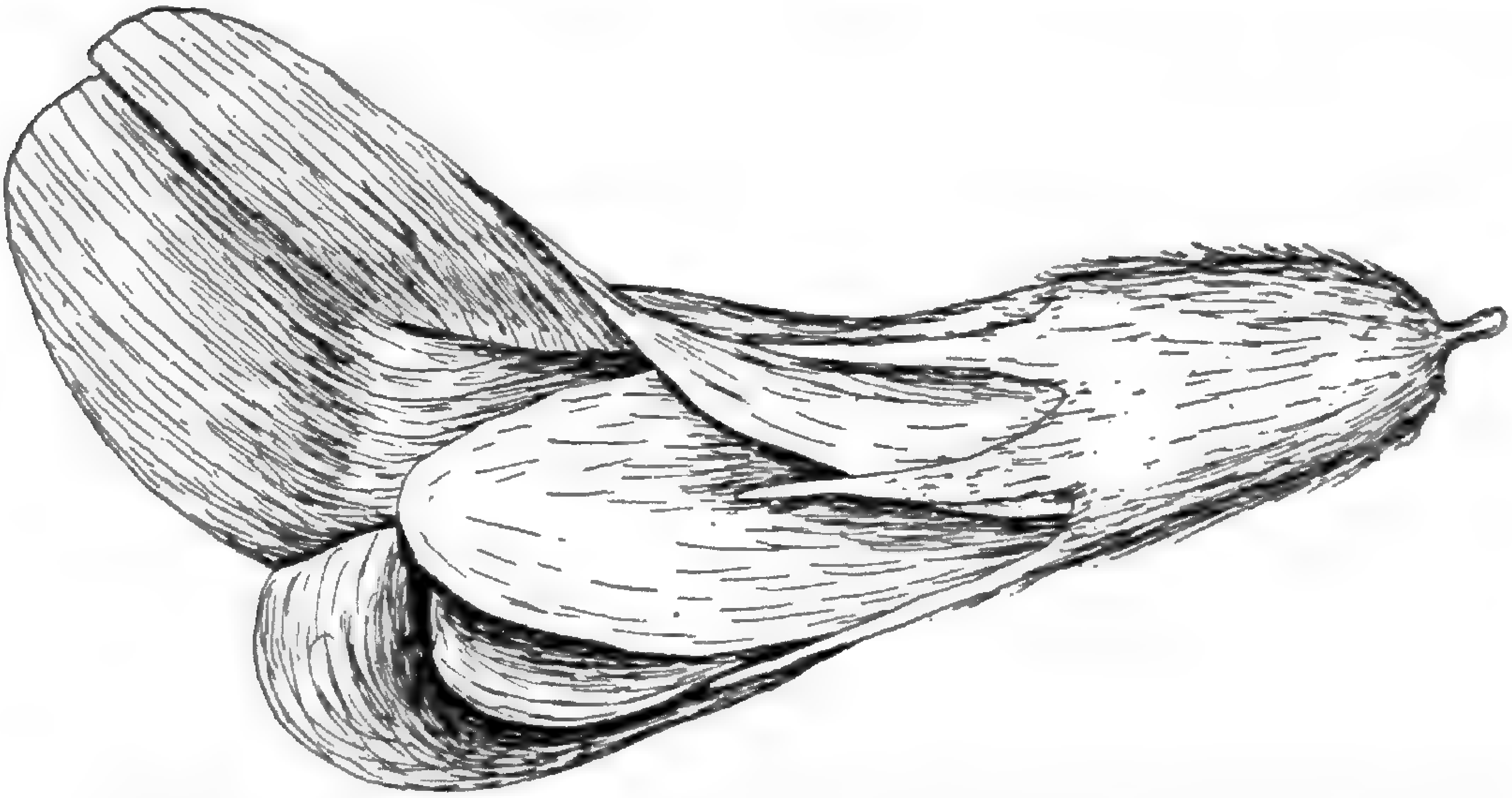


FIG. 4.—Alfalfa flower fully open and ready for tripping, representing "erect standard" stage; standard fully risen and spread; wing petals are separating, showing keel within.

The ratio of buds with anthers dehisced to those with anthers intact, in the pointed bud stage, was surprisingly close. Probably twenty flowers of the straight bud stage, in which the anthers had dehisced, were discarded, since it was thought that they might have been injured by dissection, thereby causing the bursting of the anthers. In handling the flowers of the hooded and erect standard stages, care was exercised to prevent them from tripping. The standard was first removed so as to keep the anthers from striking

TABLE I

Flower length (mm.)	Anthers intact	Anthers dehisced	Stigmas pollinated
		Straight bud stage	
5	20	1	1
6	49	1	1
7	12	1	1
8	....	1	1
Total	81	4	4
		Pointed bud stage	
6	18	1	1
7	44	22	19
8	21	41	37
9	3	19	19
10	....	1	1
Total	86	84	77
		Hooded bud stage	
7	....	1	1
8	....	8	8
9	2	18	18
10	1	9	9
Total	3	36	36
		Erect standard stage	
7	....	1	1
8	....	2	2
9	....	13	13
10	....	4	4
11	....	1	1
12	....	1	1
Total	....	22	22

it and from being broken, should the flower accidentally be tripped. One of the wing petals was then pulled away and the stamens examined. Most of the flowers in the erect standard stage, as they were found in the field, had already been tripped, untripped flowers being rather difficult to secure. A total of 316 flowers was examined and recorded as follows:

Straight bud stage.......	85	Hooded bud stage.......	39
Pointed bud stage.......	170	Erect standard stage.....	22

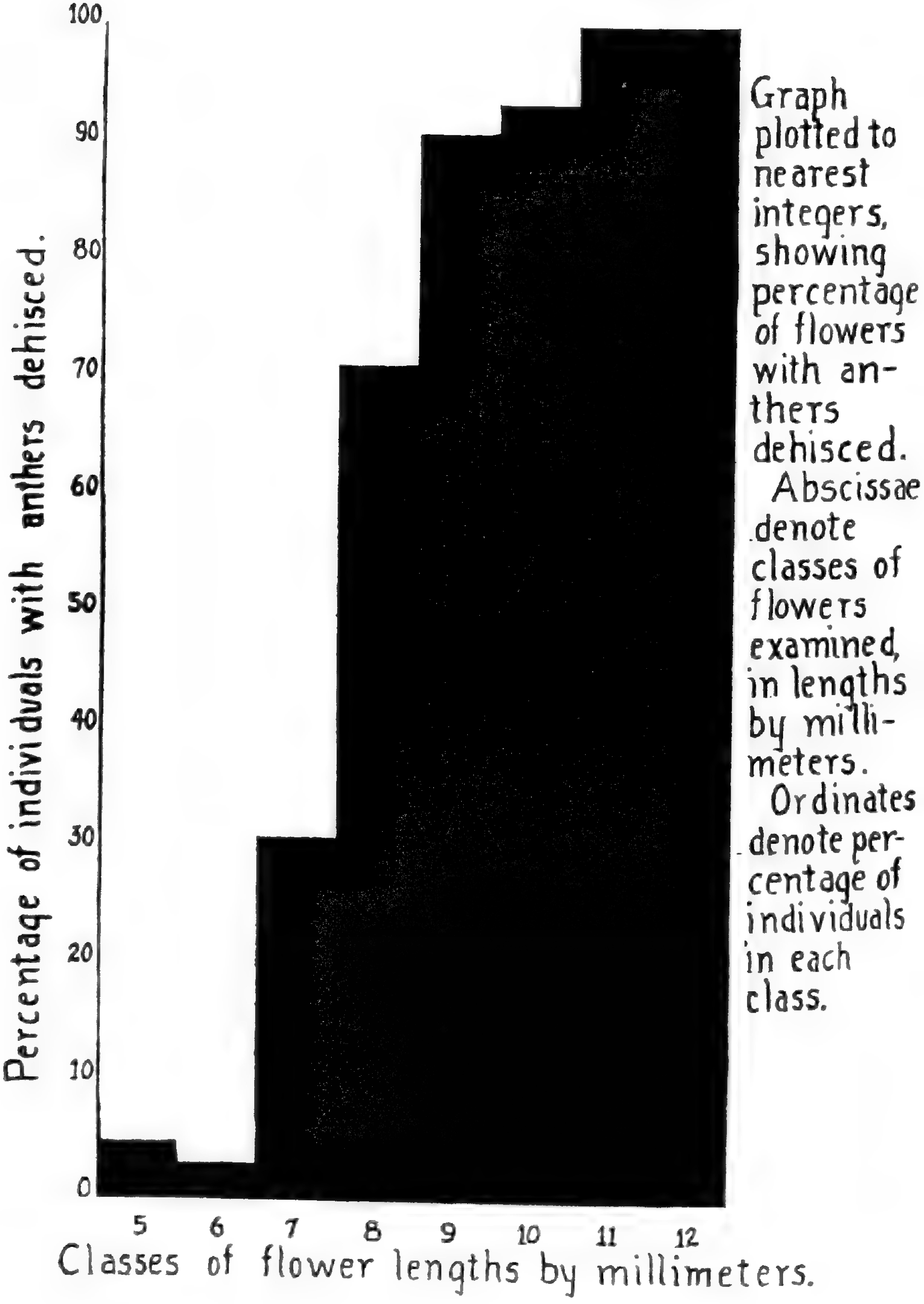
In the straight bud class, the flowers measure 5–8 mm. in length, and of the total number recorded, in only four instances had the stamens shed their pollen. The flowers in the pointed bud stage are considerably longer, being 6–10 mm. in length, and practically one-half of those recorded were pollinated. The hooded and erect classes of buds do not differ greatly from each other in size, varying from 7 to 12 mm. in length. But three flowers in the hooded bud stage had not been pollinated, while no unpollinated erect standard blossoms were found. The data as taken in this investigation, on the stages of bud development in relation to pollination, are given in table I.

From the data secured, the percentages of pollination of the flowers of different lengths, regardless of their stages of development, are given in table II.

TABLE II

Flower length (mm.)	No. examined	No. with anthers intact	Percentage with anthers intact	No. with anthers dehisced	Percentage with anthers dehisced
5...........	21	20	95.24	1	4.76
6...........	69	67	97.11	2	2.89
7...........	81	56	69.14	25	30.86
8...........	73	21	28.77	52	71.23
9...........	55	5	9.09	50	90.91
10...........	15	1	6.67	14	93.33
11...........	1	0	0	1	100.00
12...........	1	0	0	1	100.00
Total....	316	170	....	146	....

According to table II, anthers do not seem to shed their pollen before the flowers have reached 7 mm. in length, and most of them have done so by the time they are between 9 and 10 mm. long.



Graph plotted to nearest integers, showing percentage of flowers with anthers dehisced.

Abscissae denote classes of flowers examined, in lengths by millimeters.

Ordinates denote percentage of individuals in each class.

FIG. 5

This relation between dehisced anthers and flower length is shown graphically in fig. 5.

From the data secured as the result of this preliminary investigation, it appears that practically all alfalfa blossoms shed their pollen during the pointed bud stage, and before the hooded bud stage is reached. Judging by this, it would seem that tripping is not essential to pollination.

The alfalfa flower apparently begins to shed its pollen while yet comparatively small, being only about 7 mm. in length, which in hybridization operations would necessitate emasculation being accomplished while the blossom is still in the straight bud stage, in order to eliminate all possible danger of self-fertilization. As a matter of fact, this is a practical impossibility from the mechanical standpoint in the field at least, the flowers being emasculated in alfalfa crossing work by tripping them not earlier than the hooded bud stage and generally later, the pollen then being washed out with an atomizer spray. It is impossible to emasculate the flowers earlier than the hooded bud stage. The writer believes, however, that there is great danger of pollination of the stigma and of consequent self-fertilization, before the time when the flowers are ordinarily tripped in alfalfa by hybridizing operations.

This investigation was carried on in connection with graduate work under the direction of Professor H. F. Roberts, of the Department of Botany, Kansas State Agricultural College. The illustrations are from pp. 202–203 of his paper referred to herein.

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PROTECTIVE POWER AGAINST SALT INJURY OF LARGE ROOT SYSTEMS OF WHEAT SEEDLINGS

W. F. GERICKE

That wheat plants can be made to grow very large root systems as compared with the growth of their tops by certain properties of nutrient solutions, has been shown in a previous paper.[1] The writer has also shown that under certain conditions large root systems of wheat seedlings 4–6 weeks old play an important rôle in the number of tillers[2] the plant may produce. These observations suggested further experimentation where differences in the extent of the root systems of the plants would enter as the variable factor. It seemed plausible to expect that the relative physiological values or growth efficiencies of different nutrient solutions, and the tolerance of plants to salts, were not inconsiderably affected by the extent of the root development of the test plants when placed in the media. The present paper bears upon an investigation on these points.

Three different kinds of nutrient solutions were selected for the tests. These were solutions whose relative values as growth media had previously been obtained. The composition, molecular concentration of the salts, and the relative physiological values of these solutions stated as "good," "medium," or "poor" were as follows:

Solution no. 1.—0.0102 mol. KH_2PO_4; 0.0057 mol. $Ca(NO_3)_2$; 0.0062 mol. $MgSO_4$. Good.

Solution no. 2.—0.014 mol. K_2SO_4; 0.002 mol. $Ca(NO_3)_2$; 0.002 mol. $Mg(H_2PO_4)_2$. Very poor.

Solution no. 3.—0.016 mol. $MgSO_4$; 0.002 mol. $Ca(NO_3)_2$; 0.002 mol. KH_2PO_4. Poor if air temperature and transpiration for growth were high; medium if air temperature and transpiration for growth were relatively low.

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[1] GERICKE, W. F., Root development of wheat seedlings. BOT. GAZ. 72:404–406. 1921.

[2] ———, Certain relations between root development and tillering in wheat. (To appear in Amer. Jour. Bot. 9:1922.)

Sets of eight containers (Mason jars) of one-half gallon capacity were used for each of the different solutions and for each of the two different classes of wheat seedlings. These classes of seedlings were distinguished by a difference in the extent of root growth from that of top growth which the plants had when placed in the nutrient solutions. The method employed to obtain seedlings with large root systems was that referred to in the earlier paper. This consisted in allowing the cultures to grow in one quart Mason jars filled with tap water for five weeks before the cultures were placed in the nutrient solutions to be tested. The plants at this time had a root mass 70–80 cm. long, and had about one-half of their total dry matter in the roots. They were transferred from the tap water directly to the three nutrient solutions to be tested. To grow contemporaneously with these, other seedlings (young plants just germinated and therefore having small root systems) were set out in other sets of containers filled with the nutrient solutions to be tested. The seedlings in this latter case were 6–8 cm. high, with roots 8–10 cm. long, about 20 per cent of the dry weight of the plant thus being roots. Subsequent treatment of all cultures was alike, and this included additions of a small amount of $FeSO_4$ to each culture at regular intervals, also regular additions of distilled water to make up the loss of water by transpiration. The test period was six weeks. The experiment was carried on in the greenhouse during parts of July and August, the range of temperature being 20°–32° C. The relative humidity of the greenhouse did not permit excessive transpiration. At the end of the test period, the plants were harvested, dried, and weighed. Table I gives the data obtained.

In taking up the data in detail, it may be noted that the cultures which had large and extensive roots (class *A*), when placed in the "good" nutrient solution no. 1, produced less than one-half as much total dry matter as did the cultures which were started with comparatively small roots (class *B*). The latter class of cultures at the end of the test period had the largest root growth, exceeding by more than 76 per cent that of the next largest. The cultures of class *B* are to be considered as normal plants when set out, the other class not. Even though class *A* had by far the larger roots when the test was started, it is obvious that these large roots did not operate as a means to secure as great a rate of growth for the plants, especially for the aerial portion, as was obtained by the plants started with relatively small roots having less surface exposed for absorption processes. On the other hand, it is not evident that the large root systems prevented the cultures of class *A* from attaining to the

TABLE I

EFFECT ON DRY MATTER PRODUCTION OF DIFFERENT ROOT SYSTEMS OF WHEAT SEEDLINGS GROWN IN DIFFERENT NUTRIENT SOLUTIONS (WEIGHT IN GM.)

	CLASS A Cultures having large root systems when placed in solution			CLASS B Cultures having small root systems when placed in solution		
	Tops	Roots	Total	Tops	Roots	Total
SOLUTION 1						
	1.22	0.42	1.64	3.00	0.73	3.73
	1.13	0.37	1.50	3.29	1.09	4.38
	1.35	0.40	1.75	2.60	0.53	3.13
	1.06	0.38	1.44	3.12	0.95	4.07
	1.38	0.48	1.86	2.95	0.70	3.65
	1.07	0.35	1.42	3.04	0.67	3.71
	1.41	0.55	1.96	3.02	0.62	3.64
	1.15	0.40	1.55	2.97	0.60	3.57
Average.........	1.26	0.42	1.64	3.00	0.74	3.74
SOLUTION 2						
	1.65	0.43	2.08	0.62	0.12	0.84
	1.42	0.35	1.77	0.60	0.10	0.70
	1.00	0.36	1.36	0.45	0.10	0.55
	1.49	0.37	1.86	0.61	0.12	0.73
	1.38	0.36	1.74	0.75	0.11	0.86
	1.10	0.39	1.49	0.55	0.09	0.64
	1.32	0.37	1.69	0.53	0.10	0.63
	1.10	0.39	1.49			
Average.........	1.30	0.38	1.68	0.59	0.11	0.70
SOLUTION 3						
	1.00	0.39	1.39	2.94	0.35	3.29
	0.98	0.35	1.33	2.96	0.35	3.31
	1.24	0.42	1.66	3.00	0.46	3.46
	1.61	0.48	2.09	2.40	0.27	2.67
	1.00	0.33	1.33	2.97	0.42	3.39
	1.07	0.30	1.37	*1.62	0.21	1.83
	1.05	0.30	1.35	2.50	0.32	2.82
	1.75	0.37	2.12	2.74	0.39	3.13
Average.........	1.21	0.37	1.58	2.64	0.35	2.99

* Two plants in this culture died.

measure of growth obtained by the cultures of class *B*. That the cultures of class *A* failed to give the measure of growth obtained by the cultures of class *B* must undoubtedly be attributed to some effects of the previous treatment, and of which the large roots in this case apparently may be considered but an incident. In this connection it can be argued that the cultures of class *A* were stunted and did not possess the same potential power or capacity for growth as did those of class *B*, and therefore regardless of any possible beneficial effect, if these large roots meant a greater surface for absorption, this could not compensate to overcome the stunted effects suffered by the plants. Undoubtedly the capacity of a plant to grow is affected by the rate of absorption of nutrients, and vice versa, the rate of absorption of nutrients is affected by the growth of the plants, so that the absorbing capacity of any comparable unit area of root surface must vary with conditions. It appears, therefore, that the data of the cultures grown in solution no. 1, taken by themselves, do not give any indication as to what effects the different root systems had in the results.

The results obtained from solution no. 2 are decidedly different. The seedlings having large roots, when placed in this "very poor" nutrient solution, produced about two and one-half times as much dry matter as did the other class of seedlings grown in this solution. The yield of the cultures of class *A* grown in solution no. 2 were approximately of the same magnitude as those grown in solution no. 1. The yield of the cultures of class *B* having comparatively small roots when placed in solution no. 1 was about five and one-half times larger than that of the corresponding cultures grown in solution no. 2. The explanation for the differences in growth obtained from the two classes of seedlings grown in solution no. 2 seems to be due to differences in the extent of the root systems these cultures had when placed in the media. It is quite obvious that the great difference in total dry weight obtained from the two classes of cultures grown in solution no. 2 is due to the very small growth made by the cultures with small roots, and not to any exceptionally good growth made by the cultures with large roots. The effect of solution no. 2 upon the one class of seedlings (class *B*) was to prevent its making such a measure of root growth as could be necessary to enable the plants to make even a moderate measure of top growth.

The injury to these seedlings was relatively great, therefore, but this was not the case with seedlings having large root growth.

It appears that there are several reasons that can be offered as an explanation for this relatively good growth obtained from the cultures with large roots grown in solution no. 2. The roots of these cultures, presumably because of their age, had much suberized tissue. This could inhibit the entry of excessive amounts of salts. It could also cause the precipitation of some of the salts in the root mass without doing injury to the plants, and, also in a selective way prevent or retard the absorption of toxic ions. On the other hand, this large root system was beneficial to the plant growth in this poor solution, in that it still permitted sufficient absorption of the essential ions. The greater surface exposed to absorption of nutrients, therefore, could compensate for the decrease in the rate of intake of essential nutrients per unit area of root surface.

Results obtained from solution no. 3 show that the plants having comparatively small root systems, when placed in the solution, produced approximately 90 per cent more dry weight than did the other class of cultures grown in this same kind of solution. The dry weight of the cultures of class *B* was also more than four times larger than that of the corresponding cultures of solution no. 2. The growth obtained from class *B* cultures in this solution (no. 3), in which $MgSO_4$ composed eight-tenths of the total salt concentration, must be considered as very good. Had growth conditions prevailed that would have induced a higher rate of transpiration, these cultures would not have attained to the value they held in this test. It is to be noted that these cultures had the lowest percentage of dry matter in the roots of all sets, being 11.7 per cent of the total growth obtained, and constituting a very low value for wheat plants six weeks old. One effect of this solution was to retard root growth in the cultures of class *B* as compared with the growth of top. Under conditions of excessive transpiration this condition would have acted harmfully to the plants. As it was, the cultures of class *B* having the smallest root systems as compared with the tops of the plants appeared to have been the most efficient, if ratio of root growth to top be taken as the criterion.

The dry weight of all the cultures having large root systems (class *A*) when placed in the three different kinds of solutions were of approximately equal value, having increased approximately five times in weight during the six weeks' period of growth. This test shows that these three solutions, markedly different in composition, must be considered as of equal value as media for the growth of wheat seedlings five weeks old having large root systems, when placed in the solutions and grown for six weeks. These same solutions, however, must also be considered markedly different physiologically, when the test plants are wheat seedlings 6–8 cm. high with small roots 8–10 cm. long.

All the cultures with large roots may be considered as having been injured by the treatment of the first five weeks' growth in tap water, because they fell short in attaining the maximum growth rate obtained by the cultures with small roots (class *B*) in solution no. 1. Whether any treatment that can induce large root growth of a wheat plant, either at the expense of top growth or not, can subsequently be made to operate as a means to secure a greater growth rate for the plant as a whole, because of a greater root surface exposed for absorption, needs further investigation. It is probable that in the present experiment the treatment to obtain large roots was too severe, and that exposure for a less time to the conditions by which large roots were obtained would have given a larger measure of growth when set into these solutions than was obtained.

The extent of the root system appears as an important factor that affects the magnitude of growth obtainable from a given nutrient solution. It is conceivable, therefore, that extent of the root systems of plants plays an important rôle when plants are grown in the field. That some plants are more resistant than others to certain untoward conditions, such as excessive amounts of salts in alkali regions, may not be due to any peculiar genetic factor of the plant, but simply be the response from differences in root development occasioned by certain conditions in the environment in the field. The common observance in the field of a greater tolerance for salts of older plants than young ones apparently can well be accounted for in their root systems. This, however, does not mean that differences in extent of root systems any given kind of plant may have are due only to causes operative in the external environment. Differences in root systems may also be due to genetic factors.

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A NEW FRUIT ROT OF TOMATOES[1]

R. FRANK POOLE

(WITH PLATE VII)

During the summer of 1921 a fungus growth following cracking of the fruit was noted on tomatoes in several localities of Burlington and other counties in New Jersey (fig. 1). The cracking was obvious on both green and ripe fruit of the Stone, Baltimore, and Bonny Best varieties, but was especially prominent and severe on the latter. The cracking is apparently due to one or more physiological causes. An examination showed a very dense fluffy growth of *Oidium lactis* Fres. in the open cracks of ripe fruit lying on the ground and those hanging on the plant to a height of several inches above the soil. This fungus, under field conditions, penetrated the interior of the tomato, and reduced the fruit to a soft rotten mass in from two to five days. The disease was very common throughout the tomato ripening period in the fields under observation. No infection was noted on uninjured ripe fruit, cracked green fruit, or other parts of the plant.

CAUSE.—The rot is due to *Oidium* or *Oospora lactis*. The fungus is repeatedly isolated from infected tomatoes. It causes rapid decay of ripe fruit at 20° C. in a moist chamber. The mold is grayish white, fluffy, and dense (fig. 2). The mycelial growth is more important than spore production. If, however, a diseased tomato be broken open and spread out in a moist chamber for twenty-four hours at 20° C. (fig. 3), the fungus appears very similar in growth to the *Saccharomyces*. In this form the spore production is abundant, while the mycelial growth is subdued. These two factors of mycelium and spore production may be considered as distinguishable characteristics of this fungus from other fruit rot organisms. The fungus grows abundantly on a large variety of culture media. Its only known method of reproduction is non-

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[1] Paper no. 60 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Plant Pathology.

sexual, typically by means of conidial spore chains. The spores are hyaline, round or oval, and smooth. They vary in size from 2 to 6 $\mu \times 6$ to 40 μ (fig. 5).

SYMPTOMS.—The fungus causes a typical soft rot of injured, ripe tomato fruits. In some cases there is a fermentation action, due to the fungus, which causes the cracks to widen and the juice to flow out (fig. 2). The inner tissue is destroyed, while the peeling is not noticeably attacked, but dries out and remains on the field as a dry, hard shell. In advanced decay the symptoms are not easily distinguished from those of a bacterial soft rot of ripe tomato fruit. The odor is at first agreeable, but becomes very offensive before decay is complete. Decay of cracked green fruit was not observed in the field, but the fungus caused slow decay (fig. 4) in fruit that had begun to ripen.

INOCULATIONS.—Ripe, semiripe, and green tomatoes were placed in running water for an hour, treated fifteen minutes with bichloride of mercury, washed with sterilized water, and placed in dry sterilized glass chambers prepared for inoculation. No infection was obtained by spraying spores on uninjured fruit. The fungus caused rapid decay of sliced ripe tomatoes in four to six days at room temperature, 18–20° C. (fig. 4*a*). It grows very slowly on green or semiripe fruit (fig. 4*b*).

Spores of *Oidium* or *Oospora lactis* were introduced into the solid ripe tomatoes by means of a platinum needle. In forty-eight hours at room temperature there was good growth in all places where inoculated (fig. 2*b*). The growth was abundant for a similar period on deep slices made in the tomato (fig. 2*a*). Ripe tomatoes which were punctured but not inoculated did not become infected in the same chambers where other tomatoes were inoculated (fig. 2*c*).

DISTRIBUTION, PREVALENCE, LOSS.—While no definite data are available to show accurately the distribution of the fungus, THOM[2] states: "The mold variously known as *Oidium* or *Oospora lactis* is another cosmopolitan organism. The same or almost indistinguishable forms are found upon decaying vegetables and fruits, which may give reason for the statement that the odor produced of

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[2] THOM, CHAS., Fungi on cheese ripening Camembert and Roquefort. Bur. Animal Ind. Bull. 82. 1–40. *figs. 3.* 1906.

Oidium is that of rotten cabbage." There is considerable literature, too extensive to mention here, dealing with *Oidium lactis* in milk products, particularly the relation of the mold to the flavor of Camembert cheese. Very little of this literature deals with the fungus in relation to plant products. PEROTTI and CRISTOFALETTI,[3] however, have briefly reported the fungus as a parasite appearing in spots on tomatoes in Italy. They suggested that the fungus be called *Oidium lactis solani*.

The largest losses were noted near Moorestown, Burlington County. The disease was prevalent in other localities, and losses were more or less regular over the entire tomato growing area in localities where the disease was observed. While the loss was not serious at any one period, there was a rather high loss for the season.

TABLE I

BONNY BEST TOMATOES

SPRAY TREATMENTS	AUGUST 12			SEPTEMBER 7		
	Examined	Diseased	Percentage diseased	Examined	Diseased	Percentage diseased
1. 4-4-50	171	21	12.2	140	25	17.8
2. Check	95	17	17.7	64	13	20.3
3. 4-4-50	102	12	11.7	120	21	17.5
4. Check	120	19	15.8	65	17	26.1

Data were collected in tomato fields of the so-called second early Bonny Best tomatoes, August 12 and September 7, and from a late crop of Baltimores, September 19. This was during the maximum ripening period of each crop. The data were obtained by counting the total large fruits on ten average plants and also the number that were diseased. It will be noted in table I that the disease was slightly higher on September 7 in the Bonny Best tomatoes than it was on August 12. It is very probable that the conditions favoring infection were more prevalent September 7 than August 12.

The percentages of disease in table II were taken from four series of 4–7 applications inclusive of four liquid spray and dust

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[3] PEROTTI, R., and CRISTOFOLETTI, U., A fruit spot of tomato. Staz. Sper. Agric. Ital. 47:169–216. *pls. 3. figs. 9.* 1914.

treatments. It appears from the data given in this table that the disease was checked with wet Bordeaux and dust treatments. Such an interpretation is no doubt correct, but the difference of control on treated and untreated plots is not entirely due to treatments. There were slightly more diseased tomatoes on the untreated plots than were formed on the treated plots. The total yield was less on the untreated plots, because much of the fruit had prematurely ripened and was picked or had fallen. The calculation of the percentages of the disease on the untreated plots with a smaller total of fruit on the ten check plants than was obtained from ten plants on the treated plots, therefore, has resulted in a slightly increased percentage of disease in the check over the true percentage of control. It will be noted in table II, however, that there is also a difference of control in the four treatments, which indicates that some slight true control was obtained.

TABLE II

Wet spray and dust treatments	Series 1		Series 2		Series 3		Series 4	
	No. examined on 10 plants	Seven applications, percentage diseased Sept. 19	No. examined on 10 plants	Six applications, Percentage diseased, Sept. 19	No. examined on 10 plants	Five applications, percentage diseased Sept. 19	No. examined on 10 plants	Four applications, percentage diseased Sept. 19
1. Check	166	4.2	110	11.8	123	10.5	130	2.3
2. ½-0-3-50	192	1.5	142	2.8	116	9.0	168	2.4
3. 4-4-50	211	2.9	124	0.8	193	2.0	184	3.2
4. Dust	211	1.9	128	1.5	193	2.0	236	.88
5. 4-3-1½-50	193	1.5	140	0.7	134	4.5	211	.95
6. Check	154	3.6	90	8.8	159	6.3	180	3.3
7. ½-0-3-50	157	2.5	83	4.8	164	3.6	140	2.8
8. 4-4-50	142	1.4	126	2.4	209	0	175	1.7
9. Dust	169	1.2	138	1.5	154	1.3	185	2.1
10. 4-3-1½-50	146	0.7	129	0.8	198	9.6	200	0.5

The treatments were planned in connection with the investigation of *Septoria lycopersici* control. The third number in the wet Bordeaux spray represents fish oil soap. The dust was composed of 16 pounds anhydrous copper sulphate, 6 pounds lead arsenate, and 78 pounds of hydrated lime. The wet treatments were applied with a 3-row, 3-nozzle traction spray machine, while the dusts were applied with a hand duster.

Summary

1. A cracking of green and ripe fruit of Bonny Best, Baltimore, and Stone tomatoes in Burlington and other counties of New Jersey, due to one or more physiological causes, was observed to be severe in 1921.

2. *Oidium* or *Oospora lactis* was isolated from infected tomatoes. Inoculations of ripe fruit with this fungus were positive.

3. *Oidium* or *Oospora lactis* is a widely distributed fungus. It is known to appear in milk products, cheeses, decaying vegetables, and fruits. On tomato fruit the fungus mycelium is dense, grayish white, and prominent, while in other cases spores are very prominently produced.

4. The treatments with wet Bordeaux sprays and dusts gave slight control of the disease.

Appreciation is expressed for the helpful suggestions offered by Dr. MEL. T. COOK, and for the identification of the organism by Miss ANNA E. JENKINS, Office Pathological Collections, Washington, D.C.

AGRICULTURAL EXPERIMENT STATION
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EXPLANATION OF PLATE VII

FIG. 1.—Cracked ripe and green Bonny Best tomatoes, showing various forms of cracking.

FIG. 2.—Ripe tomatoes inoculated with *Oidium lactis* (after 48 hours' growth in partially dry chamber, 18°–20° C.): *a*, in fresh slices; *b*, punctures; *c*, no inoculation but punctured.

FIG. 3.—Diseased ripe tomatoes spread open in moist chamber for 24 hours at 20° C.; white fungus prominent on open material.

FIG. 4.—Sliced ripe and green tomatoes inoculated with *Oidium lactis* (from 4 to 6 days' growth 18°–20° C. in partially dry chambers): *a*, on sliced ripe fruit; *b*, on green to half ripe fruit; *c*, no inoculation on sliced ripe tomato.

FIG. 5.—*Oidium* or *Oospora lactis*: *a* and *d*, mycelial branches and spores; *b*, spores of various sizes in chains; *c*, spores; *e*, spores in budding-like formation.


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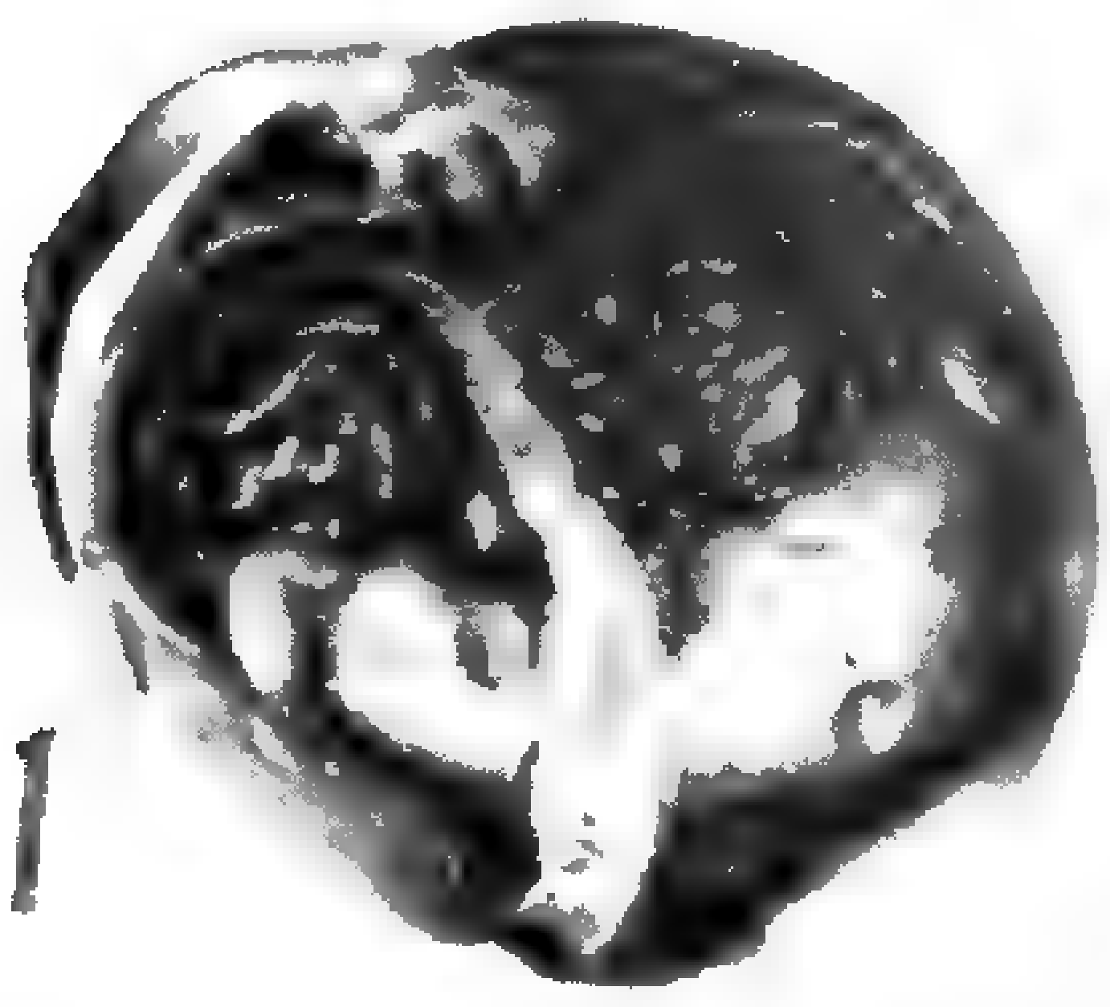
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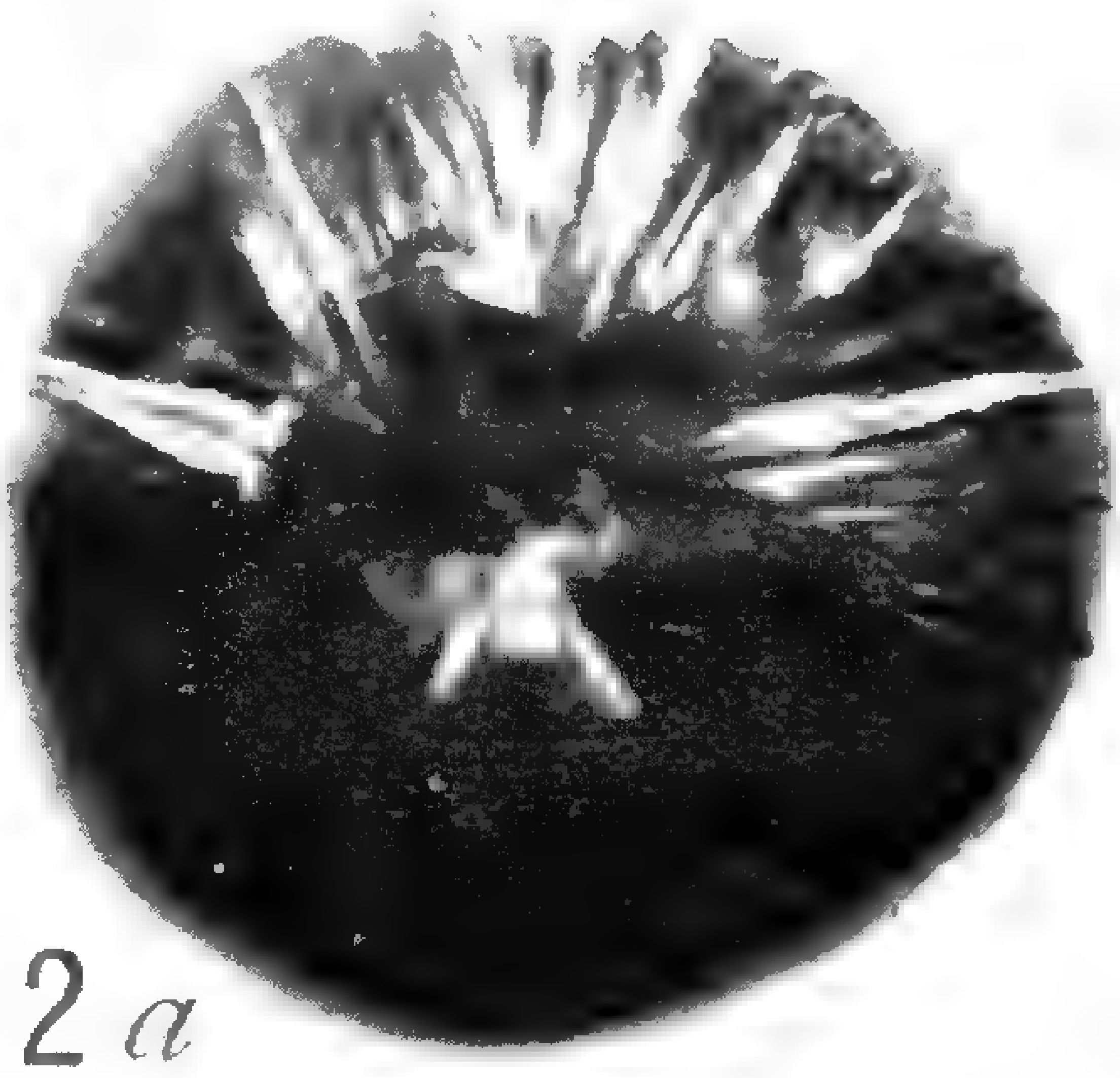
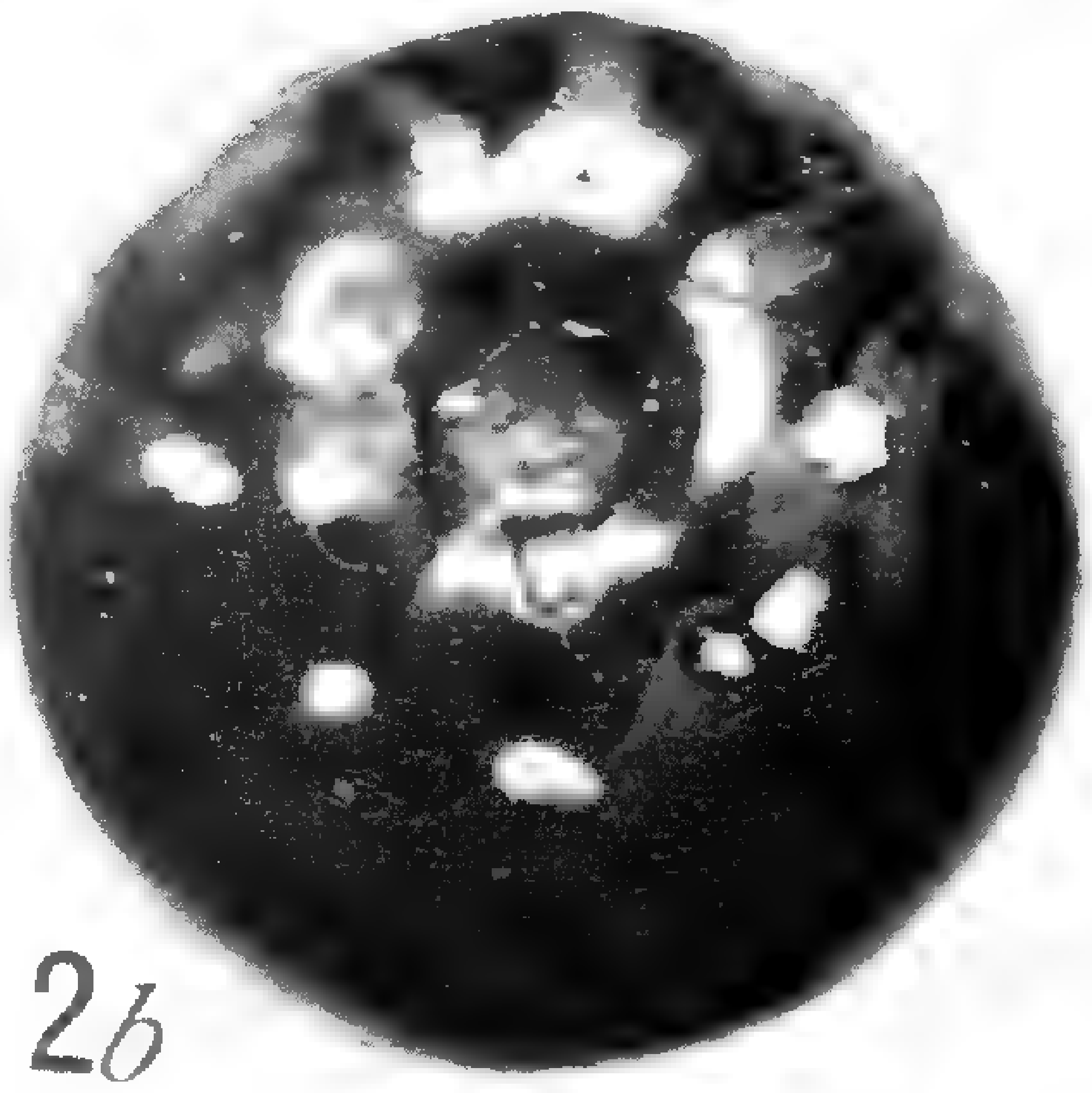
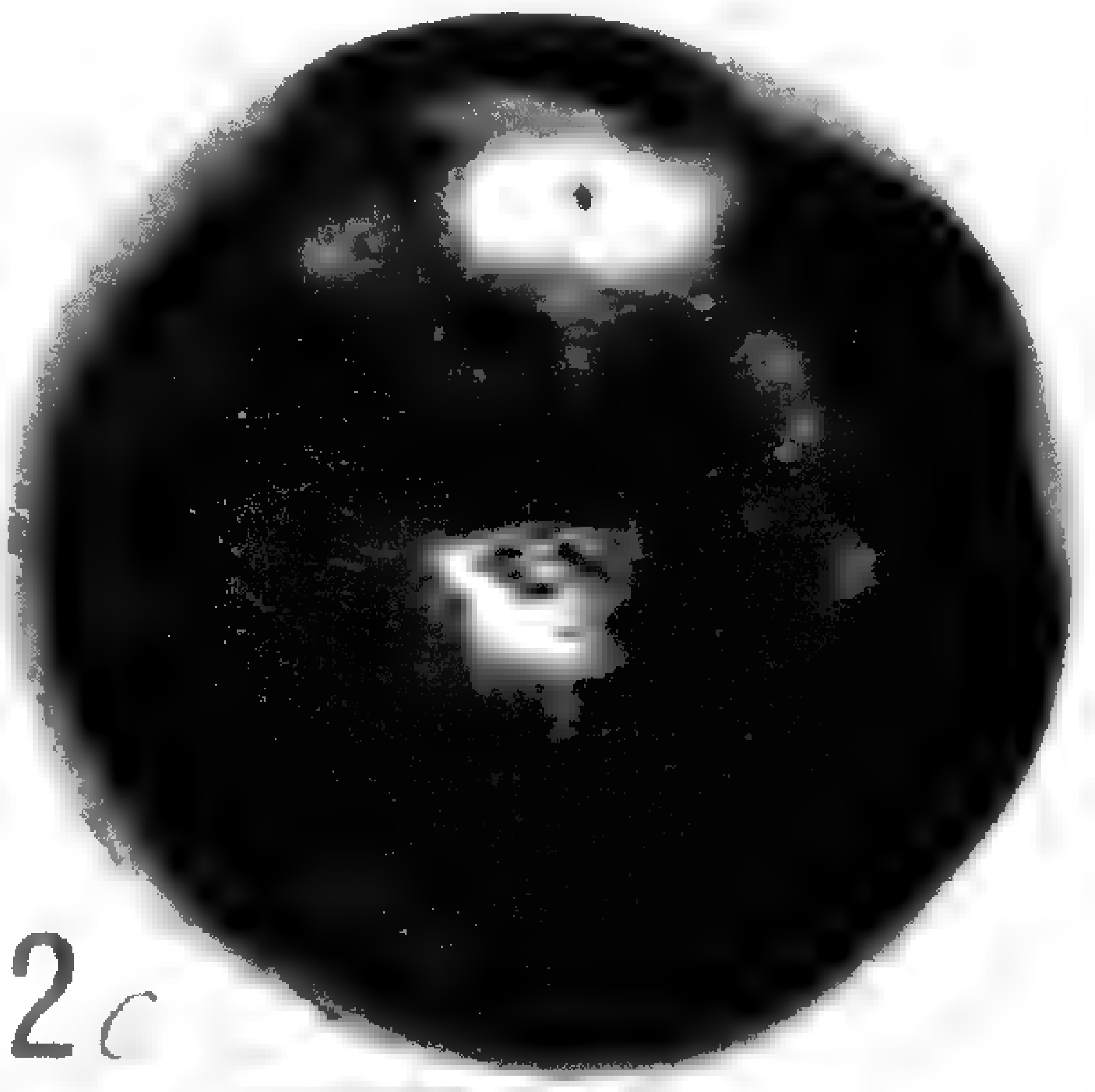
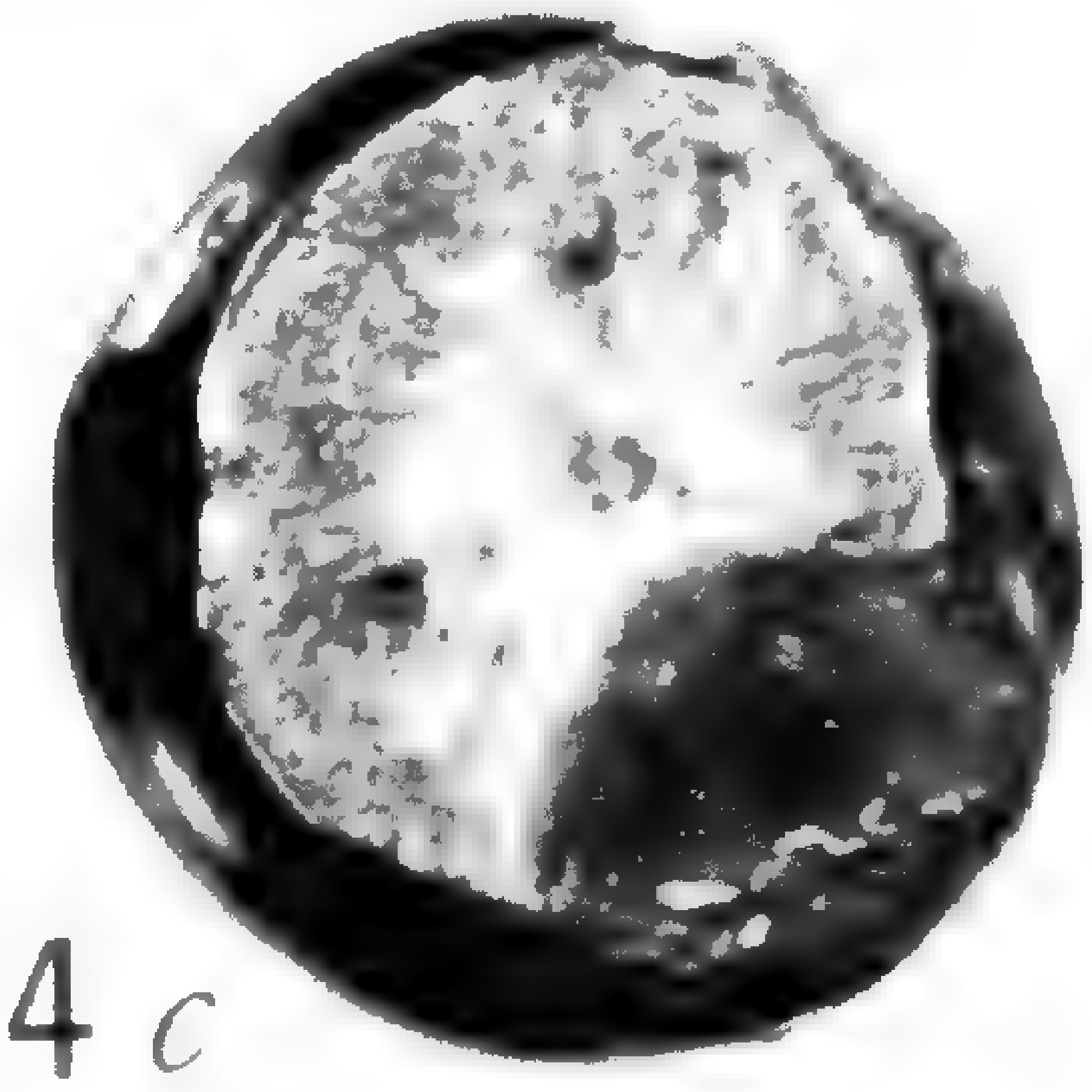

2 *a*

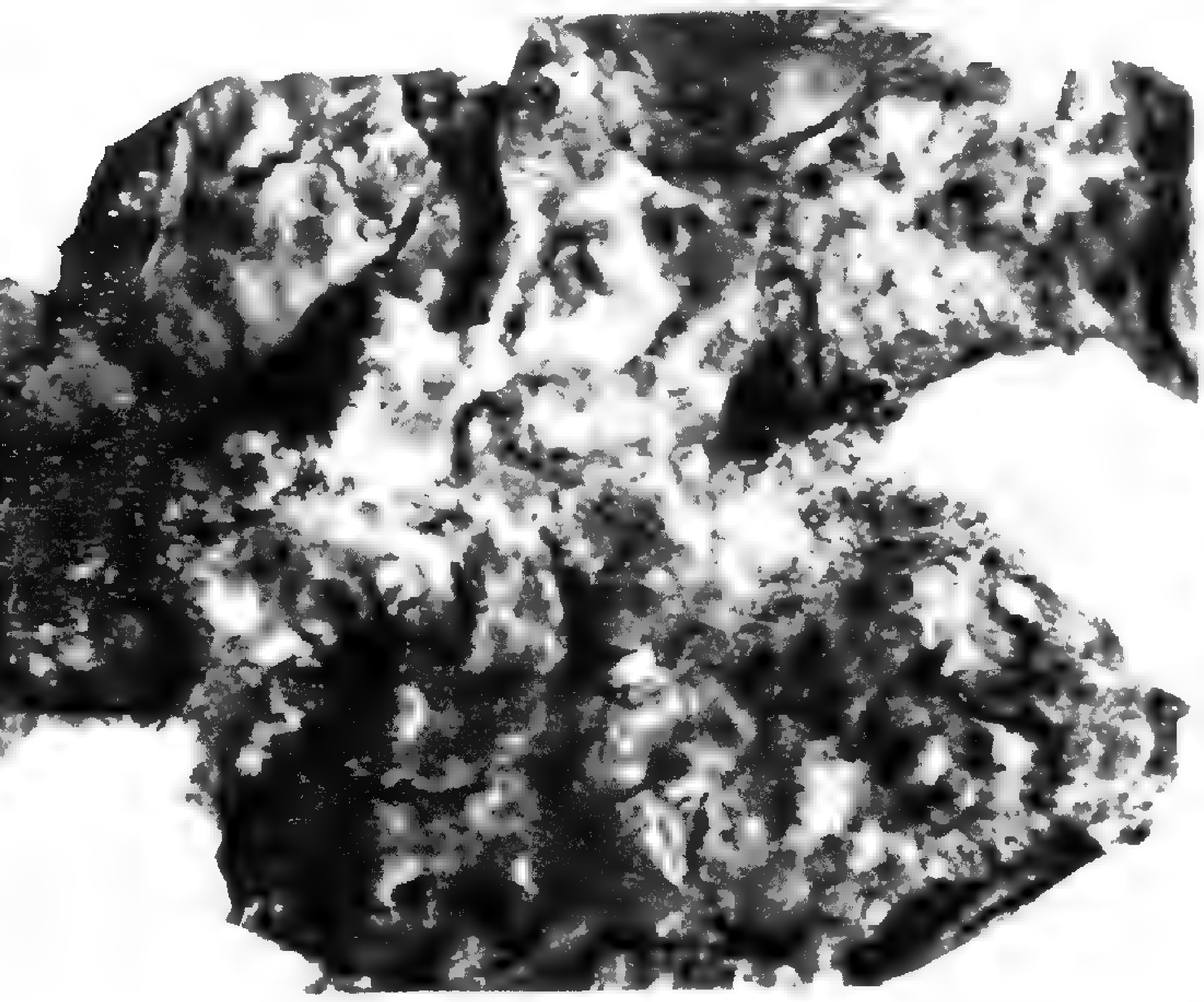

2*b*


2*c*


4*a*


4 *b*


4 *c*


3


5

POOLE on FRUIT ROT

EFFECT OF SEEDS UPON HYDROGEN-ION CONCENTRATION OF SOLUTIONS[1]

W. Rudolfs

In the course of a study on the effect of single salt solutions with definite osmotic concentration values upon absorption by seeds[2], it was found that the H-ion concentrations of the solutions in which the seeds were immersed changed markedly during the process of imbibition. The general interest and importance of this phenomenon in connection with seed studies made its further investigation highly desirable. Accordingly single salt solutions of magnesium sulphate, sodium nitrate, calcium nitrate, sodium chloride, potassium chloride, and potassium carbonate were prepared, ranging in osmotic concentration values from 0.001 of an atmosphere to 7.0 atmospheres. The seeds used in connection with these solutions were corn, spring wheat, white lupine, watermelon, Canada field peas, Japanese buckwheat, dwarf Essex rape, and alfalfa. Fifty seeds of each of the larger kind and 100 each of alfalfa and rape were placed in small bottles each containing 100 cc. of solution. The bottles were placed on a laboratory table, and the seeds were allowed to soak for a period of fifteen hours, after which the solutions were poured off and the H-ion concentrations determined by the colorimetric method, using the double tube standards of Gillespie[3] and the apparatus devised by Van Alstine.[4]

The results of these determinations are given in tables I and II in terms of P_H values. The initial P_H values of the solutions are here compared with the final values determined at the end of

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[1] Paper no. 83 of the Journal Series, New Jersey Agricultural Experiment Station, Department of Plant Physiology.

[2] Rudolfs, W., Effect of salt solutions having definite osmotic concentration values upon absorption by seeds. Soil Science **11**:277–293. 1921.

[3] Gillespie, L. J., Colorimetric determination of hydrogen-ion concentration without buffer mixtures, with especial reference to soils. Soil Science **9**:115–136. 1920.

[4] Van Alstine, E., The determination of hydrogen-ion concentration by the colorimetric method and an apparatus for rapid and accurate work. Soil Science **10**:467–478. 1920.

TABLE I

INITIAL AND FINAL P_H VALUES OF SINGLE SALT SOLUTIONS IN WHICH SEEDS WERE SOAKED FOR FIFTEEN HOURS

P_H values		Atmospheres											
		7	6	5	4	3	2	1	0.5	0.1	0.01	0.001	0.00
		$MgSo_4$											
Initial		6.5	6.5	6.4	6.4	6.3	6.3	6.2	6.2	6.1	6.0	6.0	6.0
After 15 hours	Corn	4.1	4.1	4.0	3.9	3.9	3.9	3.9	3.9	3.9	4.4	4.9	5.6
	Wheat	4.8	5.0	5.0	5.0	4.9	4.8	4.9	4.8	4.7	4.7	4.9	5.5
	Watermelon	4.9	5.0	5.0	5.0	5.0	5.0	5.0	4.9	4.8	5.1	5.8	5.8
	Peas	4.6	4.6	4.6	4.6	4.9	4.9	4.9	4.7	5.7	5.9	5.9	5.9
	Buckwheat	5.2	5.2	5.2	5.2	5.2	5.3	5.4	5.4	5.6	5.9	5.9	5.9
		$NaNo_3$											
Initial		6.6	6.5	6.5	6.4	6.4	6.3	6.2	6.1	6.0	5.9	5.9	5.9
After 15 hours	Lupine	5.7	5.7	5.7	5.6	5.7	5.7	5.7	5.7	5.8	5.7	5.7	5.9
	Rape	5.5	5.5	5.6	5.5	5.5	5.7	5.7	5.7	5.7	5.8	5.8	5.8
	Alfalfa	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.7	5.7	5.7	5.8
	Corn	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.3	4.7	4.9	5.2	5.5
		$Ca(No_3)_2$											
Initial		7.0	6.9	6.8	6.7	6.7	6.6	6.5	6.4	6.3	6.2	6.1	6.0
After 15 hours	Corn	4.2	4.2	4.2	4.2	4.1	4.1	4.1	4.1	4.5	5.5	5.5	5.9
	Lupine	5.5	5.5	5.6	5.6	5.6	4.9	4.9	4.8	5.2	5.6	5.5	5.8
	Peas	5.2	5.2	5.2	4.9	4.7	4.3	4.2	4.2	4.2	4.3	4.4	5.6
	Watermelon	4.2	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.2	4.9	5.1	6.0
		NaCl											
Initial		6.6	6.5	6.4	6.4	6.4	6.3	6.3	6.2	6.2	6.1	5.9	6.0
After 15 hours	Corn	4.1	4.2	4.1	4.1	4.1	4.2	4.2	4.3	4.7	5.1	5.1	5.7
	Wheat	4.9	5.1	5.1	5.1	5.0	5.1	5.0	4.9	4.9	4.9	5.3	5.9
	Rape	4.1	3.9	4.1	3.9	3.8	4.0	3.7	3.9	3.9	4.1	4.1	5.9
		KCl											
Initial		6.5	6.4	6.4	6.4	6.3	6.2	6.2	6.1	6.1	6.0	5.9	5.9
After 15 hours	Corn	4.1	4.1	4.1	4.1	4.1	4.1	4.2	4.3	4.7	5.0	5.1	5.9
	Wheat	5.9	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.6	5.8	5.8	5.8
	Lupine	5.7	5.7	5.6	5.7	5.7	5.7	5.8	5.9	5.9	5.9	5.9	5.9
	Peas	5.6	5.6	5.6	5.6	5.6	5.7	5.7	5.7	5.7	5.8	5.7	5.9
	Buckwheat	5.1	5.3	5.4	5.3	5.3	5.4	5.6	5.6	5.6	5.7	5.7	5.9
		K_2Co_3											
Initial		+*	+	+	+	+	+	+	+	+	8.4	5.9	5.9
After 15 hours	Lupine	7.5	7.5	7.5	7.5	7.5	6.7	6.7	6.7	5.5	5.9	5.9	5.9
	Corn	+	+	+	+	+	9.6	9.2	8.7	6.5	5.8	5.7	5.7
	Rape	+	+	+	9.4	8.8	8.2	7.5	7.5	6.2	5.7	5.5	5.9

* + = higher than 9.8.

definite time intervals, during which the seeds remained in contact with the solutions. Each of the data given represents the average of the results obtained from two or more trials made with the same number of seeds soaked in equal amounts of solution for time periods of the same duration.

From the data of table I it will be observed that in all cases the H-ion concentrations of the solutions were markedly increased by contact with the seeds, even when strongly alkaline solutions of potassium carbonate were used. The seeds immersed in solutions of different concentrations of a single salt had a tendency to bring the P_H values of the solutions to a point which was fairly constant for a given species of seed, regardless of the original salt concentration or of the initial P_H values of the solutions, except when the solutions were very dilute. The maximum reaction change produced by corn in the various concentrations of all the salts used, except potassium carbonate, brought the final P_H values of the solutions to approximately 4.1, varying only slightly above or below this value except in the very dilute solutions (0.01 and 0.001 atm.) as previously noted. While the final P_H values produced by the seeds of other species were approximately constant in the different concentrations of the same salt, they varied considerably in the solutions of the different salts. For example, the final P_H values produced by wheat in the magnesium sulphate solutions were around 4.9, in the potassium chloride solutions they were approximately 5.7. Watermelon seeds produced corresponding P_H values of approximately 5.0 in the magnesium sulphate solutions and 4.1 in calcium nitrate solutions. Seeds of other species produced similar differences in the P_H values of the solutions of the different salts.

It will be observed that the initial H-ion concentration of the solutions of each salt increased slightly with the progressive decrease in the total salt concentration, but the P_H values of the solutions after soaking the seeds in them for fifteen hours showed a striking similarity in value, except in the very dilute solutions. With a few exceptions the seeds were incapable of bringing about any marked reaction changes in the very dilute solutions (0.01–0.001 atm.), behaving in these solutions in somewhat the same manner with respect to changes in reaction as they did in distilled water.

The effect of the seeds upon the H-ion concentration of the solutions in which they were immersed is strikingly shown in the case of the potassium carbonate solutions. The P_H values of these solutions were well above 9.8. After fifteen hours' immersion, lupine seeds brought the P_H values of these strongly alkaline solutions below the neutral point in concentrations below three atmospheres, and to 7.5 in all the higher concentrations. Both corn and rape seeds immersed in the solutions of low concentration of this salt also increased the H-ion concentration to such an extent as to bring the P_H values below the neutral point.

TABLE II

SEEDS IMMERSED IN $MgSO_4$ FOR DIFFERENT PERIODS, RINSED IN DISTILLED WATER, AND THEN PLACED IN FRESH SOLUTIONS CORRESPONDING TO THE OLD

P_H values	Atmospheres											
	7	6	5	4	3	2	1	0.5	0.1	0.01	0.001	0.00
	Corn											
Initial	6·5	6·5	6·4	6·4	6·3	6·3	6·2	6·2	6·1	6·1	6·0	6·0
After 3 hours	4·1	4·1	4·2	4·2	4·1	4·1	4·1	4·1	4·0	4·1	4·9	5·9
After 5 hours	4·2	4·2	4·2	4·2	4·1	4·1	4·1	4·1	4·0	4·1	5·1	5·9
After 8 hours	4·1	4·1	4·1	4·1	4·1	4·0	3·9	3·9	3·9	4·0	4·9	5·9
After 15 hours	4·1	4·1	4·0	3·9	3·9	3·9	3·9	3·9	3·9	4·1	4·9	5·9
After 18 hours	4·1	4·1	4·0	3·9	3·9	3·9	3·9	3·8	4·1	4·2	5·0	5·7
After 21 hours	4·1	4·1	3·9	3·9	3·9	3·9	3·9	3·9	3·9	4·1	4·9	5·6
	Rinsed and placed in fresh solution											
Initial	6·6	6·5	6·5	6·4	6·4	6·3	6·2	6·2	6·1	6·1	6·0	6·0
After 1 hour	4·3	4·2	4·1	4·1	4·2	4·1	4·1	4·1	4·1	4·2	4·9	5·9
After 21 hours	4·1	4·1	4·1	4·1	4·1	4·1	4·1	4·1	4·1	4·2	4·9	5·7
After 40 hours	4·1	4·0	4·0	4·1	4·0	4·0	4·0	4·0	4·0	4·2	4·9	5·7
	Buckwheat											
Initial	6·5	6·5	6·4	6·4	6·4	6·3	6·3	6·2	6·1	6·1	6·0	6·0
After 15 hours	5·2	5·3	5·2	5·3	5·2	5·3	5·4	5·4	5·6	5·9	5·9	5·9
After 21 hours	5·2	4·9	4·9	4·9	4·7	4·9	4·9	4·9	5·1	5·3	5·5	5·7
After 40 hours	5·3	5·6	5·2	5·4	5·1	5·1	5·3	5·4	5·3	5·5	5·7	5·8
	Rinsed and placed in fresh solution											
Initial	6·6	6·5	6·5	6·4	6·4	6·3	6·2	6·2	6·1	6·1	6·0	6·0
After 1 hour	5·1	4·9	4·9	5·0	4·9	4·9	4·9	4·9	4·9	5·1	5·5	5·9
After 5 hours	4·9	4·9	4·9	4·9	4·9	4·9	4·9	4·9	5·1	5·1	5·5	5·9
After 15 hours	5·0	5·0	5·1	5·0	5·0	5·0	5·1	5·0	5·0	5·5	5·5	5·8

The maximum reaction change which the seeds were capable of bringing about in the small quantities of solution here used (100 cc.) was accomplished in a comparatively brief period of time, as is shown by the data in table II. Fifty corn seeds or fifty buckwheat seeds immersed in 100 cc. of magnesium sulphate solution, with osmotic concentration values of 7.0 atmospheres or less, produced the maximum reaction changes of which they were capable during an interval of one hour or less. The rate of reaction change of course is determined by the amount of solution used, total salt concentration, the number of immersed seeds, temperature, etc.

After the maximum reaction changes which the seeds were capable of bringing about in the solutions had been attained, the P_H values then remained approximately constant for an indefinite period. When the seeds were removed from the old solutions, rinsed with distilled water for a few seconds, and placed in fresh solutions with corresponding concentrations of the same salt, the phenomenon of reaction change again took place and continued until the maximum H-ion concentration was the same as that previously produced. The final P_H values with corn in magnesium sulphate solutions were 3.9 to 4.2, except in the very dilute solutions, always varying slightly on account of differences in temperature and other environmental factors. The seeds may thus be immersed several times successively in fresh solutions, the reaction changes taking place each time, but always bringing the final P_H values to approximately the same point, which is fairly definite for each species in the solutions of a given salt, until finally the absorptive capacity of the seeds is exhausted, and equilibrium is established between seeds and solution.

The exact cause or causes of the rapid reaction change of the solutions as indicated by changes in the P_H values has not been determined with absolute certainty. There are without doubt several contributing factors, but all the experimental evidence thus far produced appears to indicate that the primary factor, and the only one which could account for the rapid reaction changes in the single salt solutions here used, is that directly related to ion absorption by the seeds, the H-ion concentration increasing as the cations are removed from solution by absorption at a more rapid rate than the anions. This is in accord with the work of PANTANELLI,[5] who concludes from his extensive researches that salt intake by the cells of living plants is an absorption phenomenon of single ions, and he attributes the reaction changes of solutions in contact with the roots of green plants to the fact that some ions are absorbed at a more rapid rate than others.

That the materials excreted by the seeds in contact with the solutions here used can have little influence in bringing about the rapid increase in H-ion concentration, is indicated by the fact that seeds immersed in distilled water, under conditions similar to those under which they were immersed in the single salt solutions, did not bring about any marked reaction changes, even when the seeds were in contact with the solutions during a period of forty hours. This is shown by the data in the last columns of tables I and II.

The rates at which these reaction changes take place and the factors influencing them, together with a study of salt solutions of a wider range and of a number of mineral and organic acids will be the subject of a later report.

LABORATORY OF PLANT PHYSIOLOGY
N.J. AGRICULTURAL EXPERIMENT STATIONS
NEW BRUNSWICK, N.J.

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[5] PANTANELLI, E., Über Ionenaufnahme. Pringsheim's Jahrb. Wiss. Bot. **56:** 689–733. 1915.

CURRENT LITERATURE

BOOK REVIEWS

Biology of aquatic plants

The writer of this interesting volume[1] has rendered a service to botanists in summarizing and bringing together the extensive but widely scattered work that has been done on aquatic seed plants. Mrs. ARBER'S work, however, is far more than a compilation, for every chapter contains interpretive portions, and in many of them the summary of the literature is but a scaffolding for further records or conclusions. Certain chapters are greatly enriched by the author's own experimental and philosophical conclusions.

Members of this biological group are of special interest because of their double specializations, first as terrestrial plants that have achieved flowers and seeds, and secondly because of the more or less marked modification of structure and reproductive method as now related to the aquatic environment. The volume will be of interest to all botanists, for the subject matter is ably grouped around fundamental biological topics, many of them most favorably illustrated by aquatic Angiosperms.

Part I deals with water plants as a whole, and takes up typical life histories, starting with a biological classification of hydrophytes in relation to water and substratum. The author then proceeds to discuss the vegetative structure of typical aquatics, including the marine Angiosperms, which are discussed in the concluding chapter of this section. In Part II the anatomy of leaves, stems, and roots is considered, together with modifications of the external form of these organs. The development of turions, or winter buds, is discussed, and the later portion of this section takes up flowers, pollination, fruits, and seed dispersal. Part III presents an analysis of the physiological conditions of the water habitat and the application of these to aquatic plants. This section is concluded by a chapter on the ecology of hydrophytes, which is conservatively handled, a topic which in the hands of some might perhaps have given title to the book. Part IV is concerned with philosophical discussions dealing with the dispersal and geographic distribution of water plants, their possible origin, and their relations to the theory of natural selection. The concluding chapter involves a summary of the author's work on the phyllode theory.

The volume is attractively printed and illustrated by 171 text figures, one-third of them original. The bibliography occupies seventy-two pages, and is made fully available by means of an appended index to the genera and

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[1] ARBER, AGNES, Water plants, a study of aquatic Angiosperms. 8vo. pp. xvi+436. *figs. 171.* Cambridge University Press, England. 1920.

families of plants named in the literature. This is in addition to the general index for the volume as a whole.—R. B. WYLIE.

Chemistry of vegetable cell

A valuable service has been rendered to students of plant physiology by GRAFE,[2] who has prepared a textbook dealing with the biophysics and biochemistry of the processes of cell life in plants. The work was ready for publication when the war broke out, and has been delayed almost eight years by the conditions in Europe. This does not mean that the text is already old, for the delay has permitted a revision since the war, and much new material has been incorporated, particularly that which has been the result of discoveries in the laboratories of Europe. Any failure to note foreign results is excused by GRAFE on the grounds of the great difficulty experienced in keeping in touch with the scientific production of other nations.

The subject is presented under five main headings: the physicochemical laws of cell phenomena; light and heat as energy factors; the cell wall; the protoplasm; and dynamic chemistry. The first two sections occupy about a fourth of the text, and survey the problems of diffusion and osmosis, colloidal state, imbibition, adsorption, electrolytic dissociation, energy transformations, and catalysis. The third section is the briefest one, and deals with the structure, composition, and chemical transformations and modifications which occur in the walls of cells during development and maturation.

The section on protoplasm considers its colloidal structure, chemical constitution, the enzymes, toxins, precipitins, and pigments of the cell, particularly the chlorophyll and anthocyanin pigments. The final section is the longest and most important one, and considers the chemical transformations of cell activity. The author reverses the usual order of presentation by considering the utilization of energy and the respiratory processes first, then a brief consideration of stimulus and response, closing with the constructive energy-storing processes.

The book is intended as a general text for students, and the author has written in a style that is commendable for its clarity and directness. The literature list at the end of the volume occupies twenty double column pages, and the total number of references is nearly a thousand. Some of these references are not considered in the text of the volume, but are included for those who desire to orient themselves with reference to the literature on cell chemistry.—CHAS. A. SHULL.

Poisonous plants

A textbook dealing with the poisonous plants and weed seeds of Canada and the Northern United States has been prepared by THOMSON and SIFTON.[3]

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[2] GRAFE, VICTOR, Chemie der Pflanzenzelle. 8vo. pp. viii+420. *figs. 32.* Berlin: Borntraeger. 1922.

[3] THOMSON, R. B., and SIFTON, H. B., A guide to the poisonous plants and weed seeds of Canada and the northern United States. 8vo. pp. 169. *figs. 40.* University of Toronto Press. 1922.

It is intended to meet the needs of the veterinarian, the farmer, the stockman, and to a more limited extent the physician and the general public. The high standing of the authors and the fact that they are botanists insure accuracy so far as the plants are concerned.

The book is divided into four sections, the first three dealing with the plants mainly responsible for fatalities among animals, grouped on the basis of their occurrence in the animal's feed, whether found in hay (section 1), in pasture (section 2), or in concentrated feed stuffs (section 3). The fourth section deals with the plants which, although poisonous, seldom cause the death of animals. The larger number of poisonous plants, including those mainly responsible for poisoning in man, belong here. The forty illustrations are original and will enable the amateur to identify the plant responsible in an ordinary case of poisoning. There is a "symptoms" key, not claiming to be precise, but which will facilitate diagnoses by suggesting the plants which should be looked for when certain symptoms are observed and plant poisoning is suspected.

The authors emphasize the need for research on poisonous plants, both along the lines of chemical analysis and feeding experiments. The book is interesting and well written, and many of the observations, which could have been made only by experienced botanists, indicate the propriety of issuing such a book from a botanical department.—C. J. CHAMBERLAIN.

Constitution of vascular plants

CHAUVEAUD,[4] in a small volume, has presented his views as to the constitution of vascular plants, based upon an investigation of ontogeny. He calls attention to the disagreement between current theories and the facts he has observed in his investigations. His conclusion is that the body of vascular plants is built up by the successive appearance of fundamental units, or "phyllorhizes." As one passes from Pteridophytes to Spermatophytes, the disappearance of the root element ("rhize") is more and more frequent, and becomes the rule in Dicotyledons, where a rhize appears in connection with the first two phyllorhizes, but appears in connection with later phyllorhizes only in exceptional cases, as an adventitious root. In consequence of being thus reduced to a single member, the root has acquired the power of enlarging indefinitely and of persisting as long as the stem itself.

It is shown, also, that in the development of the apparatus of conduction there is a complete parallelism between internal and external morphology, since the vascular apparatus of the plant is built up by the repeated formation of elementary systems, each one corresponding to one of the phyllorhizes. Each bud is the beginning of a new phyllorhize to be added to the phyllorhize complex. In short, plants with roots are formed from elementary plantules or phyllorhizes, and the constitution of these is the unit of morphology.—J. M. C.

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[4] CHAUVEAUD, GUSTAVE, La constitution des plantes vasculaires révélée par leur ontogénie. 8vo. pp. 155. *figs. 54.* Paris: Payot. 1921. 10 fr.

NOTES FOR STUDENTS

Taxonomic notes.—LINDAU[5] has published the following new genera of Acanthaceae from Colombia: *Syringidium* and *Kalbreyeriella*.

NAKAI[6] has described eighteen new Japanese species of *Viola*.

HITCHCOCK[7] has published an account of the grasses of British Guiana based upon his collection during the autumn of 1919, and also upon the collection of the Jenman Herbarium. The list of grasses thus far includes 169 species, ten of which are described as new, four of them being species of *Panicum*. Most of British Guiana is covered by virgin forest, with extensive upland savannas only in the south. Of the 169 species included, thirty-three are introduced, the greater proportion of these being in the vicinity of settlements.

DIXON[8] has published an account of the mosses of the Wollaston Expedition to Dutch New Guiana in 1912–1913. He states that "the moss flora of New Guiana affords, perhaps, the most interesting field for the present day bryologist. Large tracts of Central Africa remain no doubt comparatively unexplored, and recent discoveries there show that much is still to be expected of bryological interest." The report includes also some additional mosses from British New Guiana. Among the species enumerated, twenty-three are described as new.

COMPTON[9] has published an account of the plants collected in New Caledonia and the Isle of Pines in 1914. The Gnetales and Ginkgoales are absent from New Caledonia, and the Cycadales are represented by a single species, which occurs only in the littoral zone. On the other hand, the Coniferales are developed to quite an exceptional degree, being represented by thirty-one species, an unusually large number for so small an area. Another remarkable feature of the coniferous flora is that apparently the whole of it is endemic. The range of the Taxineae is extended to New Caledonia by the new genus *Austrotaxus*, while the Cupressineae include the new genus *Callitropsis*.

DIXON[10] has described the following new genera of mosses, mostly from Africa: *Nanobryum*, *Chionoloma*, *Beddomiella*, *Œdipodiella*, *Chamaebryum*, *Physcomitrellopsis*, and *Dimorphocladon*.

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[5] LINDAU, G., Neue Gattungen der Acanthaceen. Notizblatt Bot. Gart. Berlin-Dahlem **8**:142–144. 1922.

[6] NAKAI, TAKENOSHIN, Violae novae Japonicae. Bot. Mag. Tokyo **36**:29–39. 1922.

[7] HITCHCOCK, A. S., Grasses of British Guiana. Contrib. U.S. Nat. Herb. **22**: 439–514. *figs. 77–86*. 1922.

[8] DIXON, H. N., The mosses of the Wollaston Expedition to Dutch New Guiana. Jour. Linn. Soc. **45**:477–510. *pls. 28, 29*. 1922.

[9] COMPTON, R. H., A systematic account of the plants collected in New Caledonia and the Isle of Pines by R. H. COMPTON in 1914. Part II. Gymnosperms. Jour. Linn. Soc. **45**:421–434. *pls. 26, 27*. 1922.

[10] DIXON, H. H., Some new genera of mosses. Jour. Botany **60**:101–110. *pl. 564*. 1922.

Diels,[11] in publishing an account of the Myrtaceae of Papua, describes numerous new species, and establishes the following new genera: *Xanthomyrtus* (14 species) and *Octomyrtus* (3 species). The large genera are *Jambosa* (50 species), and *Syzygium* (44 species).

Gandoger[12] has published the first part of a series of descriptions of new species from various countries of the world. This first paper includes descriptions of 348 new species, a very large contribution to taxonomy.

Smith and Evans[13] have described a new genus (*Craigia*) of Sterculiaceae from China, province of Yunnan. It is a tree or shrub, and in foliage and inflorescence suggests certain species of *Sterculia*.

Sargent[14] has described twelve new species of *Crataegus*, chiefly from Missouri and Arkansas.

Blake[15] has described forty-six new species of plants from Guatemala and Honduras, from a collection made during 1919 by members of an Economic Survey Mission sent out by the State Department. The new species are distributed among twenty-three families, and include two new genera: *Decazyx* (Rutaceae) and *Prosanerpis* (Melastomaceae).

Robinson,[16] in his further study of the Eupatorieae, has published seventeen new species of *Mikania* and one new species of *Ageratum*. He has also published [17] local revisions of *Mikania* covering certain regions of South America, as follows: Colombia (32 spp.), Venezuela (13 spp.), Ecuador (18 spp.), Peru (37 spp.), and Bolivia (28 spp.).

Nakai,[18] in continuation of the study of the woody flora of Korea, has published an elaborate account of the Caprifoliaceae, accompanied by numerous unusually fine plates. Thirty-six species are recognized, distributed among six genera, much the largest being *Lonicera*, with seventeen species. The descriptions and discussions are in both Japanese and English, so that the publication is available for all taxonomists.

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[11] Diels, L., Die Myrtaceen von Papuasien. Engler's Bot. Jahrb. **57**:356–400. 1922.

[12] Gandoger, M. Michel, Sertum plantarum novarum. Pars prima. Bull. Soc. Bot. France. **65**:24–69. 1918.

[13] Smith, W. W., and Evans, W. Edgar, *Craigia*, a new genus of Sterculiaceae. Trans. Bot. Soc. Edinburgh **28**:69–71. *pl. 1*. 1921.

[14] Sargent, C. S., Notes on North American trees. IX. Jour. Arnold Arboretum **3**:1–11. 1921.

[15] Blake, S. F., New plants from Guatemala and Honduras. Contrib. U.S. Nat. Herb. **24**:1–32. 1922.

[16] Robinson, B. L., Records preliminary to a general treatment of the Eupatorieae. I. Contrib. Gray Herb. N.S. no. 64. pp. 1–21. 1922.

[17] ———, The Mikanias of northern and western South America. *Idem.* no. 64. pp. 21–116. 1922.

[18] Nakai, Takenoshin, Flora sylvatica Koreana. XI. Caprifoliaceae, pp. 92. *pls. 42*. Seoul. 1921.

PAYSON[19] has published a monograph of the genus *Lesquerella*, recognizing fifty-two species, only two of which are new. The synonymy and specimens examined are recorded in detail. Preceding the taxonomic presentation, there is an interesting discussion of the general morphology, phylogeny, and geographical distribution of the genus.

PETCH,[20] in continuation of his studies of entomogenous fungi, has presented a very detailed account of *Hypocrella* and *Aschersonia*. In *Hypocrella* twenty-nine species are described, two of which are new; while in *Aschersonia* thirteen species are described, four of which are new. In addition to the species included in the systematic presentation, a number of species are named as not seen, doubtful, or excluded.—J. M. C.

Origin of variations.—Of extreme interest to students of genetics is a recent number of the *American Naturalist* which contains the papers presented at the Toronto meetings in a symposium on "The origin of variations." JENNINGS,[21] discussing "variation in uniparental reproduction," stresses the fact that the vast majority of observed variations in primitive organisms are strictly non-heritable. There exists, however, a very small residuum of heritable variations, and in considering their evolutionary significance, two types should be distinguished. Seemingly spontaneous changes (mutations ?) appear to have occurred in some forms during a series of asexual generations, and have been isolated by selection to produce new constant races. The cause of these changes is unknown. On the other hand, variations have been induced by environmental changes, which have always reverted to the normal type after a certain number of asexual generations. JENNINGS points out that the period of persistence of such variations evidently depends, in good part, on the number of generations through which the producing agent acted, and expresses the belief that heritable characters, as permanent as any that are known to exist, might be produced by allowing the producing agent to act over a sufficient period of time.

BLAKESLEE[22] describes his work on *Datura*, which by this time has become well known,[23] showing how striking heritable variations accompany changes in chromosome number. These changes in chromosome number may result either from non-disjunction of one or a few chromosome sets, producing "unbalanced types," or may involve a wholesale doubling of all the chromosome sets, giving

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[19] PAYSON, E. B., A monograph of the genus *Lesquerella*. Ann. Mo. Bot. Gard. **8:** 103–236. *figs. 34.* 1921.

[20] PETCH, T., Studies in entomogenous fungi. II. The genera *Hypocrella* and *Aschersonia*. Ann. Roy. Bot. Gard. Peradeniya **7:** 167–278. *pls. 2–5.* 1921.

[21] JENNINGS, H. S., Variations in uniparental reproduction. Amer. Nat. **56:** 5–15. 1922.

[22] BLAKESLEE, A. F., Variations in *Datura* due to changes in chromosome number. Amer. Nat. **56:** 16–31. 1922.

[23] BOT. GAZ. **72:** 178–182. 1921.

the "balanced" tetraploid (or indirectly, triploid) types. The tetraploid types breed true, but the others produce (in various proportions) several types of progeny, including individuals like themselves and others like the "normal" (pure diploid) original ancestors.

MULLER[24] takes up changes in the individual gene ("locus changes" or true mutations), and discusses their general characteristics. It is important to realize that the change is not always a mere loss, for clear-cut reverse mutations have been obtained in corn, *Drosophila*, and *Portulaca*. If the original mutation was a loss, the reverse mutation must be a gain. "It is generally true that mutations are much more apt to cause an apparent loss in character than a gain, but the obvious explanation for that is, not because the gene tends to lose something, but because most characters require for proper development a nicely adjusted train of processes, and so any change in the genes, no matter whether loss or gain, substitution or arrangement, is more likely to throw the developmental mechanism out of gear, and give a 'weaker' result, than to intensify it." MULLER depicts a very interesting and suggestive analogy between the gene and certain immunity reactions.

BRIDGES[25] elucidates the following very significant thesis. The characters of an organism, instead of being absolutely "determined" by a single gene, should rather be thought of as being acted upon simultaneously by many genes. Some genes tend to make a character more pronounced, and others to make it less pronounced, so that the grade of development actually realized by each particular character will be determined by the equilibrium between its modifying genes. The justification for this thesis appears from a consideration of some of BRIDGES' non-disjunctional *Drosophilas*, which exhibit previously unknown grades in the expression of a number of characters. Most startling are the cases where the character involved is sex itself; so that the fruitfly, previously the best known example of qualitative differentiation of sex on the basis of the X and Y chromosomes, now provides the most promising example of a quantitative sex mechanism with the newly discovered "intersexes" and "supersexes."[26]

EMERSON[27] presents and classifies a great mass of evidence on bud variation. He considers separately "somatic mutation of genes" and "somatic segregation," and under the latter heading "chromosome elimination," "cytoplasmic segregation," and "graft hybrids and other chimeras." This article should be of unusual interest to botanists.

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[24] MULLER, H. J., Variations due to change in the individual gene. Amer. Nat. **56**:32–50. 1922

[25] BRIDGES, CALVIN B., The origin of variations in sexual and sex-limited characters. Amer. Nat. **56**:51–63. *figs. 7.* 1922.

[26] BOT. GAZ. **72**:408–410. 1921.

[27] EMERSON, R. A., The nature of bud variations as indicated by their mode of inheritance. Amer. Nat. **56**:64–79. 1922.

GUYER[28] draws the following conclusions from his own well known experiments with white rabbits, and from the results of other investigations. Basically inheritance is mainly a question of the perpetuation of specific protein-complexes, and development the result of differential reactions of these same fundamental constituents under differing conditions of environment. There is evidently some degree of constitutional identity, probably protein homology, between the nature substance of a tissue and its correlative in the germ. Changes which can affect certain constituents of tissue cells initiate an influence which, borne in the circulating fluids of the body, evidently is able to affect the homologous constituents of the germ cells. This, of course, furnishes the basis for a Lamarckian view. The author feels that here may be a basis for progressive evolution.—M. C. COULTER.

Influence of host on parasite.—Continuing his studies on the physiology of parasitism, BROWN[29] has investigated the exosmose of substances from leaf and petal surfaces of several flowering plants, and the influence of such substances on the behavior of *Botrytis* spores. Drops of distilled water uniform in size were placed for twenty-four hours on petals of *Cereus*, *Phyllocactus*, *Gloxinia*, *Lilium*, *Papaver*, *Iris*, *Petunia*, *Tulipa*, *Rosa*, *Begonia*, *Viola*, *Lathyrus* (sweet pea), *Dahlia*, *Geranium*, *Cydonia*, *Pyrus*, and on leaves of several plants, including the broad bean. The change in the drops due to exosmose was determined by studying their capacity for germinating spores added to the drops in water suspensions, and also by electrical conductivity tests. Capacity for germinating spores was based on the average length of the germ tubes. An increase in conductivity resulted in all cases, accompanied in the greater number of plants studied by increased germination capacity, when the drops subjected to exosmose were compared with drops of distilled water of similar size. Petals difficult to wet gave lower conductivity and germination figures. In some plants, with leaves of *Tradescantia discolor*, for instance, increased conductivity was accompanied by germination capacity only equal to or less than that of distilled water, or by actual inhibition of germination. The exact source and nature of the inhibiting substance were not determined.

Attention is directed to the difference in the behavior of fungal parasites. Some, like the rust fungi, penetrate both susceptible and immune varieties of plants, their fate thereafter being determined by internal conditions. Contrasted with this is the behavior of *Botrytis* spores on the leaf of the broad bean, typical of another category of fungal parasites, in which the germination and attack depend upon the exosmose of substances into the infection drop, which can be used as a nutrient by the fungus.—J. G. BROWN.

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[28] GUYER, M. F., Serological reactions as a probable cause of variation. Amer. Nat. **56**:80–96. 1922.

[29] BROWN, WILLIAM, Studies in the physiology of parasitism. Ann. Botany **36**:101–119. 1922.

Calamites.—Impressions of casts of the external features of *Calamites* stems are still very little known in comparison with the pith casts of the same plants. In all four of the large monographs now available on the *Calamites*, by STUR, WEISS, KIDSTON, and especially JONGMANS, by far the greater number of the figures relate to the pith casts. As a rule, examples of both medullary casts and impressions showing the true external features of the stem are mixed together in confusion, and are all referred to a common genus, *Calamites*. Neither from the generic nor the specific names employed can one distinguish whether one is dealing with pith casts or with the rarer external surfaces of these stems. It is hardly necessary, however, to point out that incrustations of the external features of the stems of these plants are of an entirely different morphological nature from the medullary casts. For this reason ARBER had proposed in 1916 a new form genus, *Calamophloios*, for the external stem impressions, with the exception of the very distinct type of *Dictyocalamites* which had been established already in 1912.

ARBER and LAWFIELD[30] intend to establish a number of species of these two new types, and they suggest that the same specific name may be used for both types of preservations, the internal and external ones. They avoided adopting new specific names for the types of *Calamophloios*, therefore, as compared with the pith casts. The authors also succeeded in carrying out a correlation of a number of external surfaces and pith casts of *Calamites*.—A. C. NOÉ.

Pliocene flora of Varennes.—The Pliocene flora of Varennes is the subject of a monograph by DE LA VAULX and MARTY,[31] which is divided into the following sections: (1) geology of the fossiliferous beds of Varennes, (2) a critical study of the fossil plants of Varennes, (3) a description of new species discovered in the deposits of Varennes, (4) paleontological, stratigraphical, botanical, and climatological conclusions; also a chapter on fossil Diatomaceae of Varennes is added. Of the forty-seven genera which are described in the fossil flora of Varennes, and which belong to twenty-three families, thirty-eight specific determinations could be made. Fourteen new species were established.

Ecologically, the flora of Varennes indicates a temperate climate, because of the thirty-seven definite species found at Varennes, thirty-four belong to the temperate zone. This flora also contains more continental than insular species. Almost all the species which form the flora of Varennes, or their nearest living relatives, inhabit at present the more or less mountainous districts

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[30] ARBER, E. A. NEWELL, and LAWFIELD, F. W., On the external morphology of the stems of *Calamites*, with a revision of the British species of *Calamophloios* and *Dictyocalamites* of Upper Carboniferous age. Jour. Linn. Soc. **44**:507–530. *pls. 23–25.* 1920.

[31] DE LA VAULX, ROLAND, and MARTY, PIERRE, Nouvelles recherches sur la flore fossile des environs de Varennes. Rev. Gen. Bot. **32**:282–300; 327–336; 351–68. 1920.

of southern Europe, Asia Minor, Japan, and the central states of the United States. This zone follows approximately the fortieth degree of northern latitude, and indicates an annual average temperature of 12–14°C. Since the Miopliocene, during which the fossil plants of Varennes lived, the most of its components have emigrated toward the south. The plant deposits of Varennes accumulated in a lake, into which the ashes of a volcano fell.—A. C. NOÉ.

Fossil plants from Missoula region.—A paper by JENNINGS[32] deals with some fossil plants from beds which are believed to be of Oligocene age. The fossil plants consist of impressions of leaves and of leafy twigs, there being also some impressions of fruits and leafless twigs. The Missoula specimens are embedded in fine-grained volcanic ash which preserved the finer venation of the leaf surfaces. Twenty-one species are enumerated, ten of which are described as new, and one of which required a new name. Of the fifteen genera represented in the Missoula flora, all but two are also represented in the Florissant Basin of Colorado. The Missoula flora probably occupied the shores and surrounding slopes of a high mountain lake. The climate was warmer, and probably drier than that now prevailing at recent localities of similar geographic position, like, for instance, the Flat Head Valley; and the vegetation represented by the Missoula fossils ranged probably throughout a series of associations from wet meadow to moderately xerophytic oak forests on rocky or sandy shores. All of these vegetational associations were in close proximity to the waters of a lake. There are eleven plates with excellent illustrations in the book.—A. C. NOÉ.

Cycadofilicales.—CARPENTIER[33] presents a most interesting paper on a series of Cycadofilicales fructifications which were from the Lower Carboniferous of northwestern France. Two genera of seeds (*Lagenospermum* and *Carpolithus*) have been observed, and in a number of instances pictured also. Sporangia or microsporangia of *Telangium*, *Pterispermotheca*, and *Diplotheca* are described. CARPENTIER concludes that the small seeds of *Lagenospermum* and of related genera seem to have belonged to *Sphenopteris*, probably *S. Hoeninghansi* and *S. elegans*. While the occurrence of *Sphenopteris* together with seeds of *Lagenospermum* is frequent in the Westphalian of northern France, the seeds of *Neuropterides*, which occur frequently in the Bassin de Valenciennes, are very rare in the Bassin de la Basse-Loire. CARPENTIER also emphasizes that our knowledge of the microsporangia of the Cycadofilicales of Mouzeil and the Bassin de la Basse-Loire is still very rudimentary, only fragments of male inflorescences having been discovered. They seem to have been of a very delicate structure. *Telangium*, or a nearly related genus,

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[32] JENNINGS, O. E., Fossil plants from the beds of volcanic ash near Missoula, Western Montana. Mem. Carnegie Museum **8**:385–427. *pls. 22–23*. 1920.

[33] CARPENTIER, A., Contribution à l'étude des fructifications du Culm de Mouzeil (Loire-inférieure). Rev. Gen. Bot. **32**:337–349. 1920.

originated in the Devonian and flourished in the lower and upper Culm in Basse-Loire and during the Westphalian in the north.—A. C. Noé.

Availability of potassium.—Breazeale and Briggs[34] find that the potassium of orthoclase solutions is not available for wheat seedlings, owing, it is concluded, to the potassium being present with other elements in a complex solute molecule, which does not yield potassium ions. This conclusion is supported by the fact that oxidation with acids makes the potassium available. From the experiments recorded in the paper, the general conclusions are drawn that the concentration of a plant food in the soil solution is not necessarily a measure of its availability for the plant, and that applying finely ground orthoclase to a soil does not immediately increase the available potash content of the soil. While the conclusions are probably justified, it must not be concluded from experiments of this kind that plants cannot get the needed potassium from finely ground orthoclase applied to the soil or from orthoclase found naturally in the soil. The nature of the root system and the conditions of its functioning are probably quite different in the solution than in the soil.—S. V. Eaton.

Indian Gondwana plants.—A great majority of the specimens described in this volume were figured by Feistmantel in the *Palaeontologia Indica*. A revision[35] of the material brought to light some new features, and in several instances has revealed inaccuracies in the illustrations accompanying Feistmantel's descriptions. Numerous text illustrations and seven plates in folio with excellent drawings and photographs enable the reader to judge Seward's revision of Gondwana plants. Seward was ably assisted by Sahni, who promises to become an authority on Indian paleobotany.

The Gondwana system is an extremely interesting geologic period of high paleobotanic importance. It corresponded to the Permo-Carboniferous of Europe, and is distinguished by paleozoic glaciation features. The Gondwana flora is characterized by a wealth in gymnosperms, especially Cycadophyta. The present volume describes eight species of Bennettitales, and seven species of Nilssoniales; also numerous Cordaitales, Ginkgoales, and Coniferales are represented, but the pteridophytes are rather scarce. No *Glossopteris* is mentioned.—A. C. Noé.

New method of vegetative multiplication.—Dastur and Saxton[36] have described a method of vegetative multiplication in a perennial species of

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[34] Breazeale, J. F., and Briggs, L. J., Concentration of potassium in orthoclase solutions not a measure of its availability to wheat seedlings. Jour. Agric. Res. **20**:615–621. 1921.

[35] Seward, A. C., and Sahni, B., Indian Gondwana plants: A revision of Palaeontologia Indica. New Series **7**:1–42. *pls. 1–7*. 1920.

[36] Dastur, R. H., and Saxton, W. T., A new method of vegetative multiplication in *Crotalaria burhia*. New Phytol. **20**:228–233. *figs. 4*. 1921.

Crotalaria (*C. burhria*) which differs from anything previously described. The plant has a very long tap root, and when about a year old the axis becomes ribbed, the ribs beginning at the transition region between stem and root and extending in both directions. The ribbing is associated with the development of an accessory bundle system, and the gradual separation of branches which become established as separate plants. In this way, "when the main axis perishes, a circle of branches separated to below the ground level is already established." It was also observed that although the plant flowers during most of the year, it seems seldom to develop seeds.—J. M. C.

Sexual evolution.—SCHAFFNER[37] has presented his conception of the evolutionary stages of sexual expression, defining what may be called twenty-three steps in evolutionary progress, each one illustrated by examples. He is convinced that sex "cannot be associated primarily with special chromosomes." The general conclusion is reached that "the specific structures and functions developed in the ontogeny of an organism appear to be conditioned on the interaction of four fundamental influences: (1) hereditary factors, (2) influence of environment, (3) progression of senility, and (4) presence of sexual states in the living substance."—J. M. C.

Mesozoic flora.—BERRY'S[38] fourteenth contribution to the Mesozoic flora of the Atlantic coastal plain deals with the floras of the Eutaw and Ripley formations. The article comprises an advance paper of the fuller material to be described and illustrated in a professional paper of the United States Survey (no. 112) which has meanwhile appeared. The larger publication includes the Tuscaloosa formation besides the two groups mentioned.—A. C. NOÉ.

North American flora.—Part I of volume 6 begins the presentation of Phyllostictales by SEAVER. This ordinal name is used in place of Sphaeropsidales, because the generic name *Sphaeropsis* "goes out of the order," and the ordinal name becomes untenable. In this first part the genus *Phyllosticta* is presented, 300 species being recognized, only three of which are described as new.—J. M. C.

Fossil woods of Queensland.—SAHNI[39] describes and gives microphotographs of a number of fossil woods which range from fern stems through gymnosperms to angiosperms. The paper is a valuable contribution to the study of Mesozoic woods.—A. C. NOÉ.

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[37] SCHAFFNER, J. H., Progression of sexual evolution in the plant kingdom. Ohio Jour. Sci. **22**:101–113. 1922.

[38] BERRY, E. W., Contributions to the Mesozoic flora of the Atlantic coastal plain. XIV. Tennessee. Bull. Torr. Bot. Club **48**:55–72. 1921.

[39] SAHNI, BIRBAL, Petrified plant remains from the Queensland Mesozoic and Tertiary formations. Queensland Geol. Survey. Publ. no. 267. pp. 48. *pls. 5. figs 10.* 1920.

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PHYSIOLOGICAL STUDIES OF EFFECTS OF FORMALDEHYDE ON WHEAT[1]

W. M. ATWOOD

(WITH TWELVE FIGURES)

Introduction

Copper sulphate and formaldehyde have been most commonly used as fungicides in the treatment of seed wheat. The choice between the two has often been determined by local custom and prejudice, while in other cases climatic differences have been thought to be worthy of consideration in the selection. During the twenty years, approximately, that formaldehyde has been used as a dip or spray, as a gas (**44, 53**) or with steam (**39**), reports have differed radically in the degree of favor with which it has been viewed.

One group of experimenters has reported injury to germination or seedling vitality, or both, following the use of formaldehyde. STEPHENS of the Sherman County Branch Experiment Station at Moro, Oregon, has consistently reported injury in his station reports since 1913 (**50**). In 1917 he noted that 18.5 per cent of the seed wheat was killed. He has made the further important observation that in many cases seedlings may progress in development but with lessened vegetative vigor. HEALD and WOOLMAN (**31**) found germination reductions at concentrations of 30–40 gallons of water

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[1] The project on which the present paper is based was financed under the Adams Fund, to which acknowledgment is hereby made.

to the pint of formaldehyde. In Utah, STEWART and STEPHENS (**52**) noted vitality reductions in wheat, barley, and oats, but thought the advantages outweighed the injury. MACKIE (**37**) of California noted that seed stored after treatment uniformly showed poor germination. Even with proper drying the tissues appeared hardened, causing retardation and distortion of the young seedlings. Varying degrees of injury have been reported by many different investigators (**14, 19, 20, 21, 22, 53, 57, 58**).

On the other hand, formaldehyde has been approved in varying measures by different investigators, some of whom recognized the dangers and injury in some cases, but have felt that the advantages outweighed the injury. The War Emergency Board of American plant pathologists found little injury from formaldehyde except when the concentrations were higher than the usual 1 part to 320 parts of water, or when the other common precautions in treatment had not been observed. This work was based on the reports of seventeen experiment stations, and is probably the most complete and uniformly secured set of data available from so large an area of country (**35**). Many other workers in America and Europe have reported in similar vein (**6, 16, 32, 36, 38, 40, 45, 51, 55**).

Within the past two years two most interesting papers have appeared, in which the possibilities of avoiding injury from formaldehyde treatment have been suggested. BRAUN (**13**) finds the injury apparently much diminished by not treating the grain until imbibed with water. It is believed that exterior disinfection is thus attained, and a much less amount of formaldehyde enters the grain under these conditions. Miss HURD (**33**) believes that when seeds are treated in formaldehyde and subsequently allowed to dry, the polymer paraformaldehyde is deposited on the seed coat with serious eventual injury. Here, instead of the "pre-soak," we have the recommendation of washing in water subsequent to treatment to avoid the harmful paraformaldehyde deposits. With the work of Miss HURD there appears to be a better explanation than formerly of the source of the injury of formaldehyde to seeds. Amid a wide diversity of opinions as to the value of the disinfectant, and with differing recommendations for reduction in treatment injury, it seemed altogether desirable that something be learned as to the exact nature of the effect exerted by formaldehyde on the physiological processes of seeds, as shown by wheat. Accordingly, the Oregon Experiment Station has been occupied in such studies, during parts of the past three seasons, and inasmuch as local conditions have necessitated the temporary discontinuance of this work, it was thought well to report the results already obtained.

Experimentation

Wheat for these studies was kindly furnished by Mr. STEPHENS of the Oregon Branch Experiment Station. In order that the behavior of this wheat in relation to formaldehyde might be known, it was thought advisable first to determine the effect on germination of varying the concentration and also the time of treatment. The formaldehyde used was the ordinary commercial material, the strength of which was determined according to the method outlined by HAYWOOD and SMITH (**30**), and found to contain 39.3 parts per hundred by volume of the formaldehyde gas.

In the studies of the effect of varying the time of treatment, the period was varied from 5 to 300 minutes of soaking in formaldehyde 1–320. The number of seeds used was 10,800, one-third being grown in blotters in the customary manner, one-third in soil in porous clay germinators indoors, and one-third outdoors in pots of soil exposed to the weather and a temperature between 40° and 60° F. The indoor samples were grown in the laboratory, and, as might be expected, germination was much more prompt at the higher temperature. It was found that the time of dip between twenty and forty minutes only slightly reduced the germination percentages. A somewhat greater drop in the curves (figs. 1, 2) occurs as the time is lengthened up to four hours, although the drop is not great in most cases. The seeds germinated in soil displayed a somewhat greater percentage of injury, as measured by appearance above soil, than was true of the samples grown in blotters. This difference between the behavior of formaldehyde treated seeds when germinated in soil and in blotters was noted by CRANEFIELD (**20**) in studying the effect of the fungicide on oats. He found the injury in oats grown in the soil averaged four times greater than that of seed grown in blotters. The explanation of this difference in apparent injury was given by WALLDEN (56), who thought injuries to the coleoptile, making it difficult to pierce the soil, do not prevent the germination of seeds in blotters. Miss HURD (33), after making a similar observation, expresses preference for the blotter studies, which she believes show more clearly the distortion incident to injury, even though the percentage stand which would be attained under field conditions by this method could only be estimated.

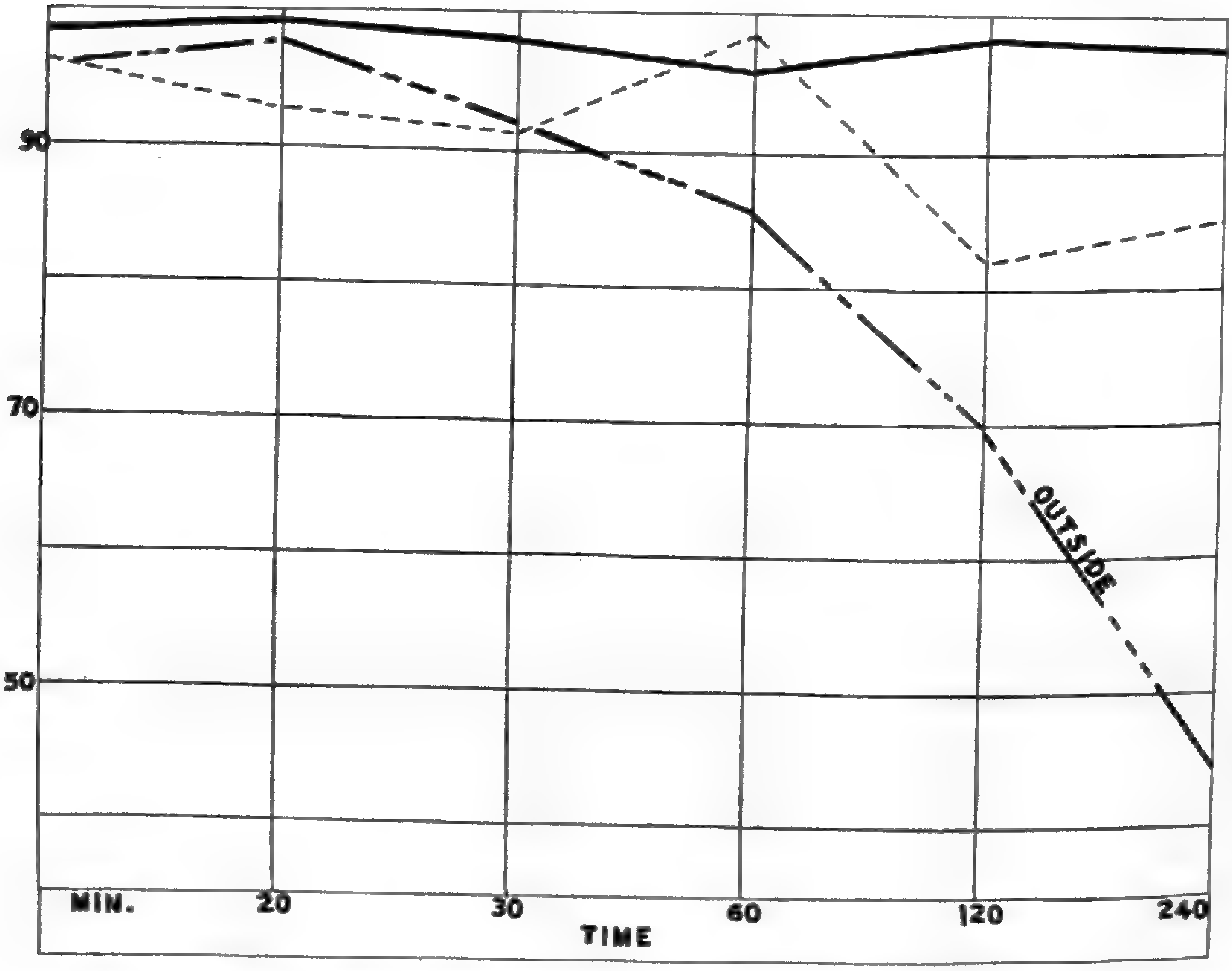

FIG. 1.—Effect of time of soaking wheat on percentage germination, Hybrid 128 wheat from Moro, Oregon: solid line shows percentage germination in blotters, broken lines in soil indoors and outside; formaldehyde mixed 1–320 parts of water; summary of 3600 seeds tested.

In varying the concentrations of formaldehyde, treatment was for ten minutes at 20° C., and the concentrations were varied from 40 to 320 parts of water to 1 part of formaldehyde. Uniform dropping in germination occurred in all cases with increasing concentrations. As compared with the water dipped controls, there was little injury apparent at the usual concentration of 1–320; but with a concentration of 1–160 the germination curves began to fall, and at 1–40 the germination was cut from 40–60 per cent, both in the blotters and in the soil. Here again, as in the previous series, the injury was greatest in the outdoor soil, less in soil indoors, and least of all in the blotter tests (figs. 3, 4).

Formaldehyde readily forms various polymers (**8, 25**). On standing in the cool a flocculent white precipitate forms readily, or on concentration of the commercial solutions. This is ordinarily referred to as paraformaldehyde, although the various polymers are probably often found more or less associated, and means for the identification of the various forms are not well known. Efforts have been expended toward developing methods to prevent such polymerization (**28**), but these methods have not been adopted in general. If wheat is dusted with the white flakes of this so-called paraformaldehyde, serious injury results. Turkey Red so treated

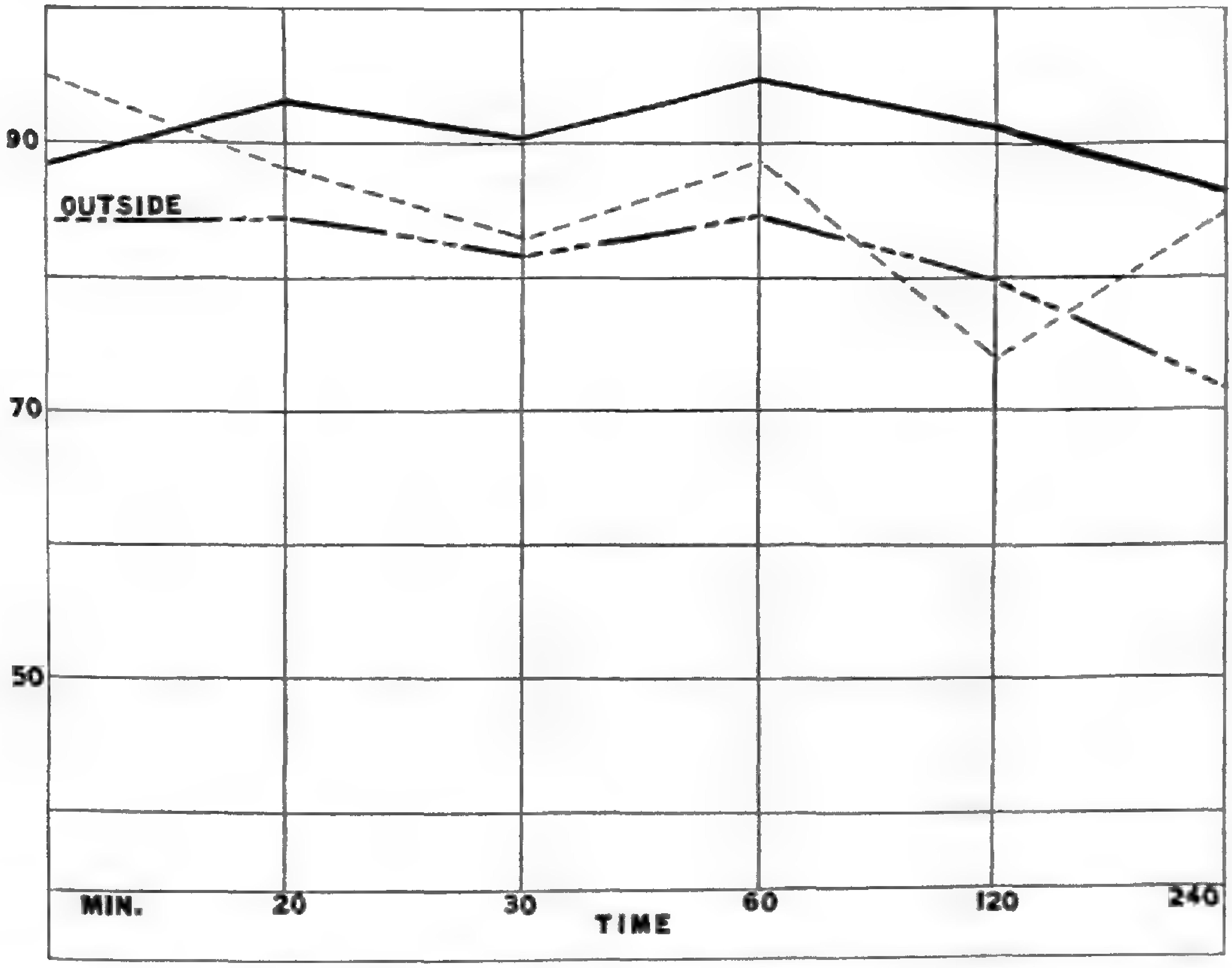

FIG. 2.—Effect of time of soaking wheat on percentage germination, Turkey Red wheat from Moro, Oregon: solid line shows percentage germination in blotters, broken lines in soil indoors and outside; formaldehyde mixed 1–320 parts of water; summary of 3600 seeds tested.

gave in one series of tests 9.5 per cent germination in blotters and 15 per cent in soil, as compared with 93.5 and 93 per cent respectively for the controls. This effect of the white polymer on the grain was noted by COONS and MCKINNEY (19), who found that it does not readily air out of grain but persists on it, so that its presence could be demonstrated by an indicator after the grain had been exposed to the air of the laboratory for many months. Miss HURD (33) later has emphasized the extreme importance of the polymer as the possible channel through which injury from formaldehyde ordinarily results.

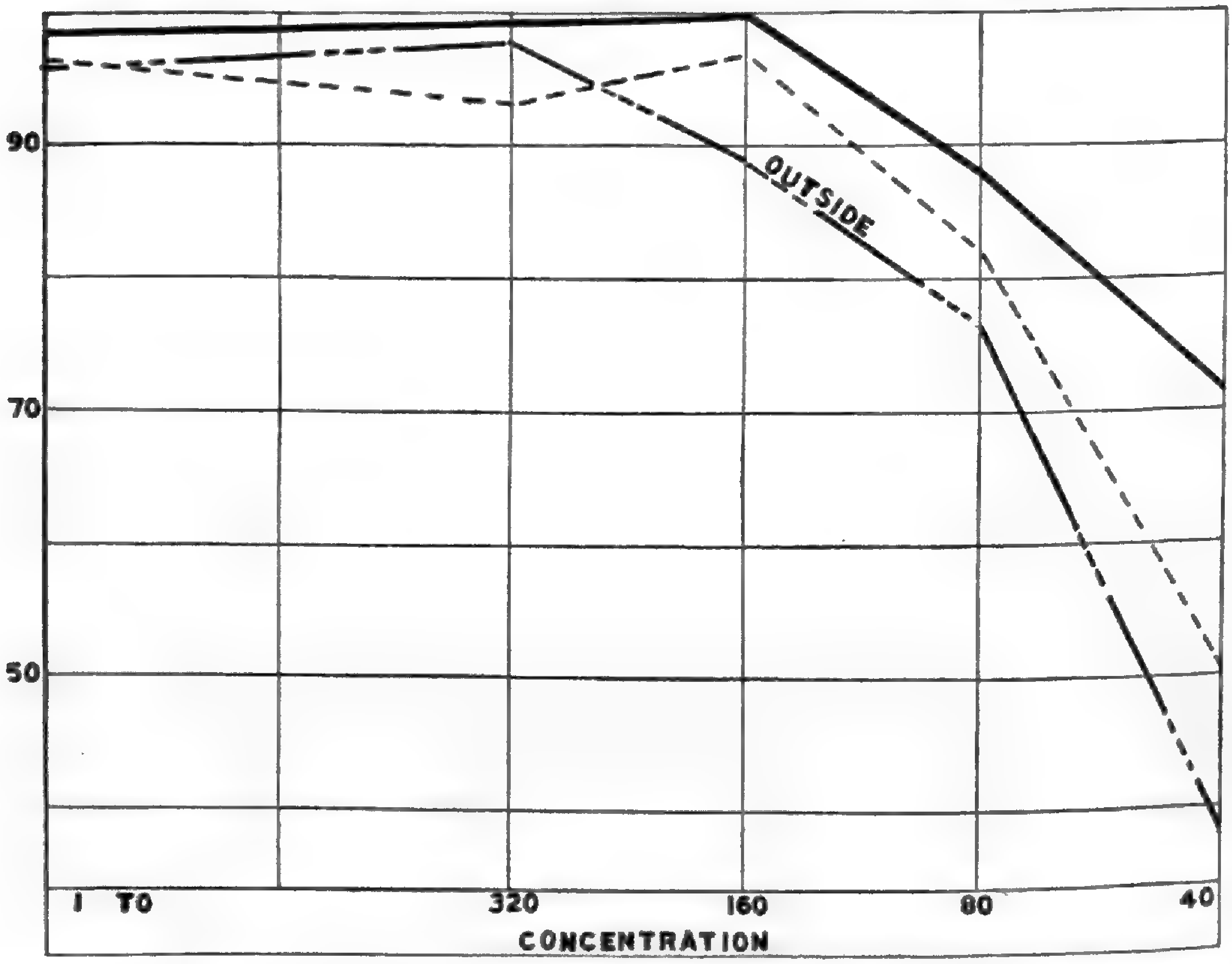

FIG. 3.—Effect of varied concentrations of formaldehyde on percentage germination, Hybrid 128 wheat from Moro, Oregon: solid line shows percentage for seeds germinated in blotters, broken lines for seeds in soil outside and indoors; ten minutes of soaking; summary of 3000 seeds tested.

PERMEABILITY

It was recognized that it must be determined whether formaldehyde actually penetrates the coat of wheat. It has long been known that the seed coats of many seeds exhibit varying powers of exclusion. BROWN (15) showed this to be conspicuously the case for barley, while SHULL (49) found semipermeability of seed coats a rather general situation. SCHROEDER (47) showed that the coat of wheat is permeable to the entry of mercuric chloride, iodine, alcohol, ether, chloroform, and acetic acid when in water solutions. Injury to the seed coat destroys this seed coat power of exclusion.

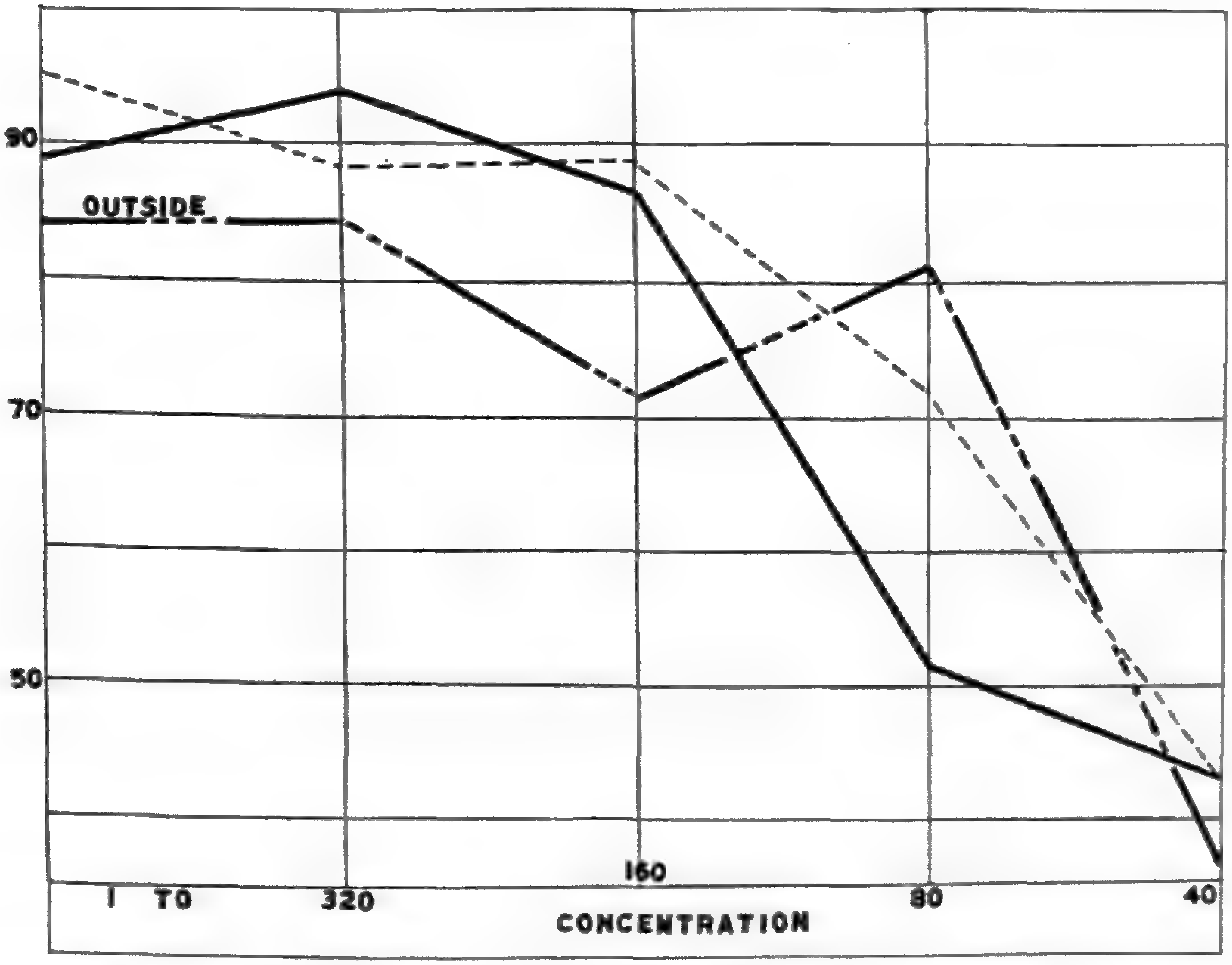

FIG. 4.—Effect of varied concentrations of formaldehyde on percentage germination, Turkey Red wheat from Moro, Oregon: solid line shows percentage for seeds germinated in blotters, broken lines for seeds in soil indoors and outside; ten minutes of soaking; summary of 3000 seeds tested.

Miss HURD (34) found that injuries from the entry of fungicides are much worse when seed coat cracks exist over the embryo.

Two methods were employed in studying the relation of the seed coat of wheat to formaldehyde entry. After various difficulties in technique, at the suggestion of Dr. E. M. HARVEY, the method was finally adopted of sealing the seeds one at a time to the end of small glass tubing, into which formaldehyde solution was placed. After allowing the seed to be in contact with the solution for 3–4 days, the dry tip of the grain exterior to the tube was sectioned and treated directly with the Schryver formaldehyde reagent (29). With long periods of exposure to high concentrations of formaldehyde (1–8) penetration appears to be possible at either tip of the grain or on either face. The second method employed was to measure the degree of semipermeability of the seed coat indirectly by determining the weight increase of the seeds when soaked in distilled water and in formaldehyde respectively. Formaldehyde of high concentration (1–8) was used, in order to make more conspicuous any semipermeability differences of the coat toward the water and formaldehyde. In harmony with the results of Brown (15), if seeds with semipermeable coats be placed in salt solutions, water will be taken up by imbibition until the inward force is offset by the equal outward osmotic force incident to the solution outside. If the two forces just balance each other, and if the coats be perfectly semipermeable, further soaking of the seeds in solution will not cause a rise in the curve indicating percentage weight increase, and the curve will continue horizontal. On the

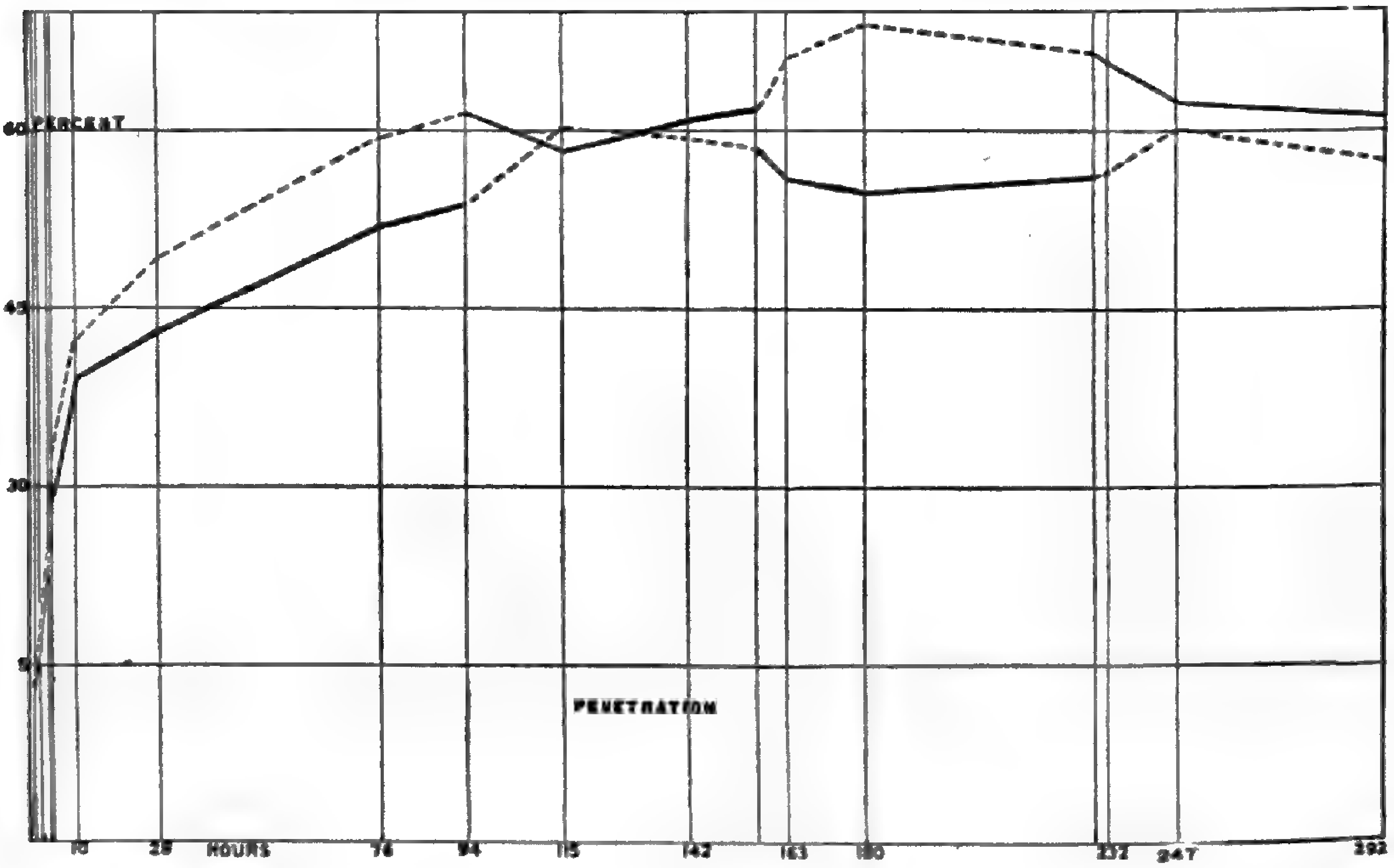

Fig. 5.—Comparative absorption by wheat of water and formaldehyde mixed 1 to 8 parts of water, Hybrid 128 wheat from Moro, Oregon: solid lines indicate percentages of weight increases when seeds were soaked in formaldehyde, broken lines when soaked in water.

other hand, if there is a gradual entry of the salt into the seed, the curve will flatten out quickly, and after changing the seeds back into pure water, the sudden and extensive rise of the curve as the water enters will be followed by a subsequent sinking as the salt gradually diffuses out of the seed again. In this way, by long and careful soaking of seeds in water and solutions of higher osmotic concentration, it is possible to determine whether the substance in solution is entering the seed coat. Frequent weighings are essential, each seed lot being dried of surface moisture with filter paper at each weighing. After the curves approach equilibrium, the seeds in either solution are transferred to the other one, with special attention to the behavior of the curves at this reversal of solutions. The weighings were made in triplicate with 3.5 gm. samples. Seventeen intervals, covering 292 hours of soaking, were followed by computation of percentages. A study of the resultant curves (fig. 5) leads to the conclusion that formaldehyde slowly penetrates the seed coat, and that when the grain is again transferred to distilled water, the formaldehyde gradually diffuses outward. These results are shown in table I. The variations at the time of reversal are by no means so great, even with this high concentration of formaldehyde,

TABLE I

DATA ON PENETRATION AS SUMMARIZED IN FIG. 5; PERCENTAGE WEIGHT INCREMENTS AT VARYING PERIODS OF SOAKING OF WHEAT

No.	Air dry weight	Hours of soaking																
		1	2	4	4.75	10	28	76	94	115	142	157	163	180	229	232	247	292
1	3.5048	**11.9**	**15.7**	**22.9**	28.9	**38.5**	**43.4**	**53.2**	**55.1**	61.4	60.0	60.1	**57.3**	**55.4**	**57.1**	57.8	61.0	58.0
2	3.478	**11.9**	**15.6**	**23.6**	29.2	**39.2**	**43.4**	**51.6**	**53.3**	60.8	60.0	58.9	**56.4**	**54.7**	**56.5**	56.5	60.6	58.2
3	3.4106	**12.3**	**15.9**	**24.2**	30.1	**40.7**	**44.1**	**52.3**	**54.5**	60.0	59.5	58.6	**55.5**	**55.1**	**55.7**	57.1	59.8	57.2
4	3.2846	13.0	17.5	28.0	**32.8**	42.5	50.1	60.8	62.6	**59.5**	**62.1**	**63.1**	68.3	70.5	68.6	**66.4**	**63.2**	**61.4**
5	3.5406	13.3	18.1	27.9	**32.7**	42.9	49.6	59.4	61.6	**58.8**	**60.4**	**61.5**	65.9	68.9	64.4	**66.2**	**63.3**	**61.8**
6	3.487	13.2	17.5	27.5	**32.4**	41.9	49.1	58.9	61.1	**57.6**	**60.7**	**61.7**	65.7	68.2	67.8	**65.4**	**62.1**	**60.8**

* Figures printed in heavy type indicate soaking in formaldehyde; others in water.

as the writer (7) in earlier work has found to maintain with other grains in the presence of gram molecular solutions of sodium chloride. From the consistent behavior of these curves we must conclude that formaldehyde penetrates the coat of wheat, although such entry is slow. The work of BAKKE and PLAGGE (10) offers interesting confirmation of these conclusions. In their work the rate of entry of water and of 1–320 formaldehyde was compared. After a dip of fifteen minutes they found the weight increase about the same for the two, and concluded that water entry from 1–320 is not greatly different from the absorption of distilled water. The question of the comparative entry of water and of formaldehyde solutions becomes specially interesting in the light of COLLINS' (18) work with barley, which indicated that the entry of solutions, and hence the seat of selectivity, is in the germ end of the grain.

DIASTASE

In order to determine the effect on starch digestion of the presence of formaldehyde, a series of twenty-six test-tubes was filled each with 10 cc. of 25 per cent soluble potato starch solution. To all but two of the tubes 1 cc. of a filtered solution of Merck's medicinal diastase of 0.5 per cent concentration was added. To the test-tubes was then added 4 cc. of a formaldehyde solution varying in concentration from 1–1000 through 1–400, 1–320, 1–240, 1–160, 1–80, 1–40, 1–20, 1–10, 1–1, and pure 40 per cent commercial formaldehyde solution. Each condition was run in duplicate. These test-tubes were then incubated for 1.25 hours at 40° C. It was presumed at the beginning of these tests that it would be essential to determine the percentage of reducing sugars as a measure of the degree of digestion. It was found, however, that by modifying the methods used by APPLEMAN (3) and SHERMAN (48), it was easily possible to detect comparative differences in the amount of digestion by the deepness of coloration of the solution upon the addition of iodine. The stock solution of iodine as used eventually by dilution 10 cc. to 100 cc. of water was prepared with 1 gm. iodine, 5 gm. potassium iodide, and 50 cc. water. In the series enumerated the gradation of color was so obvious, from the deep blue of the check to the clear solutions where digestion was complete, that the experiment was tried of giving to each tube at the end of the test a number value. No digestion (as in the tubes lacking diastase) was indicated by 10, complete digestion (no starch) by 0, and intermediate shades proportionately in between. It is not claimed that this method equals the accuracy of colorimetric technique, yet the differences were so pronounced that on checking over results with other observers who were unfamiliar with the conditions presented, it was thought that the situation did not justify the other method. Referring to fig. 6, in which these values are presented graphically, it will be observed that with the higher concentrations of formaldehyde the digestion is not greater in amount than that occurring in the check containing no diastase. Commencing with the concentration of 1–20 of formaldehyde, and running from that point down to

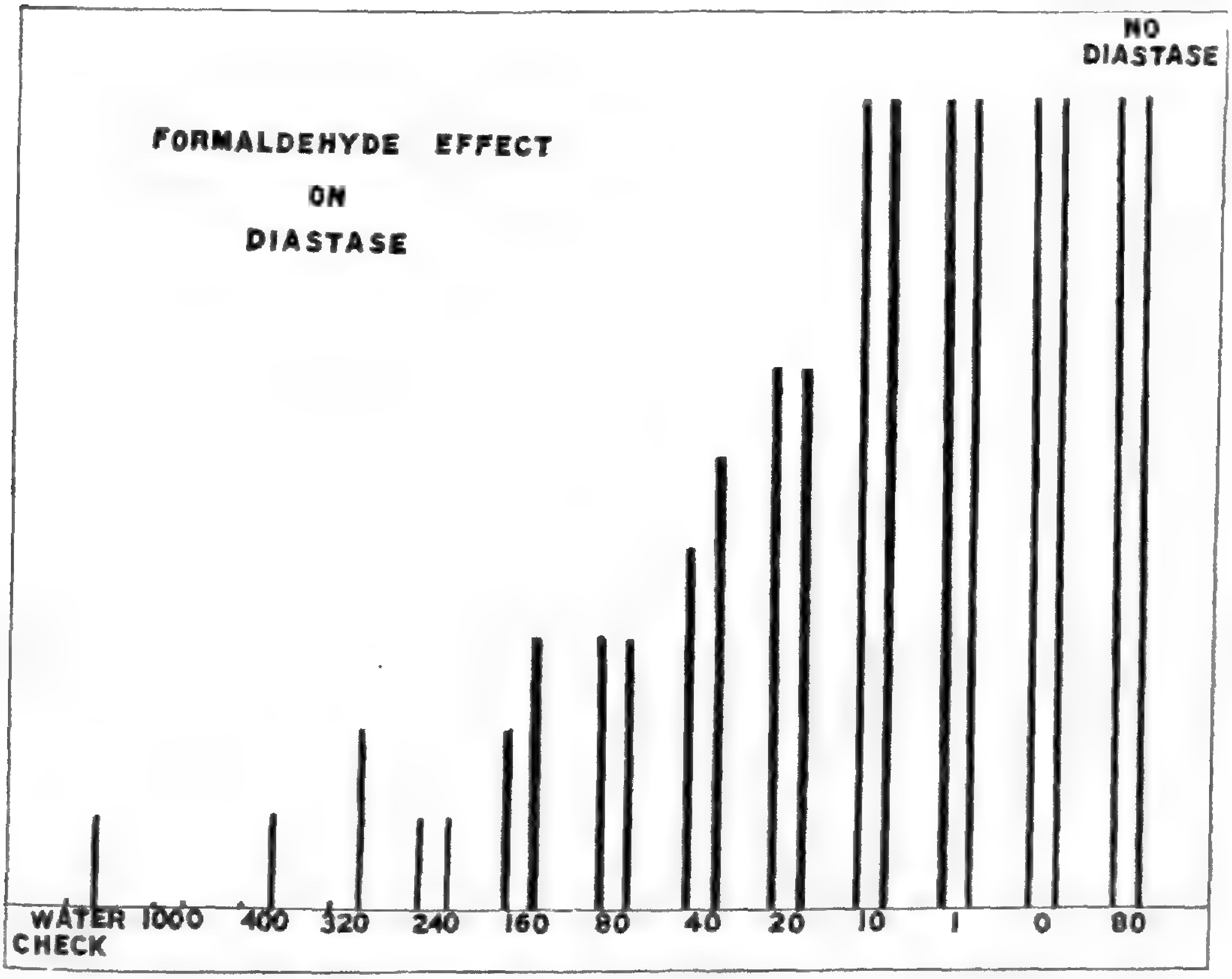

FIG. 6.—Effect of varying concentrations of formaldehyde on starch digestion: height of lines indicates amount of starch remaining undigested (all conditions shown in duplicate); 1 cc. 0.5 per cent solution Merck's diastase, 10 cc. 25 per cent soluble starch solution, and 4 cc. of varying concentrations of formaldehyde used in all but controls.

1–400, there is increasingly greater digestion found (less starch remaining). The question immediately arose as to whether the result was inhibition or a retardation of the rate of digestion. This was answered by running two series of digestions over a period of four hours at the same temperature as in the previous tests. To all the test-tubes of one series was added 1 cc. of the diastase solution, to one-half of the tubes was added 4 cc. of formaldehyde 1–40, and to the other half (control) an equal amount of water. Ten cc. of the 25 per cent soluble starch solution was placed in all tubes. The second series was prepared in the same manner, except that formaldehyde of 1–320 concentration was used. Four test-tubes from each series were removed every fifteen minutes, two contain-

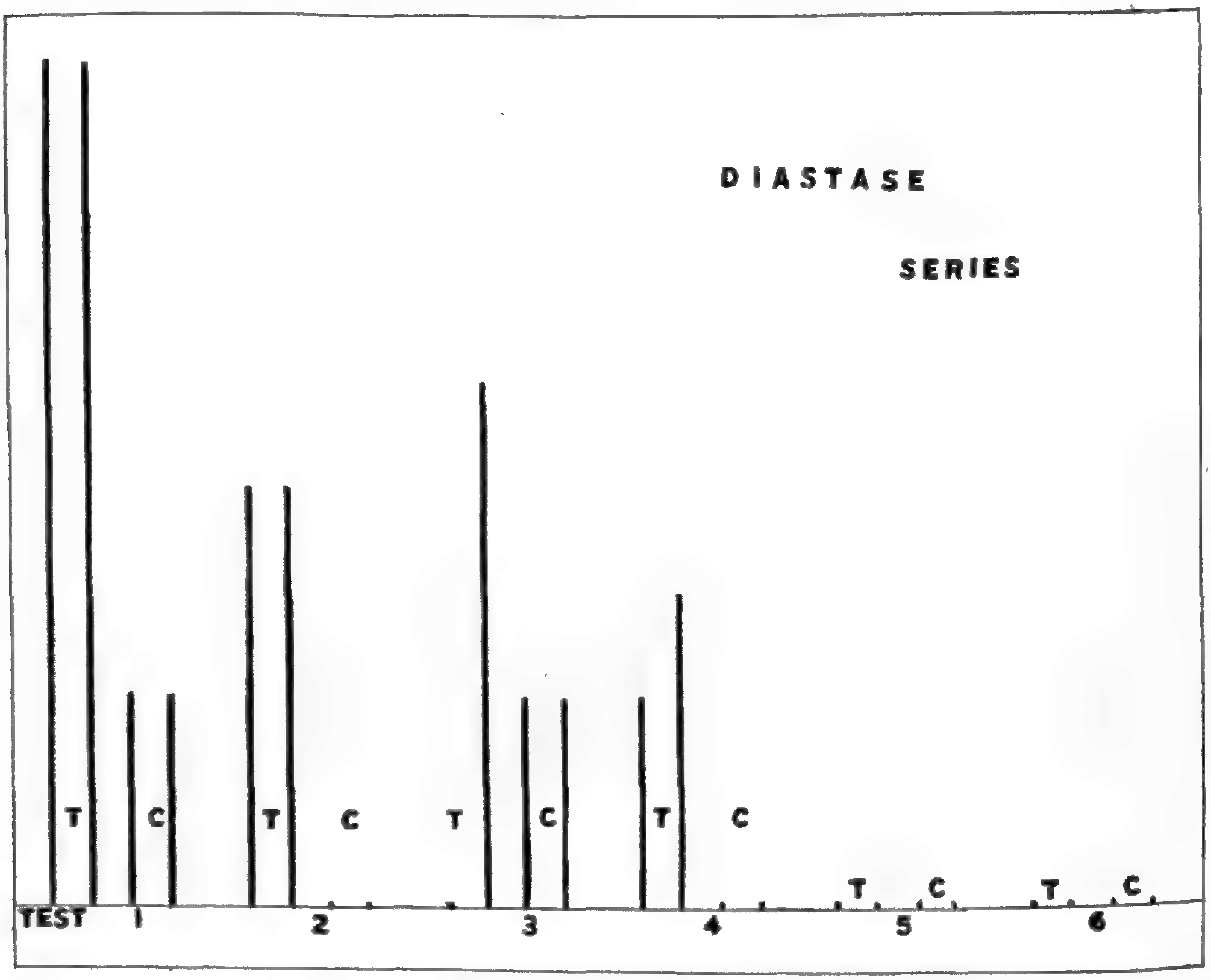

FIG. 7.—Time factor as related to starch digestion in presence of formaldehyde mixed 1–40 parts water: height of line indicates amount of starch remaining undigested (all conditions shown in duplicate); 1 cc. 0.5 per cent solution Merck's diastase, 10 cc. 25 per cent soluble starch solution, and 4 cc. formaldehyde used in tests (*T*), and a like amount water in controls (*C*); time periods in hours tests 1 to 6 respectively, 1.25, 1.5, 1.75, 2, 3, and 4 hours.

ing formaldehyde and two controls, and the iodine test applied and results evaluated. Figs. 7 and 8 show that although digestion is markedly checked by 1–40 formaldehyde at the end of the first one and a quarter hours, digestion proceeds with further intervals of time, so that by three hours the digestion which had been complete in the controls in half that time, has also occurred in the presence of the formaldehyde. The series in the presence of 1–320 (fig. 8) formaldehyde was not so striking, but nevertheless shows satisfactorily that formaldehyde does not entirely inhibit the action of diastase, but retards the same. Turning to the effects on the starch digestion in living wheat of the concentrations of formaldehyde 1–320, 1–240, 1–160, and 1–80, a considerable quantity of

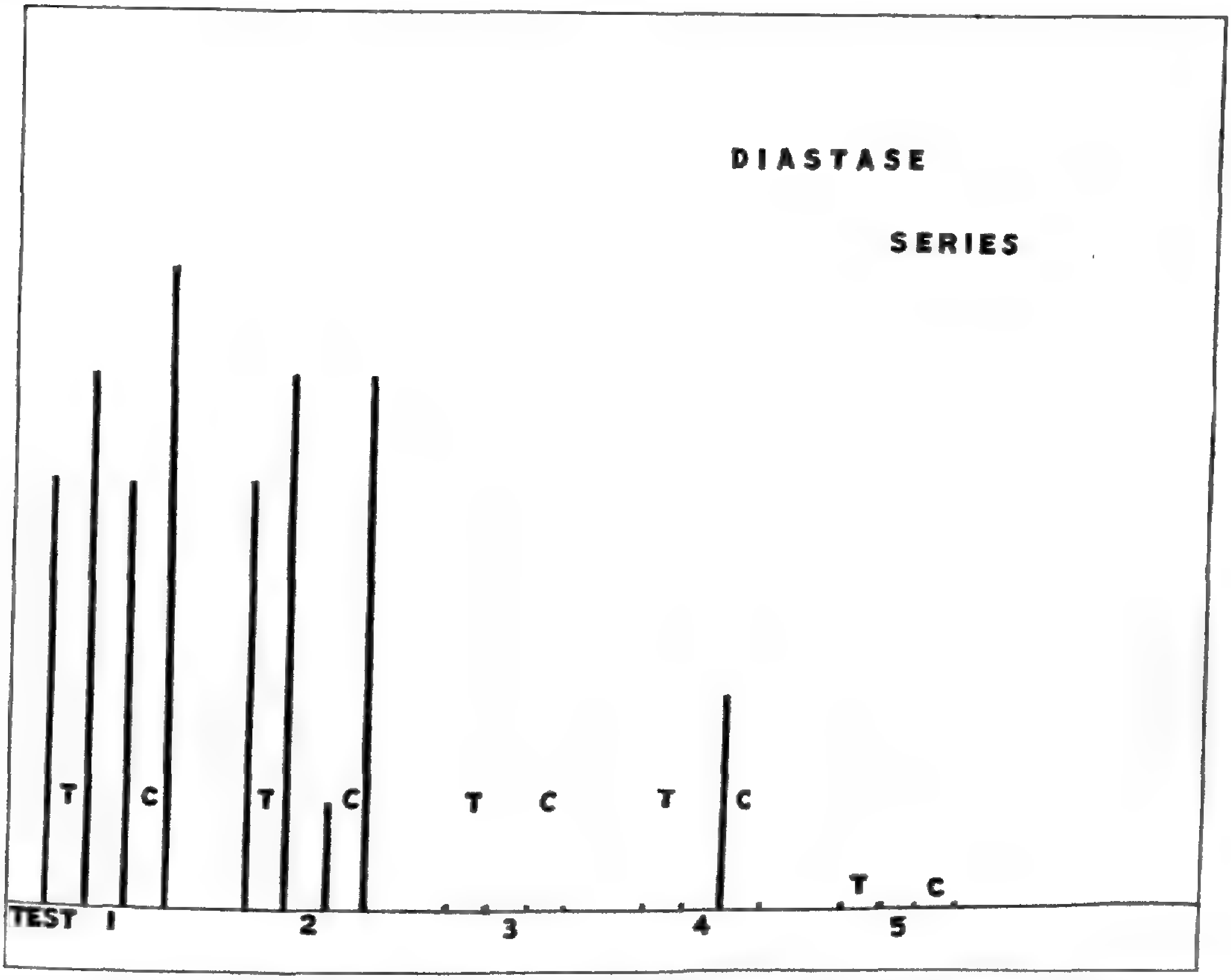

Fig. 8.—Time factor as related to starch digestion in presence of formaldehyde mixed 1–320 parts water: height of line indicates amount of starch remaining undigested (all conditions shown in duplicate); 1 cc. 0.5 per cent solution Merck's diastase, 10 cc. 25 per cent soluble starch solution, and 4 cc. formaldehyde used in tests (*T*), and a like amount of water in controls (*C*); time periods in hours tests 1 to 5 respectively, 1.25, 1.5, 1.75, 2, 2.5 hours.

grain was treated to each concentration ten minutes, allowed to drain, stand moist for two hours, and then thoroughly air dried before an electric fan. The grain was then thoroughly ground in a mill, and extracts of 8 gm. lots made in 100 cc. of redistilled water. Ascending quantities of the water extract were then added to test-tubes each containing 5 cc. of soluble starch prepared as for the other tests. After incubation for one hour at 40° C. complete digestion had occurred in the controls containing 5, 10, 15, and 20 cc. respectively of the extract of untreated seed. Fig. 9 shows that despite a few unexplained irregularities, the general trend is obviously a reduction in the amount of starch digestion, with a rise in the concentration of the formaldehyde originally used in treatment of the seed. This holds for 5, 10, 15, and 20 cc. seed extract tests

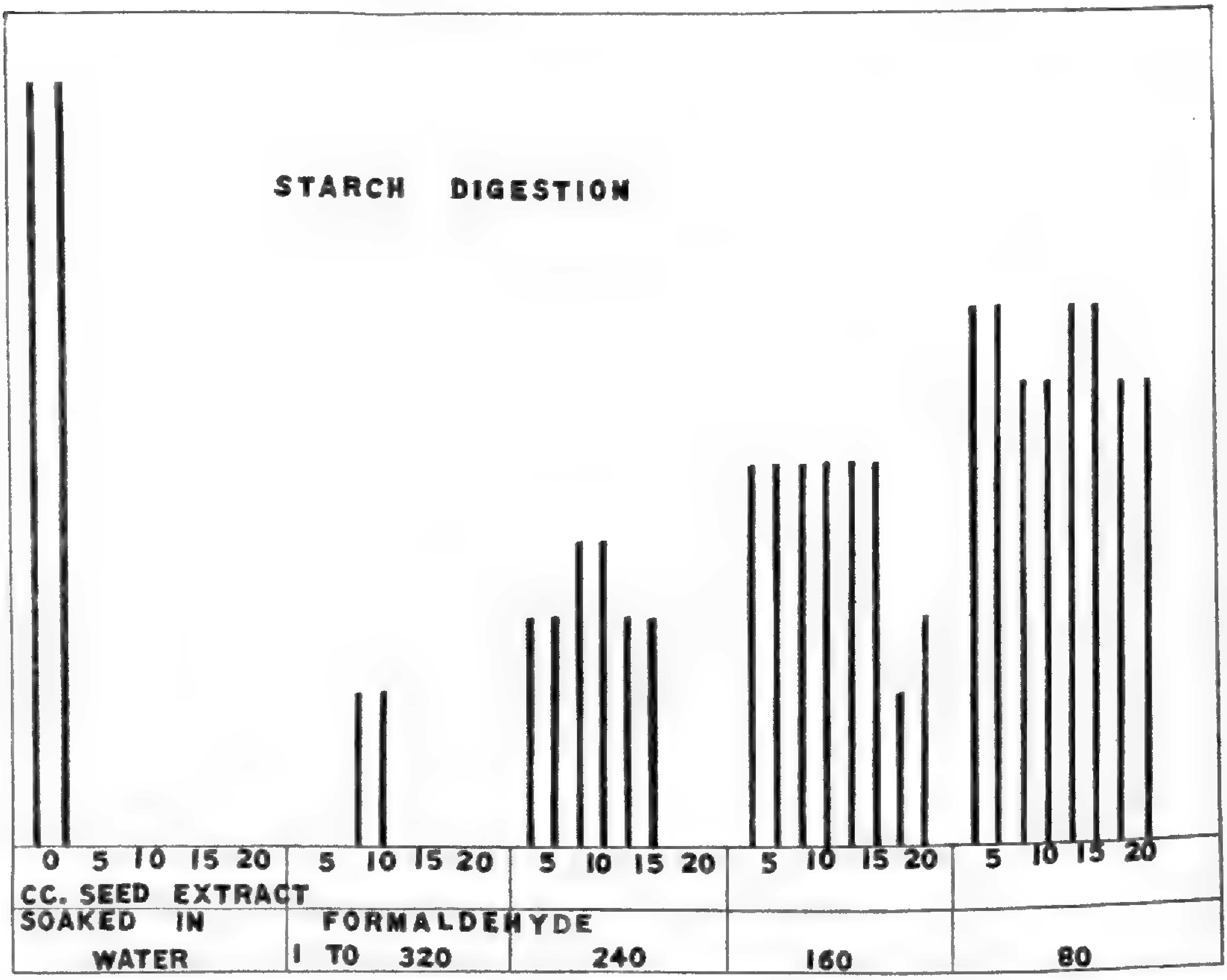

FIG. 9.—Effect of formaldehyde treatment on diastatic activity of wheat extract, seeds treated 10 minutes; concentration of formaldehyde used in treatment varied, and also cubic centimeters of seed extract used: height of lines indicates amount of starch remaining undigested after incubation one hour at 40° C.; 5 cc. 25 per cent soluble starch solution used in each test (all conditions shown in duplicate).

under all the conditions. From these data it would seem certain that treating wheat with formaldehyde retards the availability of carbohydrate to the germinating seedling. BOKORNY (12), reviewing the work of NEUBERG, in which variations showed in the inhibitory effects of different concentrations of formaldehyde on various enzymes and of the effects of the same concentration on different enzymes, explains differences in behavior on the theory of the molecular structure of the enzymes causing different linkages with the formaldehyde. Inasmuch as enzymes are commonly known to be associated at least with proteins, and as formaldehyde is known to react quantitatively with amino acids, as in the Sörensen titration, it is not surprising that effects of formaldehyde upon enzyme behavior should be observed.

AMINO ACIDS

In the light of the results of the tests on the diastatic activity effects of formaldehyde, it was thought well to determine the relationships to amino acids. Miss CHOATE (17) found amino acids occurring in ungerminated wheat and increasing in amount on germination. Miss ECKERSON (27) found only slight amounts of asparagine in the ripened grain, although histidine, leucine, asparagine, and arginine occurred during ripening. The chemical constitution of the wheat grain involves several linked amino acids according to OSBORNE (42), while ABDERHALDEN and SAMUELY (1) in a list of the amino acid constituents of gliadin of wheat flour give alanine, tyrosine, and glutamic acid as among those highest in amount.

As a preliminary test, known quantities of pure amino acids in water solution were determined by the Van Slyke method, both with and without the presence of varying amounts of formaldehyde. Alanine was secured from the organic laboratories of the Eastman Kodak Company, while glutamic acid hydrochloride and tyrosine were purchased from the Special Chemicals Company of Highland Park, Illinois. Careful checking through over 200 tests indicates that such linkages as are formed by formaldehyde and amino acids are broken by the Van Slyke process, and no reduction in nitrogen yields occurred incident to the presence of formaldehyde. The common use of the formol titration in the determination of amino acids is based on our knowledge that such linkages do occur. It had been assumed that in case such a combination between formaldehyde and amino acids of the germinating seedling does occur the nitrogenous nutrition might easily be disturbed. It is very much hoped that the opportunity may be afforded later to check further upon this point, and also to determine the comparative amount of amino acids liberated in autolysis of treated and untreated seeds.

RESPIRATION

Much effort has been expended in the determination of the effects of seed treatment upon the respiration. PEIRCE and co-workers (43) correlated germinative vigor with respiratory activity. Carbon dioxide has often been recognized as a measure of the activity of the metabolism in the tissues liberating the gas. It was desired to determine whether varying concentrations of formaldehyde, showing varying effects on viability, also affected carbon dioxide releasal in the same manner; in other words, whether the measure of formaldehyde injury may be had by the comparatively accurate carbon dioxide measurements.

Seed lots of 75 gm. each in duplicate were soaked in water as a check, and lots in duplicate in the varying concentrations of formaldehyde, period of soaking being ten minutes, after which they were drained and sealed in respiratory chambers submerged in a constant temperature bath at 28° C. for two hours before beginning the determination. Large museum jars were used for respiratory chambers, equipped with ground glass tops with openings for two-holed stoppers. The seeds were suspended on wire gauze six inches above the bottom of the chamber, while the tubing by which the gases were withdrawn from the chamber during the tests extended to the bottom of the jars. The water bath was 1.5 by 3 feet, and deep enough to permit the tall museum jars to be completely submerged in the water. Under the water bath were placed six porcelain resistance units connected to the lighting system of the laboratory. About 6 feet of small bore glass tubing was bent so as to be submerged in the bath, and filled with mercury, which served to conduct current from two gravity cells to a telegraphic relay, which at the desired temperature turned off or on the heat under the bath. This arrangement permitted control of the temperature within 0.2° C. Careful checking of the temperature at different points in the bath indicated that stirring devices were not necessary, other than the convection currents from the bottom of the bath upward. Two chambers were used for water soaked wheat (controls), two for the treated wheat, and two blanks to permit checking against leakage.

After setting up the apparatus completely and before making a determination, each of the six complete trains was tested by suction as to its ability to hold up a column of mercury 10 inches high without small leaks permitting the column to settle back again. During the tests a gentle stream of air freed from carbon dioxide was drawn through each outfit for the entire period of hours of the run. In order that the rate of aeration might be uniform in the various outfits, and ample to provide for several complete changes of the air in the respiratory chambers during the course of the experiments, the suction secured from a water pump was conducted to the various chambers through tubing, connected to manometers in such a way that after careful calibration of the separate manometers, the rate of air flow could instantly be determined by a glance at the height of the paraffin oil surface in the manometers. Gas meters of this type were developed in connection with the chemical investigations incident to the recent gas warfare work, and are described in detail by BENTON (11). For each of the six trains air was drawn respectively through 50 per cent potassium hydroxide, a *U*-tube of moist soda lime, and through a barium hydroxide indicator to detect any failure of previous absorbents to remove all carbon dioxide. Air entered the respiratory chamber at the top and was removed from the bottom under the wheat arranged as described. The air then containing the carbon dioxide released by the wheat was drawn immediately through a bead tower containing fourth normal barium hydroxide, out and over another barium hydroxide indicator before passing to the tube connected to the water pump.

The amount of suction was regulated by ground glass stopcocks between the pump and the last indicator flask. With the six complete trains to provide for, it was not deemed practicable to apply the type of automatic pipette arrangement used by Bailey and Gurjar (9). Instead, a large bottle was thoroughly cleaned and aerated with carbon dioxide-free air and filled with the standard alkali. This reservoir was connected by tubing with a burette, and communication with outside air protected by soda lime traps. At the top of each of the bead tower columns was placed a separatory funnel guarded by a soda lime trap. Before each running the required number of cubic centimeters of the alkali were run directly into the separatory funnel previously washed free of carbon dioxide.

When the whole outfit was ready to make a running, carbon dioxide-free air was run for a sufficient period through the bead tower column to remove all carbon dioxide, before admitting the alkali from the separatory funnel directly into the bead column. This method of determination of carbon dioxide is essentially that described by Truog (54). Varying periods of aspiration in these measurements were employed, although experience showed that most satisfactory results could be obtained by employing a period of from four to five hours. No results were considered worthy of recording for second runs of any one lot of samples, as experience showed the need of extreme care to avoid introducing errors incident to the growth of saprophytes upon the check samples, particularly if they were retained at 28° C. longer than one day. Bailey and Gurjar (9) allowed their moist wheat to stand several days before removing the stagnant air for carbon dioxide determination. Had they used the temperature of 28° C., and had their seed possessed a moisture content of 35–43 per cent, as was the case in these tests, it would have been impossible to avoid questioning the secondary factor of saprophytes which these experiments showed increased tremendously the output of carbon dioxide. Disregarding the fact that they failed entirely to keep their chambers aerated during the course of their work, however, it must be said that they incubated their seeds at 37.8° F., and worked with seeds of moisture contents much lower, in general between 12 and 20 per cent. Nabokich (41) concluded that part of the carbon dioxide obtained in plant respiration is incident to the same microorganisms that vegetate on leaves and seeds. It was hence a source of much concern to avoid any fluctuations in respiratory results incident to the gaseous exchanges of saprophytes which might easily be confused with the results of seed treatment. NABOKICH, however, determined that the respiration of microorganisms on seeds may be disregarded during the first day, counting from the time of wetting the seeds. It is thus believed that the data given here eliminate the errors incident to such secondary factor.

Throughout the work over forty runnings were made, representing over 160 different seed lots. As regards the possible criticism that the several per cent variation in moisture content based on dry weight might make the results incomparable, it must be borne in mind that absolute carbon dioxide yields of different runnings are not to be compared with each other, but only the four lots used in any one run. Careful analysis of the variations in carbon dioxide output as related to moisture content has indicated that these variations may not be ascribed to moisture content differences, these observations being made in duplicate independently for the four seed lots of any one running. Fig. 10 and table II summarize the entire results of the investigations on respiration. In all of the work care was taken to have present in the flasks and bead towers at least twice as much of the alkali as would be neutralized by the carbon dioxide liberated by the seeds during any one run. The graphs are expressed in terms of the percentages of the barium hydroxide neutralized. In each case 25 cc. of fourth normal barium hydroxide was used, and at the close titrated against fourth normal hydrochloric acid in the presence of phenolphthalein. If, for instance, 12.5 cc. was neutralized the graph would express 50 per cent values.

Fig. 10 shows marked depression of the respiratory rate for the highest concentrations (1–80) as compared with the water soaked controls. The depression of the respiration rate is evident, although decreasingly so, at 1–160, 1–240, and down to 1–320, the concentration usually used in seed treatment. At 1–400 and 1–1000 the difference between the controls and the treated samples was neither so great nor so constant as to indicate any marked effects of the formaldehyde on metabolism. Special care was used in checking out the situation at the 1–320 concentration, at which point

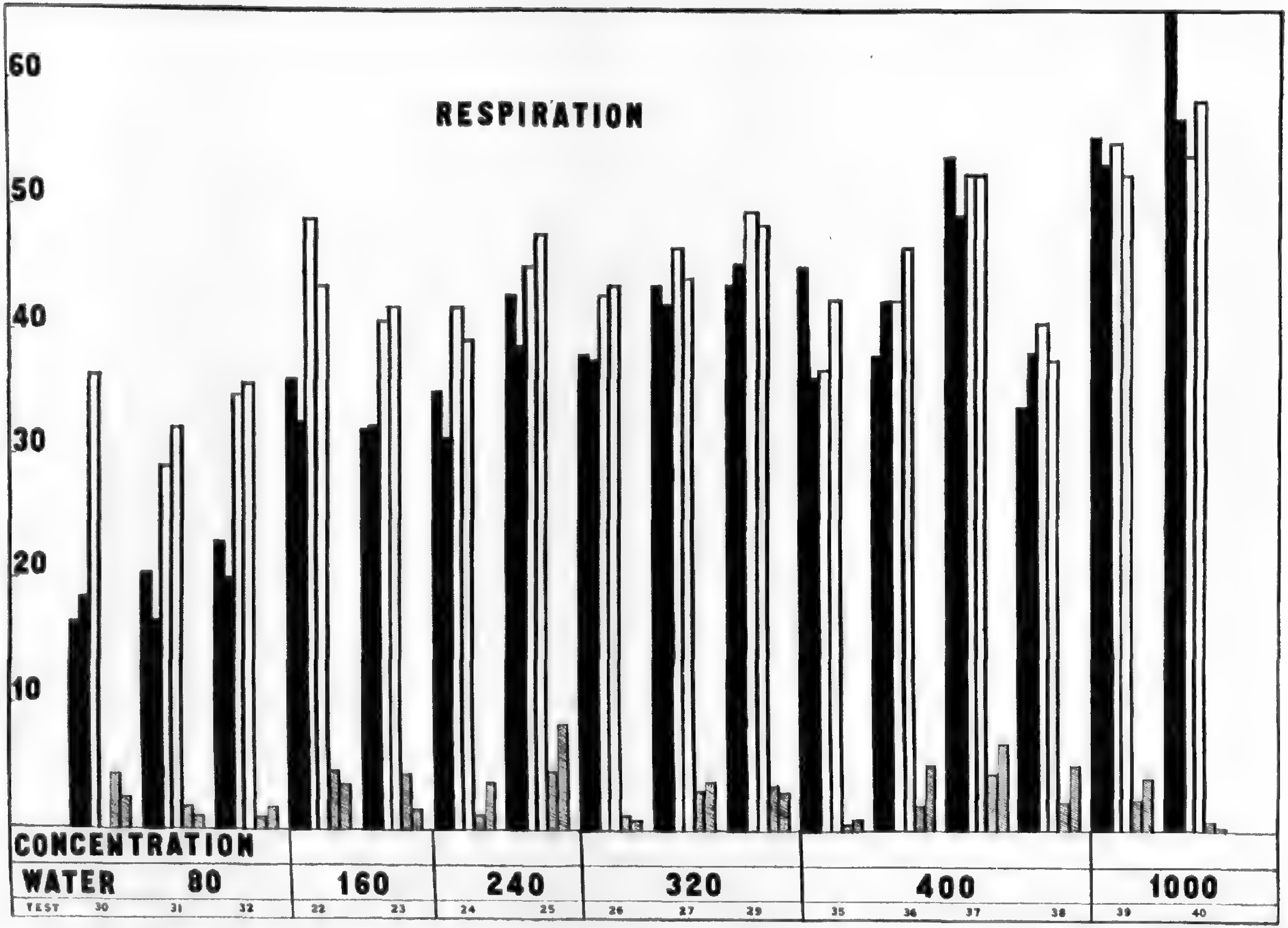

FIG. 10.—Respiration of wheat as influenced by treatment with formaldehyde of varying concentrations; results expressed in terms of percentage of neutralization of total number of cubic centimeters of alkali by carbon dioxide released by seeds; absorption in each case in 25 cc. N/4 barium hydroxide solution; six determinations for each test shown: solid black bars indicate carbon dioxide trapped from treated seeds, white bars indicate magnitudes for water-soaked control seeds, short bars with cross-lining indicate carbon dioxide from empty controls (error).

TABLE II

DATA ON RESPIRATION TESTS AS SHOWN IN FIG. 10

No. of test			30	31	32	22	23	24	25	26	27	29	35	36	37	38	39	40
Hours in respirometer before beginning test			2	2	24	2	2	2	2	2	2	2	5.5	2	2	2	3	24.5
Hours test			4.5	4	4	5	4	4.75	5	4.25	5.5	5	4.5	5	5	4	5.5	4
Hours seeds stood moist after treatment			2	2	2	2	2	2	2	2	2	2	5.5	2	2	2	3	24.5
Parts water to one part of formaldehyde			80	80	80	160	160	240	240	320	320	320	400	400	400	400	1000	1000
		Test																
Treated wheat	Percentage moisture at end of test	1	40.15	38.85	38.85	......	42.25	36.09	36.92	37.90	39.30	39.38	39.20	38.70	40.50	......	43.60	43.60
		2	39.20	36.85	36.85	......	40.23	35.25	37.70	39.70	38.80	37.60	35.65	36.35	38.80	......	41.00	41.00
	Cc. barium hydroxide combined	1	4.10	5.11	5.72	8.95	7.97	8.95	10.59	9.45	10.77	10.81	11.16	9.44	13.36	8.48	14.02	16.33
		2	4.63	4.16	5.01	8.12	8.03	7.76	9.58	9.34	10.44	11.17	8.95	10.48	12.21	9.50	13.17	14.12
	Percentage cc. hydroxide combined	1	16.48	20.52	23.00	36.10	32.10	35.10	42.70	38.10	43.42	43.60	44.90	37.92	53.70	33.91	55.14	65.70
		2	18.60	16.72	20.14	32.72	32.40	31.30	38.65	37.62	42.10	45.10	36.10	42.20	49.10	38.20	53.00	56.80
Water-soaked control	Percentage moisture at end of test	1	......	36.40	36.40	......	41.60	36.40	37.00	41.00	37.30	37.80	37.88	39.90	38.40	......	40.00	40.00
		2	37.40	36.85	36.85	......	39.67	35.20	40.18	41.48	40.00	......	36.60	42.28	39.48	......	38.45	38.45
	Cc. barium hydroxide combined	1	......	7.24	8.63	12.14	10.08	10.37	11.15	10.59	11.55	12.25	9.13	10.44	12.97	10.03	13.61	13.33
		2	9.03	8.01	8.91	10.75	10.40	9.72	11.80	10.82	11.14	11.98	10.53	11.51	13.01	9.33	12.98	14.41
	Percentage cc. hydroxide combined	1	......	29.10	34.70	48.90	40.70	41.80	45.00	42.70	46.60	49.4	36.70	42.00	52.20	40.38	54.70	53.63
		2	36.30	32.20	35.80	43.4	41.90	39.2	47.6	43.6	44.95	48.40	42.40	46.30	52.30	37.5	52.20	58.00
Empty controls (error)	Cc. barium hydroxide combined	1	0.65	0.48	0.26	1.19	1.15	0.30	1.17	0.60	0.72	0.90	0.13	0.48	1.16	0.56	0.61	0.18
		2	1.13	0.28	0.46	0.92	0.42	0.95	2.12	0.20	1.00	0.78	0.08	1.33	1.76	1.31	1.06	0.08
	Percentage cc. hydroxide combined	1	2.61	1.93	1.05	4.80	4.64	1.21	4.72	1.14	2.90	3.63	0.52	1.93	4.66	2.25	2.45	0.72
		2	4.54	1.13	1.85	3.71	1.70	3.83	8.55	0.81	4.03	3.15	0.32	5.35	7.08	5.27	4.26	0.32

evidently the concentration is near the border line of injury, since 1–400 does not definitely display such uniform depression of respiration. It was desired to compare the respiratory rates of young seedlings from treated and untreated wheat, but as yet technique has not been devised which avoids the luxuriant development of *Rhizopus* in the warm moist atmosphere of the respirometer. In these tests, as in those with the seeds alone, special care was used to avoid air infection of the respirometers. Just previous to a running, the interior of each jar was wiped out with cotton moistened with mercuric chloride (1–1000). The further precaution was taken of flaming the gauze on which seeds and seedlings were placed. KARCHEVSKI, as quoted by BAILEY and GURJAR (**9**), found the energy of wheat respiration as measured by carbon dioxide releasal twelve times greater for the embryos than for the entire seeds. This seems to indicate that the data may be more largely influenced by factors affecting the embryos than otherwise. The facts that formaldehyde denatures proteins, that the embryo is rich in proteins, and that the respiration data show the effects of formaldehyde treatment, make it possible that the injurious effects of formaldehyde are intimately connected with injury to the embryo itself. This is in harmony with the findings of COLLINS (**18**), in a study of the coat of the barley grain, that the entry of solutions and hence the seat of selectivity is in the germ end of the grain.

It is of interest in this connection to note that although Miss HURD (**33**) believed the injurious effects of formaldehyde to be attained by a slow absorption of the gas liberated from paraformaldehyde, and although these studies of the penetration of formaldehyde do not show any sudden penetration of the fungicide, nevertheless within a period of time as short as three to six hours during respiratory determinations, some effect is exerted upon the seed which very definitely modifies the respiratory rate as compared with water soaked controls. One can but wonder whether here, as in the case of the studies of CROCKER and KNIGHT (**24**), we may not have in plant responses a more delicate indicator of injury than are the chemical reactions commonly used in detecting these injurious substances.

CATALASE

The work of recent years has shown that there often seems to be a relationship between respiration of plant tissues and the catalase content. APPLEMAN (4, 5) has shown this relationship in the case of potatoes and corn, CROCKER and HARRINGTON (23) in the case of seeds. The latter workers find that this relationship is not universal, for while imbibed Johnson grass has its respiration paralleled by the catalase activity, this is not true for the seeds of *Amaranthus*. The most interesting observation is further made that in the case of Johnson grass where this parallelism is found, neither the respiratory activity nor the catalase content is paralleled by the vitality of the seeds or the seedlings. Inasmuch as seed vitality and seedling vigor are definitely related to formaldehyde treatment, and this work has linked in also the effects upon respiration, it was thought to be of interest to determine whether any effects of seed treatment could be noted upon the catalase activity of wheat.

Catalase activity in wheat was determined much after the method suggested by APPLEMAN (2), and later employed with various modifications by other workers (17). Two series of experiments were conducted, one to see whether any effects of formaldehyde could be noted immediately after treatment while the grain was still moist, and another to see whether the effects of treatment persist on grain which has been treated and air dried before an electric fan in the laboratory and subsequently exposed for about a month to the air of the laboratory. The concentrations of formaldehyde used were 1–80, 1–160, and 1–320. About 1 gm. of air dried wheat was used in each case, weighed on the analytical balance, and results computed to the basis of 1 gm. For a reaction chamber a bottle of 250 cc. capacity was used and shaken continuously during the ten minutes of the test by a mechanical shaker making 129 excursions per minute. The reaction chamber was submerged in the constant temperature bath previously described, and was kept at 28° C. Dioxygen was used and neutralized with calcium carbonate. It was found that 3 mg. of the chemically pure salt used would neutralize 5 cc. of the peroxide, and this proportion was observed throughout. The gas evolved was run into a 100 cc. gas burette, and all readings corrected for temperature and barometric pressure, the equivalent corrected volumes at 0° C. and 760 mm. being computed for 1 gm. sample. Ten cc. water and 5 cc. peroxide as neutralized were used.

In the case of the freshly treated seeds, they were soaked for ten minutes in the formaldehyde solutions, and kept moist for two or more hours, until tested. The moisture of the seeds precluded the possibility of passing the material through bolting cloth (**23**), but it was soon found that a material difference in the crop of oxygen given off resulted if the seed material was more or less glutinous and forming sticky masses, or dry enough to separate fairly well on grinding in a mortar with carborundum as an abrasive; hence all surface moisture was removed with filter paper before grinding up the seeds. Inasmuch as differing moisture contents of the differing lots would occur between the first lots tested and the last in a long series, the errors incident to this cause were avoided

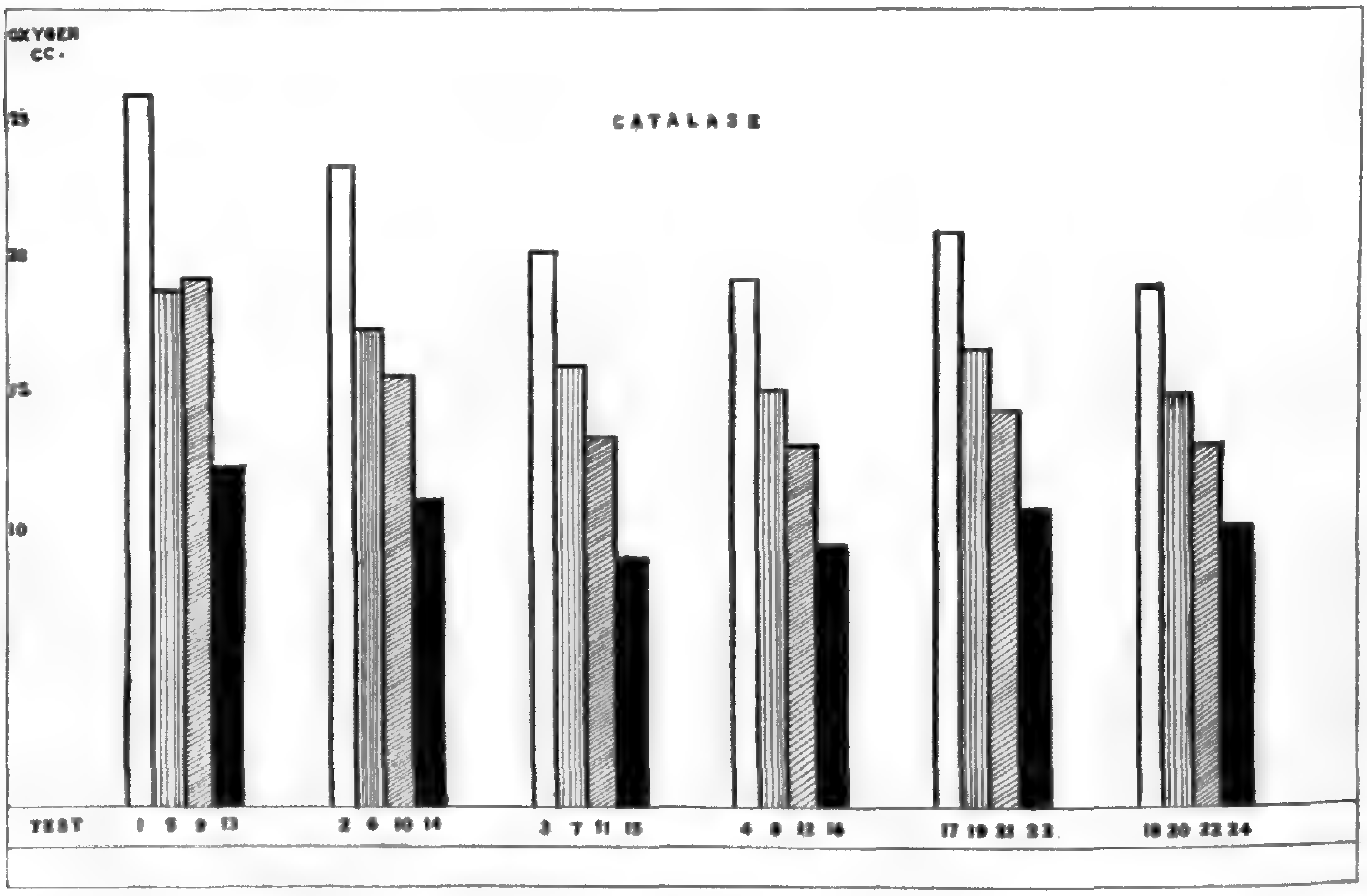

FIG. 11.—Effect of formaldehyde in varying concentrations upon ability of seed extract to liberate gaseous oxygen from dioxygen: height of lines indicates cubic centimeters of oxygen computed for 1 gm. samples and constant temperatures and pressures; extract from water-soaked seeds (controls) indicated by white, vertical lining indicates extracts from seeds treated in formaldehyde 1–320, diagonal lining formaldehyde 1–160, and solid black formaldehyde 1–80; in all cases seeds tested while still moist.

as far as possible by testing consecutively the control seeds and those treated with 1–320, 1–160, and 1–80. Such a series of four naturally fall together for comparison in one group, and many such series were made, the combined results of which may easily be seen from fig. 11 and table III. With some small variations, the results were surprisingly uniform in showing the steady depressing effect of formaldehyde on catalase activity. As the concentration of the formaldehyde rose, the catalase activity as measured by oxygen yield fell.

TABLE III

DATA ON CATALASE TESTS AS SHOWN IN FIG. 11

Test no.	Concentration formalin	Weight of sample	Volume oxygen released	Barometer	Temperature	Hours between treatment and test	Corrected volume equivalent of 1 gm. sample
1.........	Water control	1.015	30.1	748	24	2	25.9
2.........	Water control	1.005	26.9	748	24	3.5	23.4
3.........	Water control	1.006	23.5	748	25	7	20.3
4.........	Water control	1.009	22.4	748	25	8.5	19.3
5.........	1–320	1.008	21.8	748	24	2.5	18.9
6.........	1–320	1.000	20.1	748	24	3.75	17.5
7.........	1–320	1.004	18.6	748	25	7.25	16.1
8.........	1–320	1.002	17.6	748	25	9	15.3
9.........	1–160	1.006	22.2	748	24	2.75	19.3
10.........	1–160	1.015	18.3	748	24	3	15.8
11.........	1–160	1.007	15.7	748	25	7.5	13.6
12.........	1–160	1.002	15.3	748	25	9	13.3
13.........	1–80	1.010	14.4	748	24	3	12.5
14.........	1–80	1.011	13.0	748	24	3	11.3
15.........	1–80	1.006	10.6	748	25	8	9.20
16.........	1–80	1.010	11.2	748	25	9.5	9.63
17.........	Water control	1.017	24.6	747	24.5	5	21.1
18.........	Water control	1.012	22.3	745.5	25	7.75	19.1
19.........	1–320	1.007	19.4	747	24.5	5	16.8
20.........	1–320	1.010	17.7	745.5	25	8.25	15.2
21.........	1–160	1.003	16.7	747	24.5	5.25	14.5
22.........	1–160	1.011	15.5	745.5	25	8.5	13.3
23.........	1–80	1.003	12.7	747	24.5	5.5	11.0
24.........	1–80	1.013	12.1	745.5	25	8.75	10.4

In the studies of the effects of formaldehyde on catalase activity after the seeds had been dried about a month in the laboratory, comparison was made only between the control and the seeds treated in 1–80 formaldehyde. Fig. 12 and table IV show that there is a definite depressive effect of the treatment on catalase activity, but it is by no means so great as soon after treatment.

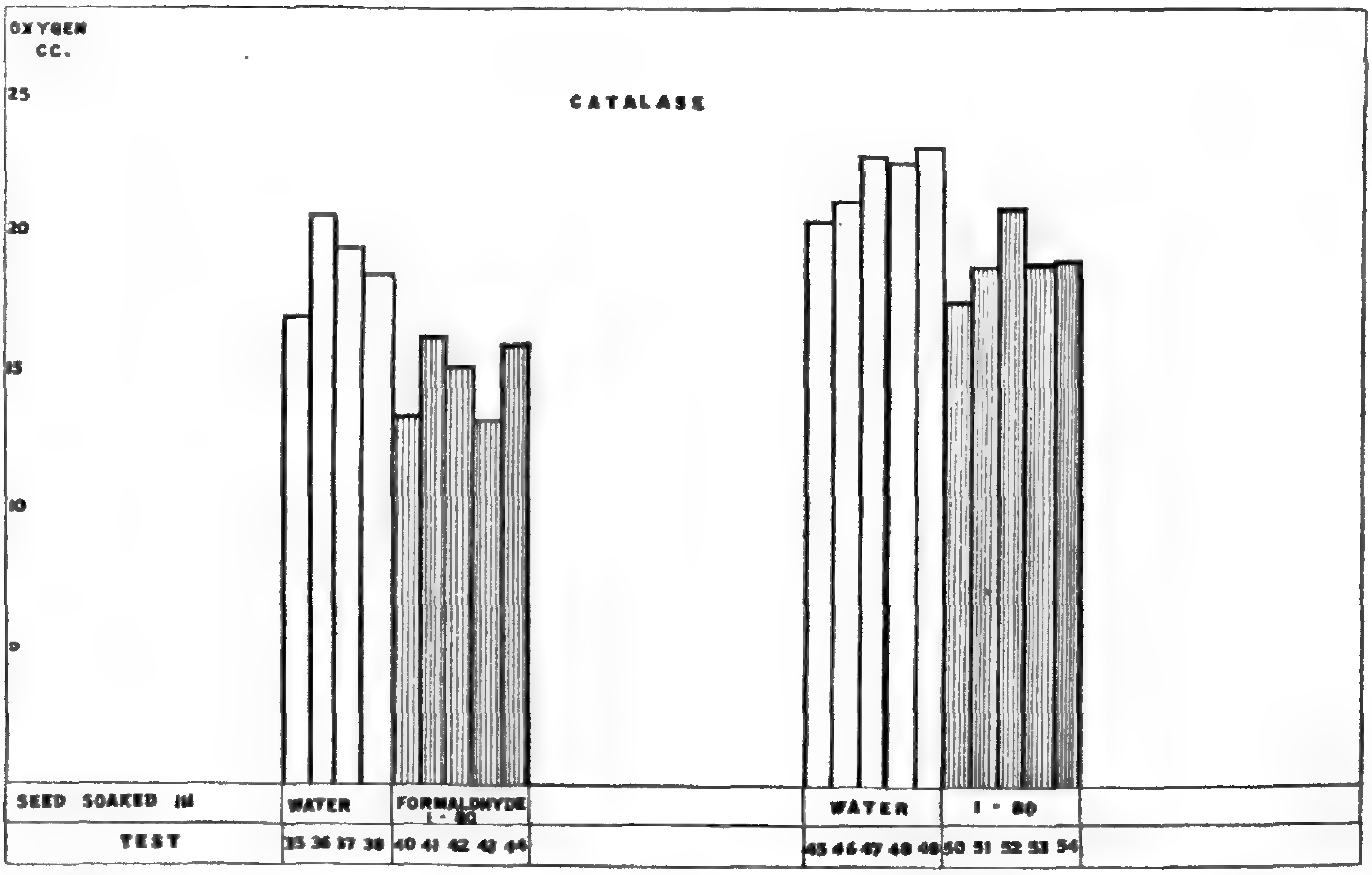

FIG. 12.—Effect of formaldehyde 1 to 80 upon ability of seed extract to liberate gaseous oxygen from dioxygen: height of lines indicates cubic centimeters of oxygen computed for 1 gm. samples and constant temperatures and pressures; extract of water soaked seeds (controls) indicated by white, treatment in 1–80 indicated by vertical ruling; in all cases seeds tested after thoroughly drying one month in laboratory after treatment.

TABLE IV

DATA ON CATALASE TESTS AS SHOWN IN FIG. 12

Test no.	Concentration formalin	Weight of sample	Oxygen (cc.)	Barometer	Temperature	Corrected volume equivalent of 1 gm. sample
35	Water control	1.014	20	743.5	25	17.10
36	Water control	1.002	24	743.5	25	20.7
37	Water control	1.005	22.5	743.5	23	19.5
38	Water control	1.006	21.5	743.5	24	18.6
40	1–80	1.014	15.7	743.5	24	13.5
41	1–80	1.005	18.8	743	23	16.3
42	1–80	1.002	17.5	743	23	15.2
43	1–80	1.020	15.5	743	23	13.3
44	1–80	1.000	18.5	743	23	16.1
45	Water control	1.011	23.5	745	21.5	20.5
46	Water control	1.011	24.2	745	20.5	21.2
47	Water control	1.012	26.1	745	20.5	22.9
48	Water control	1.014	25.9	745	21	22.6
49	Water control	1.014	26.7	745	21.5	23.2
50	1–80	1.006	20.0	745	21.5	17.6
51	1–80	1.003	21.3	745	20.5	18.8
52	1–80	1.002	23.8	745	21	21.0
53	1–80	1.011	21.8	745	21.5	19.0
54	1–80	1.001	21.7	745	21.5	19.1

This would seem to support the view that the injury is due more to exterior members retaining the formaldehyde which had been volatilized in part, than to a permanent injury to the embryo having resulted from the treatment.

General considerations

The treatment of seeds with fungicides is a process wherein one plant tissue (that of the parasite) must be destroyed, while another tissue (that of the seed) must be conserved. It is entirely probable that the points of fungicidal effectiveness and of danger to seeds are not far separated. DE ZEEUW (26), in noting this point, quotes work in which it was found that the seeds were more sensitive to formaldehyde than spores of either bacteria or fungi, when its action was deeper than the surface, as is essential to secure sterile seeds. He believes that the high concentrations of disinfectants required to care for the destruction of bacterial spores is explained by the protection afforded by the seed coat on which the spores are lodged. There is an interesting similarity between this statement and the findings of REIMER (46), who observed that in the control of the bacillus producing fire blight of pears, a disinfectant which is serviceable upon tools is ineffective when used upon the organic substrate of the wood of the tree itself.

It would seem that we have been too ready to jump to conclusions and give "recommendations" as to treatment upon the basis of germination data of more or less extent. In the light of the preceding results, it seems doubtful whether it is safe to postulate the boundary lines of safe and dangerous concentrations merely upon the basis of germination data. STEPHENS (50) has emphasized the relationship of seed treatment to subsequent lowered vitality of seedlings. It is thus entirely possible that concentrations which do not materially injure germination percentages do materially disturb the physiological processes related to germination and subsequent growth. Common agricultural practice and the findings of the War Emergency Board of American plant pathologists seem to indicate that 1–320 is at the edge of the danger zone, if indeed such zone is not here passed. If, as Miss HURD (33) believes, a polymer of formaldehyde is deposited on dried treated wheat, and subsequent injury to the grain is incident to the liberation of formaldehyde gas from the precipitate, with its resultant solution in the moisture content of the living cells, the process must be a slow cumulative one which is entirely in harmony with the definite although slow entry of formaldehyde herein shown actually to occur. Furthermore, even though the grain be treated with 1–320 formaldehyde, such a deposit of the polymers would result in the presentation to the living cells of a much stronger concentration than that of the dip used. Yet even at the concentration of 1–320 it is evident that while germination is often but lightly affected, the diastatic power of wheat is retarded, the catalases are less active, and respiration is definitely reduced. It is not impossible that such results indicate a decided tendency to a reduction in seedling vitality even in the presence of germination.

It is highly desirable that these studies be pursued further to determine the relationship of treatment to the proteolytic activities of the germinating seed, and further, to determine whether the recommended "presoak" of BRAUN (13) or the "post-washing" of Miss HURD (33) correspondingly modify the physiological activities and alleviate their injury.

Summary

1. Tests of formaldehyde entry into wheat have been made both by microchemical tests and by imbibitional studies, indicating that formaldehyde slowly enters through the seed coat.
2. Diastatic activity of the grain is retarded.
3. Respiration is slowed down.
4. Catalases are reduced in their ability to break down peroxides.

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LITERATURE CITED

1. ABDERHALDEN, E., and SAMUELY, FRANZ, Die Zusammensetzung des "Gliadins" des Weizenmehls. Zeit. Physiol. Chem. **44**:276–283. 1905. Abs. in Jour. Chem. Soc. **88**:620. 1905.
2. APPLEMAN, C. O., Some observations on catalase. BOT. GAZ. **50**:182–192. 1910.
3. ———, Physiological behavior of enzymes and carbohydrate transformations in after-ripening of the potato tuber. BOT. GAZ. **52**:306–315. 1911.

4. Appleman, C. O., Relation of catalase and oxidases to respiration in plants. Bull. 191, Md. Agric. Exp. Sta. pp. 16. 1915.
5. ———, Relation of oxidases and catalase to respiration in plants. Amer. Jour. Bot. **3**:223–233. 1916.
6. Arthur, J. C., Formalin for grain and potatoes. Ind. Agric. Exp. Sta. Bull. **77**:38–44. 1900.
7. Atwood, W. M., A physiological study of the germination of *Avena fatua*. Bot. Gaz. **57**:386–414. 1914.
8. Auerbach, F., and Barschall, H., Studies of formaldehyde. II. The solid polymers of formaldehyde. Arb. Kais. Gesundheitsamt. **27**:183–230. 1908. Abs. in Chem. Abs. **2**:1125. 1908.
9. Bailey, C. H., and Gurjar, A. M., Respiration of stored wheat. Jour. Agric. Res. **12**:685–715. 1918.
10. Bakke, A. L., and Plagge, H. H., Studies on the absorption and germination of wheat treated with formaldehyde. Ia. Acad. Sci. **26**:365–375. 1919.
11. Benton, A. F., Gas flow meters for small rates of flow. Jour. Ind. and Eng. Chem. **11**:623–629. 1919.
12. Bokorny, T., Bindung des Formaldehyds durch Enzyme. Biochem. Zeitschr. **94**:69–77. 1919. Abs. in Bot. Abs. **8**:641.
13. Braun, Harry, Presoak method of seed treatment. Jour. Agric. Res. **19**:363–392. 1920.
14. Brittlebank, C. C., The effect of formalin and copper sulphate solution on the germination of wheat. Jour. Dept. Agric. Victoria **11**:473–476. 1913.
15. Brown, Adrian J., On the existence of a semipermeable membrane inclosing the seeds of some of the Gramineae. Ann. Botany **21**:79–87. 1907.
16. Burmester, H., Comparative investigations on the effect of various methods of seed treatment on the germination of seed. Zeitschr. Pflanzenkrank. **18**:154–187. 1908.
17. Choate, Helen A., Chemical changes in wheat during germination. Bot. Gaz. **71**:409–425. 1921.
18. Collins, E. J., The structure of the integumentary system of the barley grain in relation to the localized water absorption and semi-permeability. Ann. Botany **32**:381–414. 1918.
19. Coons, G. H., and McKinney, H. H., Formaldehyde injury to wheat. 21st Mich. Acad. Sci. Rept. 321–324. 1919.
20. Cranefield, F., The influence of formalin on the germination of oats. Wis. Sta. Rept. 327–335. 1901.
21. ———, The influence of formaldehyde on the germination of oats. Wis. Sta. Rept. 268–272. 1902.
22. ———, Treatment of seed oats for the prevention of smut. Wis. Sta. Rept. 363–367. 1903.

23. CROCKER, WILLIAM, and HARRINGTON, GEORGE T., Catalase and oxidase content of seeds in relation to their dormancy, age, vitality, and respiration. Jour. Agric. Res. **15**:137–174. 1918.
24. CROCKER, WILLIAM, and KNIGHT, LEE I., Effect of illuminating gas and ethylene upon flowering carnations. BOT. GAZ. **46**:259–276. 1908.
25. DELEPINE, MARCEL, Formaldehyde in gaseous liquid and solid states and in solution. Bull. Sci. Pharmacol. **16**:146–160. 1910. Rev. in Chem. Zentr. 1909 (I). 1467.
26. DE ZEEUW, RICHARD, The comparative viability of seeds, fungi, and bacteria when subjected to various chemical agents. Centralb. Bakt. 2 Abt. **31**:4–23. 1912.
27. ECKERSON, SOPHIA H., Microchemical studies in the progressive development of the wheat plant. Bull. 139, Agric. Exp. Sta. Wash. 1917.
28. GUARCO and JEROME, Preventing polymerization of formaldehyde. Rev. in Chem. Abs. **3**:2202. 1909.
29. HAAS, PAUL, and HILL, T. G., An introduction to the chemistry of plant products. Longmans Green & Co. Citation page 149.
30. HAYWOOD, J. K., and SMITH, B. H., A study of the hydrogen peroxide method of determining formaldehyde. Jour. Amer. Chem. Soc. **27**:1183–1188. 1905.
31. HEALD, F. D., and WOOLMAN, H. M., Bunt or stinking smut of wheat. Bull. 128, Agric. Exp. Sta. Wash. pp. 24. 1915.
32. HENDERSON, L. F., Experiments with wheat and oats for smut. Bull. 53, Agric. Exp. Sta. Idaho, pp. 15. 1905.
33. HURD, ANNIE MAY, Injury to seed wheat resulting from drying after disinfection with formaldehyde. Jour. Agric. Res. **20**:209–244. 1920.
34. ———, Seed coat injury and viability of seeds of wheat and barley as factors in susceptibility to molds and fungicides. Jour. Agric. Res. **21**: 99–122. 1921.
35. JOHNSON, A. G., STAKMAN, E. C., and POTTER, ALDEN A., Preliminary review of results on seed injury tests with standard disinfectants. Report from Office of Cereal Investigations, U.S. Bur. Pl. Ind. 1918.
36. KIESSLING, L., Experiments on the germ ripening of grain. Landw. Jahrb. Bayern **1**:449–514. 1911.
37. MACKIE, W. W., Progress report on cereal smuts. Smut control measures practised in California. 1918 (unpublished).
38. MCALPINE, D., Effect of formalin and bluestone on germination of seed wheat. Bull. 12, Dept. Agric. So. Aust. pp. 21. 1908.
39. MELHUS, I. E., A quick method of eliminating seed borne organisms of grain. Science N.S. **50**:21. 1919.
40. MUELLER, H., and MOLZ, E., Experiments on the combating of stinking smut in winter wheat by means of the formaldehyde treatment. Fühlings Landw. Z. **63**:742–752. 1914. Rev. in Chem. Abs. **9**:2122. 1915.

41. NABOKICH, A. J., Über den Einfluss der Sterilization der Samen auf die Atmung. Ber. Deutsch. Bot. Gesells. **21**:279–291. 1903.
42. OSBORNE, THOMAS B., The proteins of the wheat kernel. Carnegie Inst. Publ. no. 84. pp. 119. 1907.
43. PEIRCE, G. J., DARSIE, M. L., and ELLIOTT, C., A study of the germinating power of seeds. BOT. GAZ. **58**:101–136. 1914.
44. PERDRIX, L., Bactericidal action of dry formaldehyde. Ann. Inst. Pasteur **29**:701–719. 1908.
45. PICCHIO, G., On the germination of wheat treated with some fungicides and insecticides. Coltivatore **59**:435–439. 1913. Rev. in Exp. Sta. Rec. **30**: 837. 1914.
46. REIMER, F. C., Unpublished work at the Southern Oregon Branch Station, Talent, Oregon.
47. SCHROEDER, H., The resistance of wheat and barley grains to poisons with special reference to seed sterilization. Centralb. Bakt. 2 Abt. **28**:492–505. 1910.
48. SHERMAN, H. C., KENDALL, E. C., and CLARK, E. D., Studies on amylases. I. An examination of methods for the determination of diastatic power. Jour. Amer. Chem. Soc. **32**:1073–1086. 1910.
49. SHULL, C. A., Semipermeability of seed coats. BOT. GAZ. **56**:169–199. 1913.
50. STEPHENS, D. E., Unpublished annual reports from the Sherman County Dry Farm Branch Station, Moro, Oregon.
51. STEVENS, F. L., Experiments upon the effect of formalin on the germination of oats. N.C. Agric. Exp. Sta. Rept. 30–36. 1908.
52. STEWART, R., and STEPHENS, J., The effect of formalin on the vitality of seed grain. Bull. 108, Agric. Exp. Sta. Utah, 145–156. 1910.
53. THOMAS, CECIL C., Seed disinfection by formaldehyde vapor. Jour. Agric. Res. **17**:33–39. 1919.
54. TRUOG, E., Methods for the determination of carbon dioxide and a new form of absorption tower adapted to the titrametric method. Jour. Ind. and Eng. Chem. **7**:1045–1049. 1915.
55. WALLDÉN, J. N., Damage caused by threshing of wheat and rye and its influence upon sensibility to treatment with fungicides and to storage. Sveriges Utsädesför. Tidskr. pp. 24. 1917. Rev. transl. from Dept. Agric.
56. ———, Experiments on the prevention of stinking smut on winter wheat. Sveriges Utsädesför. Tidskr. **22**:242–252. 1912. Rev. in Exp. Sta. Rec. **28**:242. 1913.
57. WINDISCH, R., Concerning the effect of formic aldehyde on germination. Landw. Vers. Stat. **49**:223–226. 1897. Rev. in Exp. Sta. Rec. **9**:955. 1898.
58. ———, On the action of formaldehyde on germination. Landw. Vers. Stat. **55**:241–252. 1901. Rev. in Exp. Sta. Rec. **13**:656. 1902.

DEVELOPMENT OF THE GEOGLOSSACEAE

G. H. DUFF

(WITH PLATES VIII–XII)

Introduction

Detailed knowledge of the ontogeny of Helvellinean fungi had its beginnings in the work of DITTRICH (7), whose paper on the complete development of *Mitrula phalloides* and *Leotia gelatinosa* (*L. lubrica*), with observations on other forms, has been the starting point for all subsequent life history studies in this group. This worker found that in the early stages of growth the ascocarps of *Mitrula* and *Leotia* possess a veil which, although evanescent, covers over the hymenium during the early part of their history, and thus renders their development endogenous. Heretofore the Helvellineae had been regarded as an essentially exogenous order, and SCHROETER (**20**) had separated them on this basis from the Pezizineae, in which group the hymenium was considered to be developed characteristically in a closed cavity which later opened. DURAND (11) confirmed and extended DITTRICH'S observations in the introduction to his excellent monograph of the Geoglossaceae. He states that he had observed the veil of *Mitrula phalloides* before learning of DITTRICH'S work. He had, in addition, noted the occurrence of a particularly well organized veil in *Microglossum viride*, and the conspicuous envelopes of *Spathularia velutipes* and *Cudonia lutea.* Of the latter he published beautiful photographs, illustrating these structures macroscopically.

The next contribution to this subject was a paper by MCCUBBIN (17) on the development of *Helvella elastica*. His work constitutes the first study in the family Helvellaceae, all the previous work having been confined to the Geoglossaceae. Here also it is stated that a membrane incloses the ascocarp in the younger stages of the form with which he worked. It is disorganized relatively early, and the last traces of it are cast off with the appearance of the paraphyses. BROWN (4) follows with an account of the development of the ascocarp of *Leotia*, but, strange to say, he makes no reference to a veil, although his material apparently included very early stages of this form. His failure to mention the veil might be due to the fact that he was chiefly concerned with the origin of the asci and the nuclear phenomena connected therewith.

From these investigations it will be seen that in two of the three Helvellinean families, the Geoglossaceae and the Helvellaceae, an endogenous origin of the hymenium has been claimed. Until the publications of FITZPATRICK (**14, 15**), nothing was known of the conditions prevailing among the members of the remaining family, the Rhizinaceae. FITZPATRICK has now shown quite conclusively that *Rhizina undulata*, the type of the family, possesses no investment at any stage of its history, and that, therefore, not only is the hymenium "exposed from the first," but the ascocarp itself is naked, that is, "gymnocarpous."

In a preliminary communication the writer (**10**) briefly outlined his findings in an examination of four Geoglossaceous forms, namely, *Cudonia lutea*, *Spathularia velutipes*, *Trichoglossum hirsutum*, and *Leotia lubrica*. The veils of *Cudonia* and *Spathularia* were found to be present practically from the first. *Trichoglossum* proved to be devoid of all traces of a veil at every stage.. On the other hand, *Leotia lubrica* showed some slight traces of a veil, which might be comparable with the one claimed for it by DITTRICH, but the series of stages which the writer had under observation did not include ones sufficiently young to make diagnosis positive, and the question must still be regarded as an open one.

Turning to the question of the sexuality of the Helvellineae, there is still less to record. DITTRICH found no highly organized sexual apparatus in either *Mitrula* or *Leotia*, and he characterizes both forms as apogamous. The fertile hyphae here are differentiated from the vegetative threads at an early stage as conspicuous, deeply staining cells, with large single nuclei.

Similarly MCCUBBIN describes and figures the multinucleate, fertile elements of *Helvella* as having their origin in the vegetative hyphae, from which they spring at a later stage than is the case with *Mitrula* and *Leotia*. No sign of any body resembling an ascogonium was discovered at any stage.

On the other hand, BROWN (4) concludes that *Leotia* possesses an ascogonium which makes its appearance early in the history of the ascocarp. From this the fertile hyphae arise, and may be seen in succeeding stages, passing upward into the cap, where after much branching they reach the hymenium and form asci. This ascogonium was seen in but one plant, and was observed only in an empty and partly degenerated condition. The manifestations of sexuality in *Leotia*, therefore, cannot be said to have been established beyond the possibility of a doubt.

It is only in the work of FITZPATRICK (15) that we find a wholly satisfactory description of sexuality in any of the Helvellineae. This worker has given a very careful and detailed account of sexuality in *Rhizina*. In this form, while the ascocarp primordia are yet small, certain multicellular hyphae near the center are transformed into procarps. This transformation entails a great increase in diameter and in the number of nuclei in the cells, the assumption of an irregular coiling habit, and the development of a somewhat elongated terminal cell which may be considered a trichogyne. No antheridia are present, and the trichogyne is functionless. When maturity is reached, the centrally situated cells of the procarp proliferate ascogenous hyphae, and into these the nuclei from all parts of the procarp migrate, passing from cell to cell through pores which have previously been formed in the transverse septa. These nuclei migrate in pairs. Evidence of nuclear fusions in the ascogonial cells or in the ascogenous hyphae is entirely lacking, and neither conjugate nor simple divisions were observed to occur in the latter.

The conditions obtaining in the species discussed in this paper have already been summarized (10). It was pointed out that no evidence of the existence of any type of procarp bodies has been found in *Trichoglossum*, in which the ascogenous hyphae arise from threads of vegetative form adjacent to the hymenium. In *Cudonia* and *Spathularia* the structures interpreted as procarps arise at a comparatively late stage in the development of the ascocarp from threads differentiated while the fructification is yet very young. They are numerous, more or less irregular in form, and while in *Cudonia* very distinct trichogynes are organized, similar structures are absent from *Spathularia*. In *Cudonia* the cells of the procarp appear to be uninucleate at first, later becoming multinucleate, while in *Spathularia* they are multinucleate from the beginning. The nuclei in the later stages of these bodies are arranged in pairs. Ascogenous hyphae take their origin in these procarps, and after engaging in crozier formation, give rise to asci.

Materials and methods

The material for this investigation was all obtained by collection, and was variously fixed, the fluids of Flemming and Carnoy, medium chrom-acetic acid and picro-sublimate, being used. It was imbedded in paraffin and cut in serial sections 2.5–10 μ thick. The usual staining methods were employed. Safranin and acid fuchsin proved to be the best general purpose stains, while Haidenhain's haematoxylin gave much the best results where details were required. In some cases the young fructifications sectioned *in situ* were stained by VAUGHAN'S (**21**) method for the differentiation of fungous hyphae and host tissues. This method gave very clear differentiation of the fungous tissue from the substratum, but had the serious disadvantage of impermanence.

As is not infrequently the case, the cells of certain tissues, while in a state of rapid growth and development, proved to be exceedingly difficult to differentiate cytologically, due to the density with which they are filled with deeply staining substances. As a result it has been impossible to answer some questions of nuclear behavior as fully as would be desirable. Otherwise the tissues appeared to be quite easily amenable to the usual microtechnical methods.

As already indicated, this paper deals in more or less detail with the four forms *Cudonia lutea*, *Spathularia velutipes*, *Trichoglossum hirsutum*, and *Leotia lubrica*. With the object of emphasizing the comparative aspects of the investigation, the contents are arranged in two parts, the first of which deals with the development of the ascocarp, with particular reference to the veil, and the second with the manifestations of sexuality in these plants.

I. Development of ascocarp

CUDONIA LUTEA

The earliest stage of *Cudonia lutea* of which we have knowledge is shown in fig. 3. At this stage the ascocarp is very minute, and measured but 84 μ from base to apex. It can be seen to consist of loosely interwoven hyphae, which are obviously mycelial elements elevated to their present position chiefly by the upward growth of a central knot of conspicuous, deeply staining threads. The latter are of peculiar interest on account of the part they play in the development of the fertile system. At first sight, because of their marked differentiation from the other threads composing the young fructification, they might easily be taken for ascogenous hyphae, or even for ascogonia. At a later stage (fig. 6) the proliferations of these threads give rise to bodies of characteristic form and history, bodies that admit of one interpretation only, namely, that they are procarps. The first appearing threads must therefore have some significance other than this.

Clearly differentiated bodies are known to originate the procarps or corresponding bodies in various Ascomycetes. In *Ascobolus carbonarius* (DODGE **8**) the procarps arise through the germination of asexual conidia borne on special mycelial branches. BROWN (**3**) found that in *Xylaria tentaculata* the Woronin hyphae are formed by the continued modification of threads that are differentiated at the center of the perithecium anlage while the latter is very young and deeply imbedded in the stroma. According to Miss DAWSON (**6**), a well differentiated thread appears in *Poronia punctata*, which at a later stage grows into a procarp.

The nearest approach to the first differentiated hyphae of *Cudonia* is to be found, however, in the disco-lichens. NIENBURG (**18**) gives a description of the development of the fruit bodies of four of these lichens, *Usnea barbata*, *Baeomyces roseus*, *Sphyridium byssoides*, and *Icmadophila aeruginosa*. It appears from his account that more or less well developed carpogonia arise in these forms, not from the ordinary hyphae of the ascomata in which they are found, but from threads which are differentiated at a considerably earlier stage. To these threads NIENBURG applied the name "Primordialhyphen" or "generativen Hyphen." In *Icmadophila* they appear before the fruit body has begun to rise above the surface of the thallus.

NIENBURG apparently was not very logical in his use of the terms "Primordialhyphen" and "generativen Hyphen." He employed the first in his study of *Usnea*, and the latter in studies of *Bae-*

omyces, *Sphyridium*, and *Icmadophila*. He drew no distinction between the so-called "Primordialhyphen" of *Usnea* and the "generativen Hyphen" of *Sphyridium* and *Icmadophila*. In *Baeomyces*, however, the carpogonia are so poorly defined that they are not separable from the "Primordialhyphen," so to the entire system in *Baeomyces* he extended the term "generativen Hyphen." Then, because of the evidently close natural relationship of *Sphyridium*, *Icmadophila*, and *Baeomyces*, he adopted the designation "generativen Hyphen" in connection with these forms. It is to be understood, therefore, that the "generativen Hyphen" of *Baeomyces* are not exactly homologous with those of *Sphyridium* and *Icmadophila*.

In the preliminary paper (**10**) the writer adopted the term "generative hyphae" rather than "primordial hyphae" to designate the pre-fertile threads of *Cudonia* and *Spathularia*. This was done because the Geoglossaceous forms appear to be more closely related to *Baeomyces*, *Sphyridium*, and *Icmadophila* than to *Usnea*. The occurrence of such a system of threads acting as precursors to the procarps is unusual, so far as the writer is aware, being found only in the disco-lichens, and so may be considered of sufficient importance to warrant emphasis.

The second developmental stage (fig. 4) shows a very definitely organized outer tissue, which has apparently been thrown up by the mycelial threads. By following through the series (figs. 4–7) this tissue may be seen to be identical with that later regarded as the veil. The veil in *Cudonia*, therefore, originates at a time when the ascocarp is nothing more than a cushion of mycelial threads with a core of generative hyphae, and can therefore be said to be present from the first. It is to be noted that at this stage a well marked clear zone separates the generative threads from the veil tissue. This clear zone contains only the remnants of the mycelial hyphae that once surrounded the generative threads. Fig. 5 illustrates the next developmental stage of *Cudonia*. Here the generative hyphae form an elongated mass of filaments of which the oldest are at the lower, and the newly formed elements, recently proliferated from them, are at the upper extremity. This upward growth of the generative threads has completely obliterated at the top the clear space that once surrounded them on three sides, and at this point they are now in a subapical position, almost in contact with the veil tissue. Remnants of the clear space still remain on both sides, but it is rapidly being filled by the growth of fundamental tissue which has arisen from the mycelium, and which has pushed up before it the mass of generative hyphae. The intrusion of fundamental tissue from below has its beginning at an earlier stage, and indications of it may be seen on both sides at the base of the ascocarp in fig. 4. Judging from these two stages, it would appear that the most rapid formation of new tissue, at least during the early stages, takes place at the base rather than at the apex of the ascocarp.

The subapical position of the generative hyphae is retained as the ascocarp increases in size, until the apex broadens out to form the cap. These hyphae then pass into the cap, and there some of their branches are transformed into procarps. This transformation entails an increase in diameter, the assumption of an irregular coiling habit, and the development of a process which passes from the procarp more or less directly through the veil to the exterior. These processes are to be regarded as trichogynes. The history of the procarps will be discussed in greater detail subsequently. While at this stage, the upper portions of the cap are densely filled with generative hyphae, procarp complexes, and their proliferations the ascogenous hyphae, interwoven in an almost inextricable tangle (fig. 6).

It is important to note that until this time no sign of a hymenium has made its appearance. Very shortly after paraphysis formation commences, however, and following this the ascogenous hyphae proceed with the proliferation of asci. Paraphyses in *Cudonia* arise from undifferentiated vegetative elements. They are multiseptate, in this respect differing from the description given for this species by DURAND, and their cells are multinucleate. At first they are straight, but later become strongly recurved at the tips, which are also somewhat enlarged.

The veil still completely invests the entire ascocarp, including the hymenium. Over the hymenium it has become separated from the tissue of the subhymenial region, with, indeed, no attachment at all except at the edges (fig. 7). Nevertheless it does not cease growth, but persists without rupturing, and keeps pace with the enlargement of the cap for considerable time. This power of independent growth is undoubtedly responsible for the persistence of the veil in this form, and is expressive of the distinct nature of the veil as an organ of the ascocarp and of its high degree of organization. Dehiscence finally takes place, by which time many of the asci are matured and their spores ready to be discharged. In the meantime, with the advent of the paraphyses the trichogynes disappear. The remaining portions of the procarps are visible for some time after, with cells empty or containing only a few strands and knots of plasm, evidently in a disorganized condition (figs. 12, 41). Finally practically all traces of them disappear.

SPATHULARIA VELUTIPES

Fig. 14 is a section of the earliest stage of *Spathularia velutipes* that has been studied. It can be seen to have been growing upon much rotted deciduous wood, and to have protruded above the substratum for somewhat less than 0.5 mm. From its size and organization it is evident that it represents a corresponding stage considerably older than the earliest *Cudonia* plants here figured. The interior of the young fruit body is composed of undifferentiated fundamental tissue, which is surrounded by a compact and well defined layer, which, as in *Cudonia*, is the investing membrane or veil.

By the use of suitable counter stains the veil of *Spathularia velutipes* may be sharply differentiated into two layers, an inner and an outer, the former compact and comparatively thin walled, and the latter looser and thicker walled. As the ascocarp increases in size, the outermost layer is split by tension into small adherent cell masses, which to the naked eye give the fruit body a velvety appearance. The power of independent growth is just as much a characteristic of the very persistent veil of *Spathularia velutipes* as it is of the membrane of *Cudonia*. In contrast to the condition of *Cudonia* at this stage, however, the interior of the ascocarp is still undifferentiated, and no threads corresponding to the generative hyphae of that form are yet visible.

By the time the ascocarp has attained the stage represented in fig. 15, conspicuous hyphae have made their appearance, and are situated as the generative hyphae come to be in *Cudonia*, just behind the apex. These conspicuous threads in the young *Spathularia* plant are also generative hyphae, for, as will shortly be seen, certain of their proliferations at a very much later stage are transformed into procarps. As the young ascocarp increases in size the pointed apex becomes broadened out, and the generative hyphae are distributed in an arch below it. This arch widens in concert with the gradual expansion of the apex into a somewhat globular cap, which as time goes on assumes the spatular shape characteristic of the mature plants of the genus (figs. 16, 17).

The expansion of the apex into the cap is marked by the appearance of paraphyses. The paraphyses of *Spathularia* arise from vegetative hyphal threads, the end cells of which become somewhat club-shaped. Various students of other species, as McCUBBIN (**17**) and BROWN (**4**), have found paraphyses arising from specialized storage cells. This does not occur here, the cells from which the paraphyses arise being insufficiently differentiated to be regarded as storage bodies. In form, septation, and nucleation the paraphyses of *Spathularia* resemble those of *Cudonia*.

While the paraphyses are still young, procarps become evident for the first time. They are scattered about in irregular fashion through those portions of the cap containing the generative hyphae, and are complexes conspicuous for their size and staining qualities (figs. 19–21, 43–45). An examination of all stages after this until the veil is shed and spores are being cast reveals their presence continuously. They vary in shape; some appear as coiled structures, some are immensely swollen, and others take the form of chains of cells. These bodies arise from the generative hyphae just as was the case in *Cudonia*, and from them ascogenous hyphae proceed directly to the hymenium, where in turn they may be seen giving rise to asci. Further growth results, as in the case of *Cudonia*, in the introduction of no new tissues or organs, but in the mere expansion of the ascocarp. The hymenium reaches a fairly well advanced state of maturity before the veil bursts. The observations of the writer have not established any regularity in the method of rupture, but DURAND states that "the veil seems to rupture by a crack running around the plant just above the stem," and exhibits photographs which illustrate his point.

TRICHOGLOSSUM HIRSUTUM

The very young fructification of *Trichoglossum hirsutum* shown in fig. 22 measures 175 μ in height, and is of simpler structure than the corresponding stages of either *Cudonia* or *Spathularia.* It is composed of compactly intertwining threads, the walls of which are comparatively thick and very dark. The hyphae all appear to be alike except for some, which, situated near the periphery and at right angles to it, extend beyond the ascocarp as straight, sharp pointed, unicellular hairs of greater diameter and thicker walls than the other threads. These setae correspond to similar ones described by BROWN in *Lachnea scutellata* (5), and by FITZPATRICK in *Rhizina undulata* (14). The setae of *Trichoglossum* are differentiated somewhat earlier than in *Rhizina,* but do not contain a glutinous substance such as is found in those of the latter form. The setae in *Trichoglossum* appear to be continuously produced over all peripheral parts of the ascocarp as it grows, but those that are earlier formed persist for some time, and come to be imbedded deeply in the new tissues that grow up around them. This is particularly evident in the hymenial region, where such growth is rapid (figs. 25, 26).

Paraphysis formation commences reatively early at the apex of the ascocarp, before it has begun to broaden out to form the cap (figs. 24, 25). The paraphyses are large, unbranched, multiseptate threads, the cells of which are usually uninucleate, but apparently may come to contain several nuclei in exceptional cases. They are strongly coiled at the tips.

Shortly after the paraphyses are organized, broadening of the apex of the ascocarp begins, and the hymenium extends over the entire surface of the globular cap as it takes shape and expands into the mature condition.

From the figures it may be seen that the hymenium is of undoubtedly exogenous origin, and, moreover, the ascocarp itself is clearly gymnocarpous. Throughout the entire series of stages careful examination has failed to reveal the presence of an investment of any kind. *Trichoglossum hirsutum,* therefore, adds one more species to those Helvellineae the hymenium of which is exposed from the first and the fructification gymnocarpous.

The vegetative threads in a well developed ascocarp of *Trichoglossum hirsutum* constitute a loosely interwoven tissue in which the individual cells of the threads are clearly distinguishable, even in thick sections. The threads pass with more or less uniformity from the base to the apex of the ascocarp. Since they are thick walled and consist of uninucleate cells, it is not difficult to see that hyphae of any other type are absent. The paraphyses make their appearance at an early stage (fig. 24), but ascus formation does not commence until the cap is well shaped. Then, just beneath the bases of the paraphyses in the subhymenium, there appear thin walled hyphae which, unlike the vegetative threads, form a very densely interlaced tissue. These constitute the ascogenous system of *Trichoglossum.* It is certain that no bodies such as the generative hyphae and procarps of *Cudonia* or *Spathularia* stand out as specific organs, nor is it possible to distinguish the homologues of procarps as distinct from the ascogenous hyphae.

The ascogenous hyphae are short, since they are differentiated in such close proximity to the hymenium. Asci arise from them in the usual manner. The formation of an ascus is preceded by the inversion of the tip of an ascogenous hypha to form a crozier, the ascus growing out of the penultimate cell. The asci are very large, with dense protoplasm, and, especially after the dark multiseptate spores are mature, form very conspicuous objects in the hymenium (fig. 26).

LEOTIA LUBRICA

Since material of this form had been collected, and since previous investigators are not in complete agreement on two important points in the life history, it was thought advisable to examine the material with a view to the possibility of shedding some further light on these points. The questions to which reference is made are those of the presence of the veil and the origin of the ascogenous hyphae, and attention was confined to these questions alone.

Two specimens at approximately the same well advanced stage showed irregular tissue fragments overlying parts of the ascocarp and the hymenium (figs. 27, 28). These specimens were not the youngest of the fruit bodies examined. In no others, however, whether younger or older than these, were any similar evidences seen. These fragments are composed of interwoven threads more or less degenerated in appearance, and in one case still connected with one another by a few hyphae, which give them the appearance of having been torn apart by the growth of the ascocarp (fig. 27). DITTRICH (7) describes the veil of *Leotia* as having a ground substance of gelatinous material formed by the progressive inward swelling ("Verquellung") of the peripheral hyphae. In this matrix are imbedded threads which are rather compactly interwoven. Apparently, therefore, so far as structure is concerned, the tissue fragments observed correspond fairly well to the veil tissue of *Leotia* as described by DITTRICH. Had very young ascocarps been available, and had such tissue been found covering them, particularly as entire structures, DITTRICH'S view that *Leotia* is at first angiocarpous would have been substantiated. On the other hand, had the material included a satisfactory series of very young stages which did not show traces of a veil, the writer would have felt justified in denying its presence in *Leotia lubrica*. The youngest stages available, however, were advanced sufficiently to show a well differentiated cap and young paraphyses, and consequently were considerably older than the youngest stages which BROWN, and probably also DITTRICH, had under observation. Under these circumstances the writer does not feel that the evidence is sufficient to lend conclusive support to either view. It would seem best, therefore, to regard the question as still open.

SIGNIFICANCE OF VEIL IN HELVELLINEAE

Ever since the appearance of DITTRICH'S (7) original paper on this subject, each succeeding investigation has served to emphasize the inadequacy of the distinction drawn by SCHROETER (20) between the Helvellineae and the Pezizineae. According to this classification, in the former group the hymenium is formed upon the surface of the ascocarp, and therefore is freely exposed from the first, while in the latter the hymenium is originally inclosed. SCHROETER'S fundamental idea appears to have been that in the Pezizas the hymenium is formed within the ascocarp in an inclosed depression, while in the Helvellas it appears upon a flat or convex surface, not closed in.

The discovery of the veil introduces a new morphological feature not contemplated in SCHROETER'S scheme. The value of the veil as an important taxonomic criterion depends, not so much upon the fact that it may at first inclose the hymenium, as upon its nature as an organ of the ascocarp. If it were simply the roof of a depression, comparable with the roof that covers over the young hymenium of a *Peziza*, and which breaks away to form the edge of the cup, then the Helvellinean forms in which it occurs would plainly be only modified Pezizas. But if, as seems to be the case, it were a distinct envelope which covers over the entire ascocarp, and perhaps incidentally also the young hymenium, then so far as we know it is a feature not represented in the Pezizineae, with the possible exception of the as yet uninvestigated Helotiaceae and Mollisiaceae.

In reviewing the conditions prevailing in the Helvellineae, it is found that the veil is absent in the only member of the Rhizinaceae which has been studied, it is present in the only member of the Helvellaceae of which we have knowledge, while at the present time the Geoglossaceae are about equally divided. In the last named family the following species may be considered as angiocarpous: *Mitrula phalloides, Leotia lubrica* (DITTRICH **7**); *Microglossum viride* (DURAND **11**); *Cudonia lutea, Spathularia velutipes* (DURAND **11**, DUFF **10**). The gymnocarpous members appear to be *Geoglossum glabrum, G. difforme, Trichoglossum velutipes* (DURAND **11**); and *Trichoglossum hirsutum* (MCCUBBIN **17**, DUFF **10**). It may be noted that the gymnocarpous species are all closely related, belonging to genera that were at one time comprised in the single genus *Geoglossum*, while the others, with the exception of *Microglossum viride*, belong to less closely related genera. This means, so far as our evidence goes, that the *Rhizina* and *Geoglossum* groups are typically gymnocarpous, while the remaining Helvellineae are angiocarpous.

In searching for a structure in other Ascomycetes which might be homologized with the Helvellinean veil, we find one in the *Baeomyces* group of the disco-lichens. Some such relationship had already suggested itself to DITTRICH, who, after emphasizing other points of similarity, goes on to say that in *Baeomyces* the upper portions of the thallus which overlie the anlage of the fruit body, and which later surround the young fructification, seem to assume the "functions" of a veil. Although he does not say so explicitly, it is a fair inference that DITTRICH considered this thalloid investment of the young *Baeomyces* fructification and the inclosing membranes of *Mitrula* and of *Leotia* homologous, for he implicitly adduces this as evidence of the Geoglossacean affinities of *Baeomyces*. DITTRICH's conclusions were based upon the description of the development of the fructification of this lichen given by KRABBE (16). NIENBURG (18) re-examined more recently some of the forms with which KRABBE worked, and the results recorded in his paper present even more interesting parallelisms to the conditions prevailing among some of the Geoglossaceae.

In *Icmadophila aeruginosa* the covering of the ascocarp is more distinct and more persistent than in *Baeomyces*. Figs. 1 and 2, taken from NIENBURG, represent sections of very young fruits of the former species. Here it may plainly be seen that the superficial layer of the thallus grows into an envelope for the young ascocarp and incloses it until it has attained considerable size, after which the growth of the ascocarp becomes too rapid for the extension of the epithecial tissue, which then ruptures. The fructification does not subsequently become covered with any other tissue which might be compared to a veil, and the hymenium therefore develops exogenously. As has already been indicated, however, the point of importance in this connection is not the origin of the hymenium, but only the occurrence of an envelope which incloses the entire ascocarp, and which constitutes a morphologically distinct organ of the fruit body. In the opinion of the writer, therefore, the envelopes of *Baeomyces* and *Icmadophila* are strictly comparable and homologous with those of the Geoglossaceae. From this it follows that in any natural scheme of classification some lichens, at least those of the *Baeomyces* group, must be brought in with the angiocarpous Geoglossaceous forms, as also possibly some members of the Helotiaceae.

II. Sexuality

CUDONIA LUTEA

It is in the youngest stages of *Cudonia lutea* that we find the beginnings of the hyphal system destined finally to give rise to asci. As we have seen, here there are differentiated from the mycelium certain threads which have been called generative hyphae, and which are the most prominent central components of the simply organized fructification. These threads are clearly differentiated in the stained preparation from the rest of the ascocarp. So vigorously do they react to stains that all details of cell structure are obscured, even when nuclear stains are applied.

The generative hyphae grow upward as the ascocarp increases in length, remaining subapically situated. When the upper part of the fruit body is differentiated into the cap these hyphae make their way into it, and in that position certain branches of this system appear that are enlarged, irregularly coiled, and deeply staining (fig. 6). These are the procarps. The procarp coils are continued upward by processes which can only be regarded as trichogynes (figs. 6, 8, 9). These are multiseptate and follow a more or less direct course toward the exterior. Where the procarps lie in a suitable position the trichogynes may be followed for their whole length, and may be seen to penetrate the veil tissue and to reach the exterior. Sometimes the procarps are deeply imbedded, however, and in these cases it is impossible to follow the trichogynes as far as the surface of the ascocarp.

The number of procarps formed in this manner in a single fruit body appears to be very large, although it is not possible to count them, on account of the way in which they are interlaced and because of the presence of masses of generative and ascogenous hyphae. Some idea of their number may be conveyed, however, when it is known that as many as seven distinct trichogynes have been counted in a single longitudinal section 10 μ thick through a region of the cap in which they are numerous. Their distribution is irregular. Some of them are formed closely under, or even partly imbedded in the veil tissue, while others are situated at a distance from the surface in the direction of the center of the cap (figs. 6, 8, 9). Sometimes they are localized in one or two restricted portions of the cap, being entirely absent from others.

Fig. 11 is a portion of an unusually loosely organized procarp. This section does not contain any of the upper portion of the procarp, but it shows most of the lower part and the generative hypha from which it arose. The latter follows a somewhat straighter course than is usual with these threads, and hence its relation to the coil is rendered very obvious. The generative hypha is more deeply stained than the surrounding vegetative threads, while the procarp coil itself is quite opaque. Figs. 32–35 represent other procarp bodies at approximately the same stage of development as that of fig. 11. Most of these figures are taken from sections cut longitudinally through the ascocarp, but fig. 34 represents portions of a procarp found in two contiguous sections of a series taken in a transverse plane. These illustrations give some idea of the diversity of form displayed by these structures. They vary from an almost straight, slightly twisted series of cells, to coils such as that of fig. 11. These illustrations also show the relationships of the procarps and trichogynes. This relationship is evident in fig. 6. Two procarps and their trichogynes appearing in this section are shown enlarged in fig. 8, where, however, on account of the thickness of the section and the meandering habit of the threads of the procarp and trichogynes, not all of these structures can be obtained in sharp focus at the same time. The protrusion of the trichogyne from the surface of the ascocarp is shown well in fig. 13, which is the first section of a series through the cap of an ascocarp at this stage. The exposed tip of a trichogyne, cut off with the first section, is clearly shown, several of its cells being visible.

No structures have been found that could be regarded as spermogonia, and no bodies that correspond to spermatia. Furthermore, the writer has no evidence that the trichogynes functioned in any way, and since they disappear very soon, is inclined to regard them as vestigial organs in *Cudonia*.

As may be seen from the illustrations, the procarps are very deeply staining bodies, and in their earlier stages it is impossible to differentiate them cytologically. Nevertheless here and there an occasional cell may be seen to contain what is doubtless a single nucleus, while a smaller number contain two (figs. 32–35). Thus what slight evidence there is points to the original condition of these bodies as being uninucleate. A little later on, however, they become multinucleate. This takes place soon after the appearance of the paraphyses, and is preceded by the disappearance of the trichogynes. At this stage the remaining cells of the procarps become more distended, and their contents do not stain quite so densely, and thus cell structures can be distinguished with a greater degree of certainty. How the originally uninucleate cells become multinucleate is not known. The nuclei are small, and are frequently unequal in size. They often appear to lie in pairs (figs. 36, 37, 39, 40).

While at the multinucleate stage, or even before they can be seen definitely to be multinucleate, the procarp cells form branches which bud out from them (figs. 36, 38, 40). These branches are ascogenous hyphae, and can be recognized directly as such from the fact that in well advanced specimens in which the formation of asci is just beginning they have been seen passing from procarp complexes, which are usually empty and degenerated at this stage, to the hymenium, and there giving rise to asci (fig. 10). Ascogenous hyphae may be seen passing from an old procarp complex to the hymenium, which is just being differentiated (fig. 10). In the subhymenium they are in direct connection with cells undergoing crozier formation preparatory to the formation of asci. The cells of the ascogenous hyphae are usually multinucleate, but may be binucleate.

The proliferation of one or more asci from an ascogenous hypha by the formation of a crozier, with its four nuclei and various evolutions, has been described by numerous workers, and need not be detailed again. It will be sufficient to say that in *Cudonia* the penultimate cell usually grows out into an ascus, but a succession of croziers may be formed, and there may be a fusion of the ultimate and the antepenultimate cells, followed by the formation of an ascus or of another crozier. Several croziers may be formed from a single cell of an ascogenous hypha, and when all these are confined to the distal end of the cell a peculiar candelabra-like arrangement of asci results.

The two nuclei which find their way into the young ascus fuse at once. The fusion nucleus enlarges as the ascus grows, and finally assumes very large proportions. Divisions of the nucleus begin when the ascus has reached about half its ultimate size, and these divisions succeed one another very rapidly. When eight daughter nuclei have been formed the spores are delimited.

Spathularia velutipes

The origin of the generative hyphae in *Spathularia* has already been described. Mention has also been made of the fact that they come to occupy such a position in the ascocarp that when the hymenium has been formed they will lie just beneath it. Apart from their somewhat larger size, conspicuous staining qualities, and restricted position in the ascocarp, the generative threads do not appear to differ from the vegetative hyphae. The cells of both contain from one to several nuclei.

After the paraphyses have been formed, procarps grow out from some of the cells of the generative hyphae. The procarps are of relatively large proportions and become very strongly chromophilous. They are curious conspicuous complexes of hyphae. Attention has already been called to them, and some of them are illustrated in figs. 19–21 and 43–45. They appear to exhibit no uniformity of structure, but their cells are all multinucleate, and frequently distinct pairing of nuclei is observable (figs. 43–45.)

Ascogenous hyphae arise from these procarps. This is very easily demonstrated where the hyphal complex lies particularly close beneath the hymenium, and where the ascogenous hyphae pass directly into the hymenium. Under these circumstances the ascogenous hyphae take on something of the staining qualities of the cells from which they arise, and they may be traced with ease into the hymenium, and there may be seen in connection with asci. Fig. 21 is a plexus of such ascogenous hyphae arising from a group of large cells, some of which are visible. This large group may be traced through a series of sections $7.5\,\mu$ in thickness. The origin and termination of these threads are not visible in all sections, but by following through the series their connections in both directions are easily ascertained.

Fig. 19 affords another illustration of this type, showing two enlarged procarp cells, somewhat depleted of their contents, one containing a single nucleus and the other two nuclei. From these cells deeply staining ascogenous hyphae are to be seen passing outward, following a very irregular course to the hymenium, and there in direct union with young asci. The group of uninucleate asci of about the same age shown in this figure appears to have arisen from ascogenous hyphae originating in the same procarp cells. More frequently, however, the hyphae passing from the procarps do not retain their staining qualities sufficiently long to make it possible to follow them to the hymenium, especially when they are much branched or when they follow a devious route. The cells of the ascogenous hyphae are multinucleate, and the nuclei appear to be paired (fig. 45).

McCubbin, who described bodies in the ascoma of *Helvella elastica* which bear considerable resemblance to those at present under discussion, took the view that they are merely storage organs. In addition to ascogenous hyphae McCubbin claims to have found paraphyses arising from these so-called storage cells. Judging from his figures, however, the paraphyses arising in this manner are so far from typical that it may be questioned whether they are actually paraphyses. Since the "storage bodies" of *Helvella* resemble the procarps of *Spathularia* in being multinucleate, in having the nuclei arranged in pairs, and in producing ascogenous hyphae, it would seem to the writer that they might also be interpreted as sex organs. Fitzpatrick (15) has already expressed this opinion. He states that "several significant facts would seem to indicate that at least part of these 'storage bodies' constitute some type of sexual apparatus, particularly the statement that they are sometimes found giving rise to ascogenous hyphae." The ascogenous hyphae of *Spathularia* behave as in the case of *Cudonia* in the evolution of asci. The description given for the latter form may be taken to include them both.

LEOTIA LUBRICA

According to Dittrich, the fertile threads of *Leotia lubrica* are differentiated from vegetative hyphae at an early stage in the history of the ascocarp. While they do not appear to be easily traced in intermediate stages, they become conspicuous again as maturity approaches, this time in the cap, where, lying just beneath the hymenium, they give rise to asci. Although Dittrich does not figure the youngest stage in which they are visible, as he does in the case of *Mitrula phalloides*, he says that these two forms are much the same in this regard. If so, the fertile hyphae appear at a very early stage, indeed, one almost comparable with the youngest *Cudonia* plants described in this paper.

On the other hand, BROWN (4) has figured the remains of what he interpreted to be an ascogonium, a structure found by him in one case only, at the base of an older ascocarp than the youngest with which DITTRICH presumably worked. The ascogenous hyphae pass up from the ascogonium, and may be traced to the cap, where they become much branched, finally finding their way to the hymenium. The writer's material did not include stages young enough to make possible a direct confirmation of either of these statements, but it did show hyphal complexes just previous to the formation of the first asci strikingly suggestive of those already described for *Spathularia*.

At this period the ascogenous hyphae appear under the hymenium as deeply staining threads in various stages of crozier formation and ascus proliferation, and since they are not numerous as yet, they are easily distinguished and recognized. Beneath them in the cap may be seen at the same time very conspicuous groups of greatly enlarged cells. Some of these enlarged cells are quite empty, some contain light vacuolated protoplasm, and others are densely filled with deeply staining contents, identical in appearance with those of the ascogenous hyphae. These enlarged cells are usually rounded, occurring singly or in groups, but they may assume any of a great variety of forms. Their distribution in the ascocarp follows no regular arrangement whatever. They contain from one to several nuclei which are variable in size and sometimes may be very large (figs. 47–53). From these large cells ascogenous hyphae arise. This is readily demonstrated wherever they occur close to the hymenial region.

BROWN has described what he regards as storage cells in the ascocarp of *Leotia*. Of these he says: "While the hymenium is being differentiated some of the vegetative hyphae give rise to large storage cells. . . . . These large storage cells are formed in rows and give rise to paraphyses. The storage cells are at first multinucleate, but the nuclei usually fuse as growth proceeds." He affirms that the nuclei of these cells, where they are more than one, may be very unequal in size, and the fusion nuclei are sometimes of extraordinarily irregular form. The writer has not been able to find storage cells which exactly correspond to those described by BROWN. The only large unusual cells noted were those already described, and it is believed that these are BROWN'S "storage cells." Careful attention was given to the possibility of paraphyses arising from these cells, but in the many sections examined for the purpose of confirming this observation, not a single clear case of the origin of paraphyses in these bodies was found.

The fact that ascogenous hyphae arise from these special cells in *Leotia* makes it necessary to consider the possibility that they represent some form of sex apparatus. Obviously, before this interpretation can actually be put upon them, it will be necessary to re-examine stages comparable with the youngest which BROWN studied in order to make sure whether or not an ascogonium occurs at the base of the young ascocarp. If there be no ascogonium there, *Leotia* would then resemble *Spathularia* in its manifestation of sexuality.

SEXUALITY IN HELVELLINEAE

The phenomena of sexuality in the Helvellineae bring forward some interesting questions. In the first place, the large number of procarps occurring in these Geoglossaceous forms is noteworthy; the overwhelming majority of Discomycetes possess but a single one. FITZPATRICK (15) drew attention to this point in his discussion of *Rhizina undulata*, which form is characterized by the production of several of these bodies. This investigator also indicated that while this character tends to separate the Helvellineae from other Discomycetes proper, it brings them closer to the disco-lichens, which are notable for their compound apothecia. FITZPATRICK quotes a number of lichen forms of which this is true, and to these may be added those which constitute the subject of NIENBURG'S (18) researches, namely, *Usnea barbata*, *Baeomyces roseus*, *Sphyridium byssoides*, and *Icmadophila aeruginosa.* It may be mentioned also that in those species in which the procarp is not obviously greatly reduced, for example in *Rhizina undulata* and in *Cudonia lutea*, it is of the same general form as that which characterizes lichens, that is, it consists of a modified, more or less coiled multicellular hypha the terminal portion of which constitutes a trichogyne. The trichogyne is unicellular in *Rhizina* and multicellular in *Cudonia.*

Another feature in common with the lichens is the fact that the procarps of the Helvellineae are not "initial organs" arising from the mycelium and later becoming surrounded by the tissue of the ascocarp, as they are in *Ascobolus*, etc., but are formed within an already well developed ascocarp. This is true of *Rhizina*, in which the fructification begins as a "wholly undifferentiated button of mycelium." It is not until the ascocarp has attained a diameter of about 1 mm. that the procarps appear. They then arise by the differentiation of certain centrally situated hyphae of the fruit body. In *Cudonia* the procarps arise at an even later stage, that is, after the ascocarp has become differentiated into cap and stem, while those of *Spathularia* are delayed still further in their appearance.

There is still another point of resemblance between these Geoglossaceous fungi and the disco-lichens in their common possession of a feature noted by NIENBURG (18), namely, that the procarps are offshoots of a unique hyphal system (designated by him "generativen Hyphen"), which makes its appearance at an early stage. NIENBURG shows that in *Icmadophila aeruginosa* the "generativen Hyphen" appear as deeply staining threads in the young ascocarp anlage before it becomes erumpent. As the fructification increases in size these hyphae proliferate to form scattered knots of threads, which are connected together, having a common origin, by "Verbindungshyphen." The latter are evidently a part of the system of "generativen Hyphen." These knots become transformed into procarps, and distinct trichogynes are formed which penetrate the tissues of the ascocarp and project into the air. Although spermatia frequently become attached to the trichogynes there is no conclusive evidence that they are functional, or that a process of fertilization takes place. Many of the procarps disorganize without producing ascogenous hyphae, but those that survive act as the source of these threads.

In *Sphyridium* the first elements of the pre-fertile system, the "generativen Hyphen," appear at a somewhat later stage than in *Icmadophila*. In this form they grow into a series of very clearly defined nests of hyphae, which, as in *Icmadophila*, are connnected together by "Verbindungshyphen." The growth of the vegetative hyphae carries these knots to the upper portion of the ascocarp near the surface and separates them from one another. The ascogenous hyphae arise from these structures. They evidently represent some form of sex apparatus, but because of their lack of conventional trichogynes and their irregular habit, NIENBURG hesitates to give them the full status of typical "carpogones." He says: "Ich glaube deshalb, dasz regelrechte Trichogyne bei Sphyridium nicht mehr angelegt werden, sondern dasz wir hier reduzierte Gebilde vor uns haben, die wahrscheinlich früher als Empfängnisapparate gedient, heute aber diese Funktion aufgegeben haben."

In *Baeomyces* the young fructification is sunken in the thallus and is distinguished as a mass of hyphae more closely interwoven than those of the thallus. At the center of this mass appear certain enlarged, deeply staining threads which are the "generativen Hyphen," and which are the source of the ascogenous hyphae. NIENBURG'S uncertainty in the interpretation of these bodies is indicated in the following quotations: "So ist zu vermuten, dasz auch hier auf sehr frühem Stadium, Askogone als Verzweigungen der vegetativen Hyphen gebildet werden, die man nur mit unsern Mitteln von den übrigen Gewebe—dem askogenen wie dem vegetativen—nicht deutlich unterscheiden kann. . . . . Vielleicht sind die dunklen Hyphen in den Knäueln der Fig. 17 solche Askogone, vielleicht sind es aber auch schon askogene Hyphen." He decides that they may represent carpogonia, but if so, that they are very much reduced in *Baeomyces*, which he regards as an apogamous form. It is to be noted, however, that ascogenous hyphae still arise from these bodies even if they are structurally reduced.

These brief reviews indicate how close is the correspondence in the history of the fertile system between some of the disco-lichens and of the Geoglossaceae. In both groups the procarps are numerous; they have, in general, the same type of structure; and in both are formed at a more or less advanced stage from a system of threads which makes its first appearance while the ascocarp is very young.

It is interesting that in both groups parallel series are found showing comparable stages in structural reduction. In the lichens we have a graded series in *Icmadophila*, *Sphyridium*, and *Baeomyces*.

In *Icmadophila* the procarps are slightly if at all reduced, in *Sphyridium* they have lost the trichogyne and become somewhat irregular in form, while in *Baeomyces* it is questionable whether such structures can be said to occur at all. Among the Geoglossaceae we have *Cudonia* illustrating a condition entirely comparable with that of *Icmadophila*, while *Spathularia* resembles *Sphyridium* in the much-reduced nature of its procarps. In *Trichoglossum* the sex organs do not stand out as morphologically distinct structures. That these stages in reduction give a picture of the direction of evolution of sexuality in both groups is the opinion of the writer. Beginning with a form of procarp in which a true process of fertilization through the medium of the trichogyne took place, the evolution of these plants seems to have been marked by the successive suppression of the male organ, the trichogyne, and finally the procarp as a distinctive morphological body.

The facts observed in the Geoglossaceous forms examined point in a contrary direction to the opinion held by BLACKMAN and WELSFORD (1), as stated in their paper on *Polystigma rubrum*. They maintain that in all cases where a degenerate ascogonium occurs, the ascogenous hyphae will probably be found to originate independently of this organ. Their own recorded observation that the well organized procarp of *Polystigma rubrum* takes no part whatever in the formation of ascogenous hyphae lacks confirmation. Indeed, NIENBURG (19), in a more recent work on this species, maintains that ascogenous hyphae do originate in the procarp. BROOKS'S (2) similar contention with regard to the part played by the procarp of *Gnomonia erythrostoma*, and FISCH'S (13) statement on Woronin's hypha in *Xylaria polymorpha*, are offset by H. B. BROWN'S (3) observation that the cells of the Woronin hypha of *X. tentaculata* actually do become ascogenic.

The parallelism shown to exist between Geoglossaceous and disco-lichen forms must surely be significant of phylogenetic relationship. The question of the relationship of these groups is of practical interest, in view of the conviction on the part of certain botanists that the lichen genera should be distributed among those of the fungi proper. It is recognized by many who favor such a procedure that the history of the reproductive tracts must ultimately be the basis of any such reclassification. The following quotation from FINK (12) exemplifies this view: "The classification of the fungi is really based in the main upon the morphological relationships of the reproductive areas, and in the lichens also these relationships surely have a greater importance in classification than the form-relationships of the thallus." This being so, such correspondences as have been disclosed here should indicate a basis of relationship between the Geoglossaceae and the *Baeomyces* group of the disco-lichens. This evidence of relationship is made stronger by the homology shown to exist between the veils which inclose the ascocarp in many of these two groups. The further extension of this relationship should aid materially in the problem of classification, and should give direction to our ideas concerning the affinities of these two interesting groups of plants.

Summary

1. The development of the ascocarp in *Cudonia lutea* and in *Spathularia velutipes* is notable for the appearance of an inclosing membrane which covers over the entire fructification, and which constitutes a morphologically distinct organ of the fruit body. Very early stages of *Cudonia* show its presence, and it is one of the first organs of the ascocarp to be differentiated. *Trichoglossum hirsutum* lacks entirely any such investment, and the ascocarp is therefore gymnocarpous. *Leotia lubrica* shows some evidences of possessing an evanescent veil, but this is a point which requires further elucidation.

2. The value of the Helvellinean veil as a systematic criterion depends more upon the fact that it is a morphologically distinct organ inclosing the entire ascocarp than that it may result in an endogenous development of the hymenium. This being so, the closest homology to the Helvellinean veil is to be found, not in the Pezizineae, but in the *Baeomyces* group of the disco-lichens. This is adduced as evidence of relationship between these groups.

3. The fertile systems of *Cudonia lutea* and *Spathularia velutipes* appear at an early stage in the history of the fructification as threads which have been called "generative hyphae." These hyphae proliferate with the other tissues of the ascocarp, and at

a later stage give rise to the procarps. The procarps of *Cudonia* are irregularly coiled, and are provided with multiseptate trichogynes which pass outward and protrude through the veil. The nuclear history is not fully known, but the original condition of the cells of the procarp seems to be uninucleate, later becoming multinucleate, and at this stage giving evidence of nuclear pairing. Ascogenous hyphae arise from the cells of the procarps, after which the procarps become emptied of their contents and finally disappear. The procarps of *Spathularia* appear somewhat later than in *Cudonia*, and are formed close to the hymenium. They are irregular, do not possess trichogynes, and are obviously reduced in structure. They are multinucleate throughout their history, and the nuclei are paired. Ascogenous hyphae arise from them.

4. In *Trichoglossum hirsutum* there is no structural differentiation of sex organs. The ascogenous hyphae arise from threads which do not differ in form from vegetative ones.

5. In *Leotia lubrica* bodies resembling in some respects the "storage bodies" described by BROWN for the same form were found to produce ascogenous hyphae. On this account it is suggested that they may represent sex organs, that is, they may be degenerate procarps.

6. It is pointed out that there is a very close correspondence in the history of the fertile systems of the Geoglossaceae and of certain disco-lichens of the *Baeomyces* group. This is cited as further evidence of relationship between them.

7. It is suggested that the progress of evolution in these plants has been from a type in which fertilization took place through the agency of the trichogyne, and has been marked by gradual reduction of the sex organs.

This investigation was conducted under the direction of Professor J. H. FAULL of the University of Toronto, to whom the writer would acknowledge his great indebtedness for assistance and criticism through the whole course of the work, and also for liberal contributions of material.

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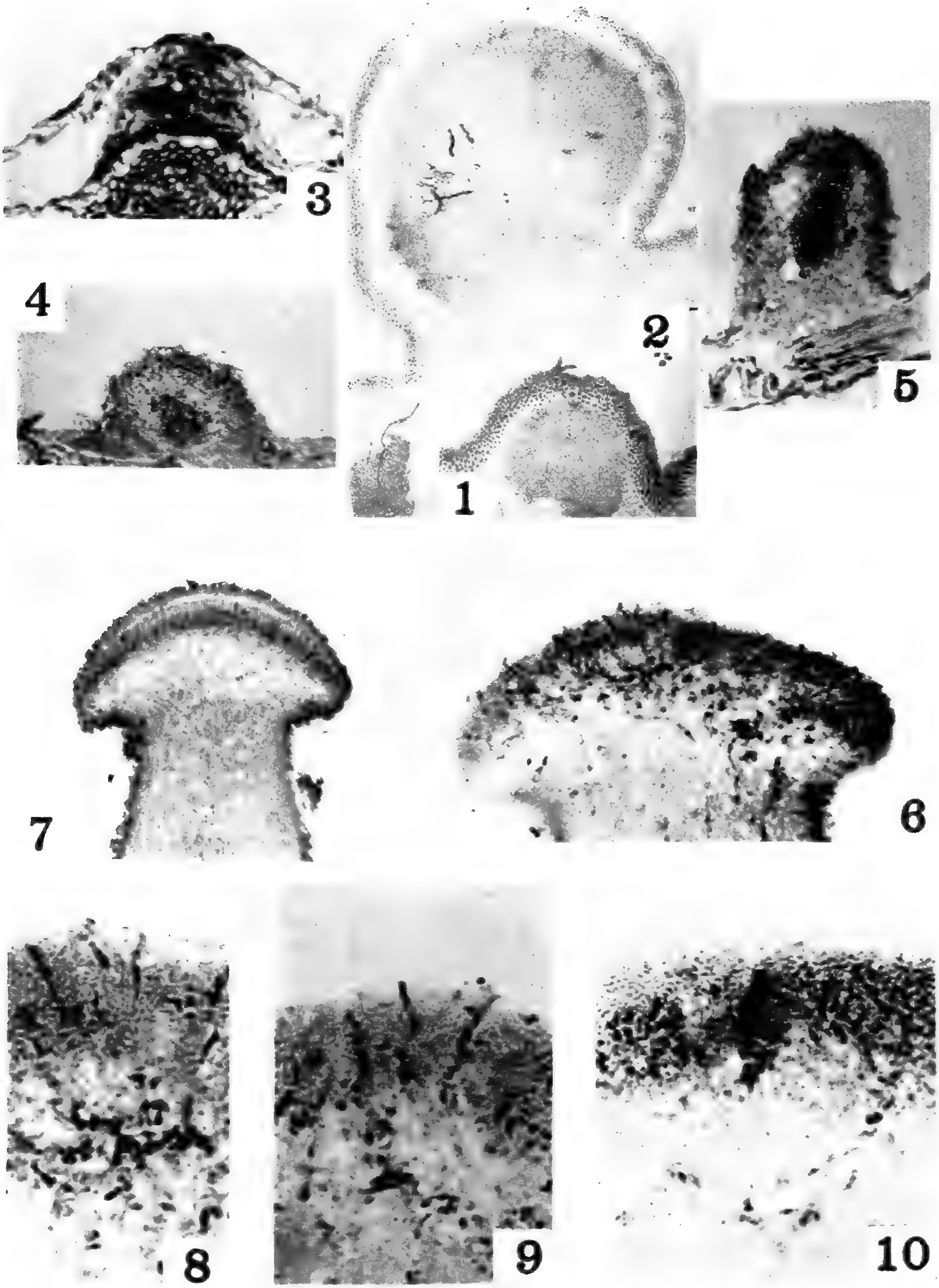
LITERATURE CITED

1. Blackman, V. H., and Welsford, E. J., The development of perithecium of *Polystigma rubrum* DC. Ann. Botany **26**:761–767. 1912.
2. Brooks, F. T., The development of *Gnomonia erythrostoma* Pers. Ann. Botany **24**:585–606. 1910.
3. Brown, H. B., Studies in the development of *Xylaria*. Ann. Myc. **11**:1–13. 1913.
4. Brown, W. H., The development of the ascocarp of *Leotia*. Bot. Gaz. **50**:443–459. 1910.
5. ———, The development of the ascocarp of *Lachnea scutellata*. *Ibid.* **52**:273–305. 1911.
6. Dawson, Maria, On the biology of *Poronia punctata*. Ann. Botany **14**:245–260. 1900.
7. Dittrich, Gustav, Zur Entwickelungsgeschichte der Helvellineen. Cohn's Beiträge **8**:17–52. 1898.
8. Dodge, B. O., Artificial cultures of *Ascobolus* and *Aleuria*. Mycologia **42**:218–222. 1912.
9. ———, The morphological relationships of the Florideae and the Ascomycetes. Bull. Torr. Bot. Club **41**:157–202. 1914.
10. Duff, G. H., Development of the Geoglossaceae. Bot. Gaz. **69**:341–346. 1920.
11. Durand, E. J., The Geoglossaceae of North America. Ann. Myc. **6**: 388–477. 1908.
12. Fink, Bruce, The nature and classification of lichens. I. Views and arguments of botanists concerning classification. Mycologia **3**:231–269. 1911.
13. Fisch, C., Beiträge zur Entwickelungsgeschichte einiger Ascomyceten. Bot. Zeit. **40**:865–870, 875–882. 1882.
14. Fitzpatrick, H. M., The development of the ascocarp of *Rhizina undulata* Fr. Bot. Gaz. **63**:282–296. 1917.
15. ———, Sexuality in *Rhizina undulata* Fries. *Ibid.* **63**:201–226. 1918.
16. Krabbe, G., Entwickelung, Sprossung, und Theilung einiger Flechtenapothecien. Bot. Zeit. **40**:65–83, 89–99, 105–116, 121–142. 1882.
17. McCubbin, W. A., Development of the Helvellineae. I. *Helvella elastica*. Bot. Gaz. **49**:195–206. 1910.
18. Nienburg, W., Beiträge zur Entwickelungsgeschichte einiger Flechtenapothecien. Flora **98**:1–40. 1908.
19. ———,. Zur Entwickelungsgeschichte von *Polystigma rubrum*. Zeit. Bot. **6**:369–400. 1914.
20. Schroeter, J., In Engler und Prantl. Die natürlichen Pflanzenfamilien. 1894.
21. Vaughan, R. E., A method for the differential staining of fungus and host cells. Ann. Mo. Bot. Gard. **1**:1914.



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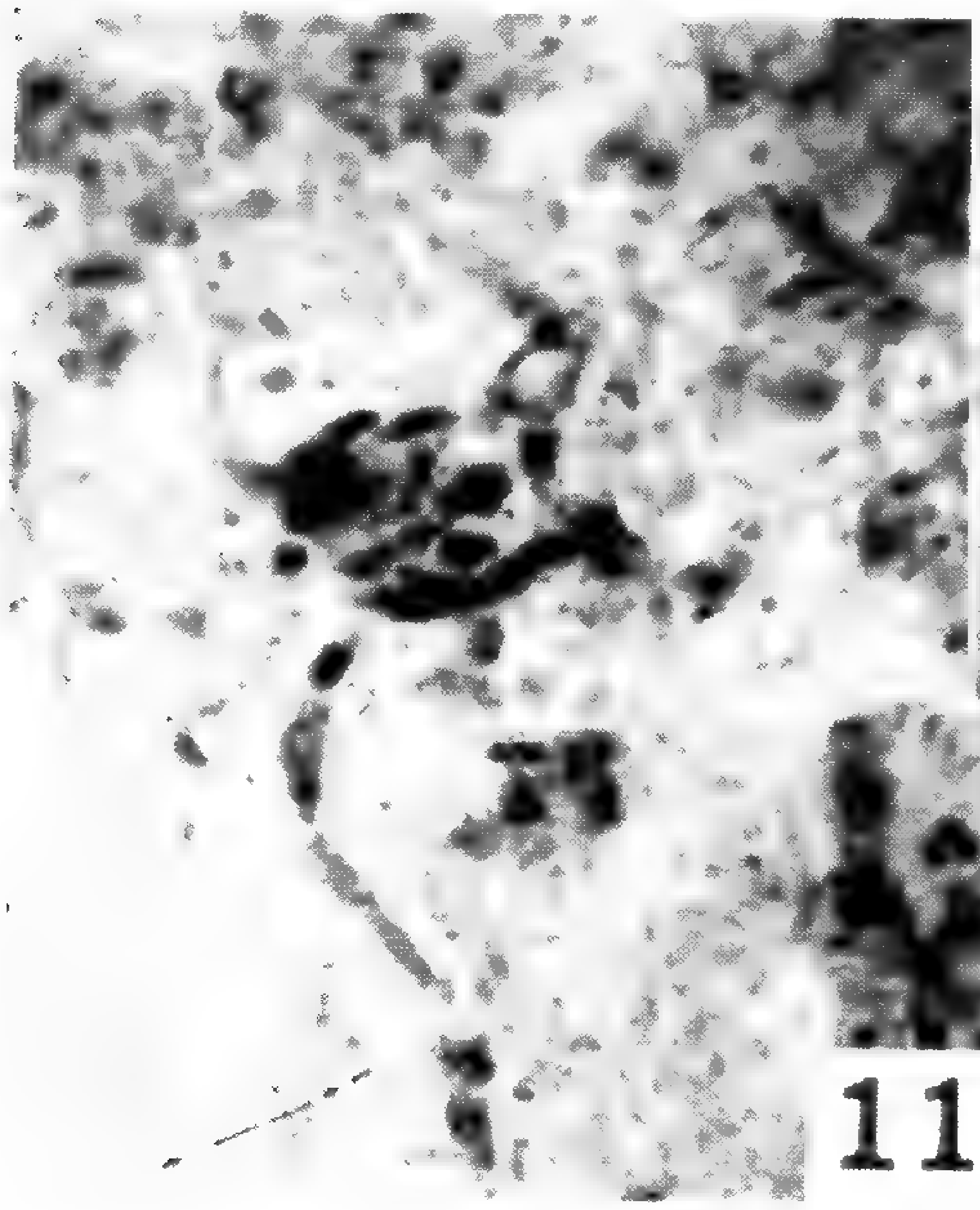
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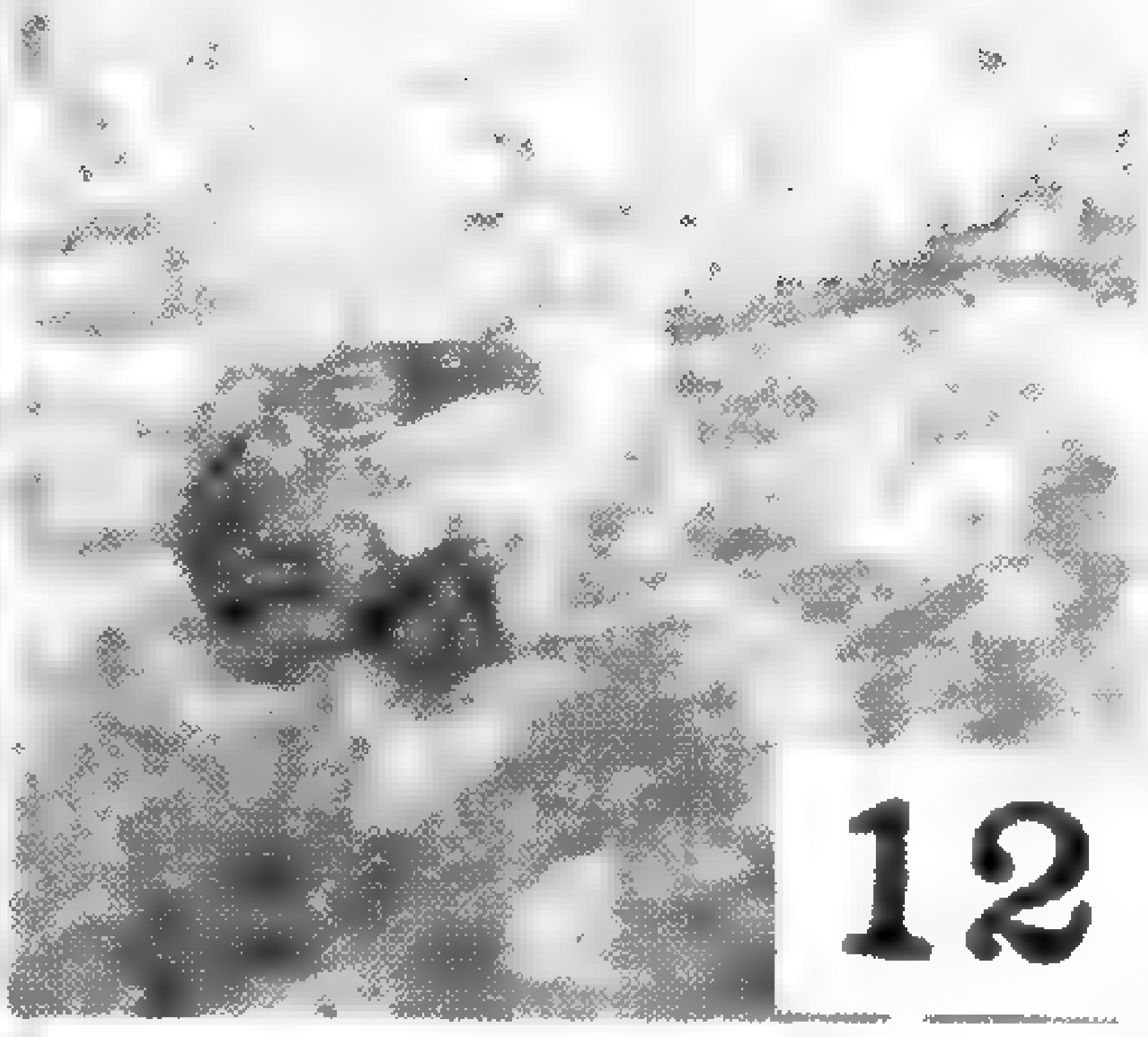
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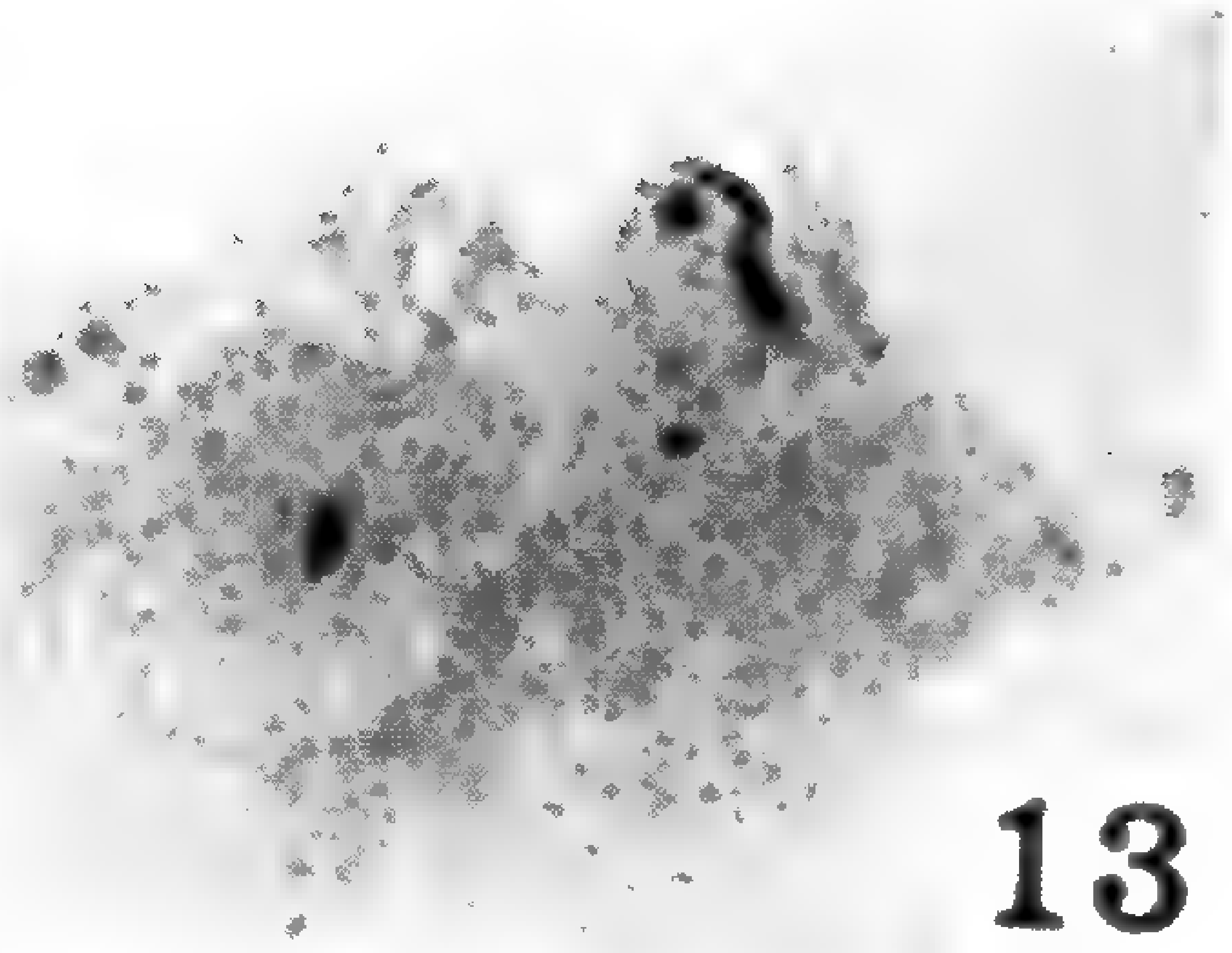
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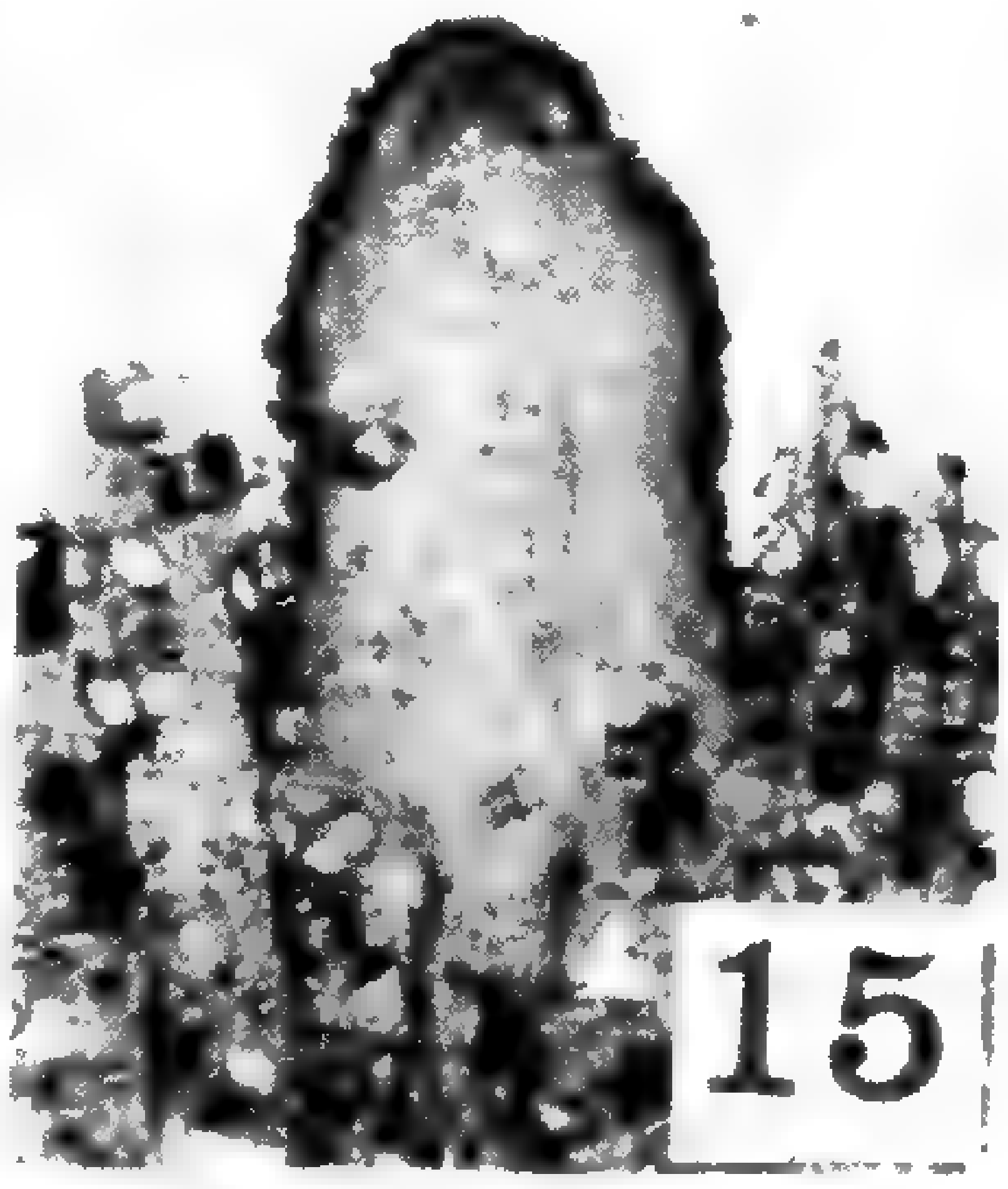
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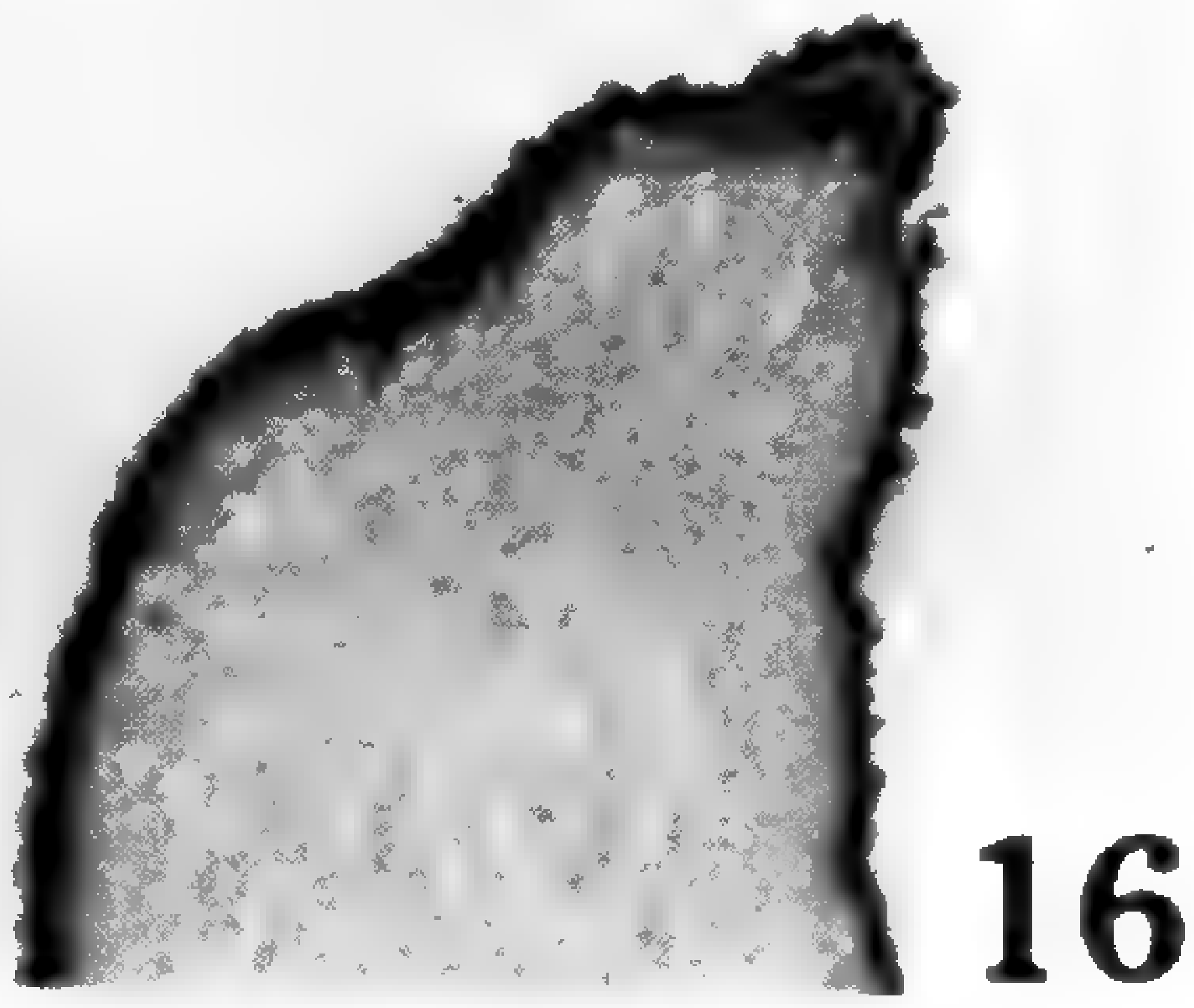
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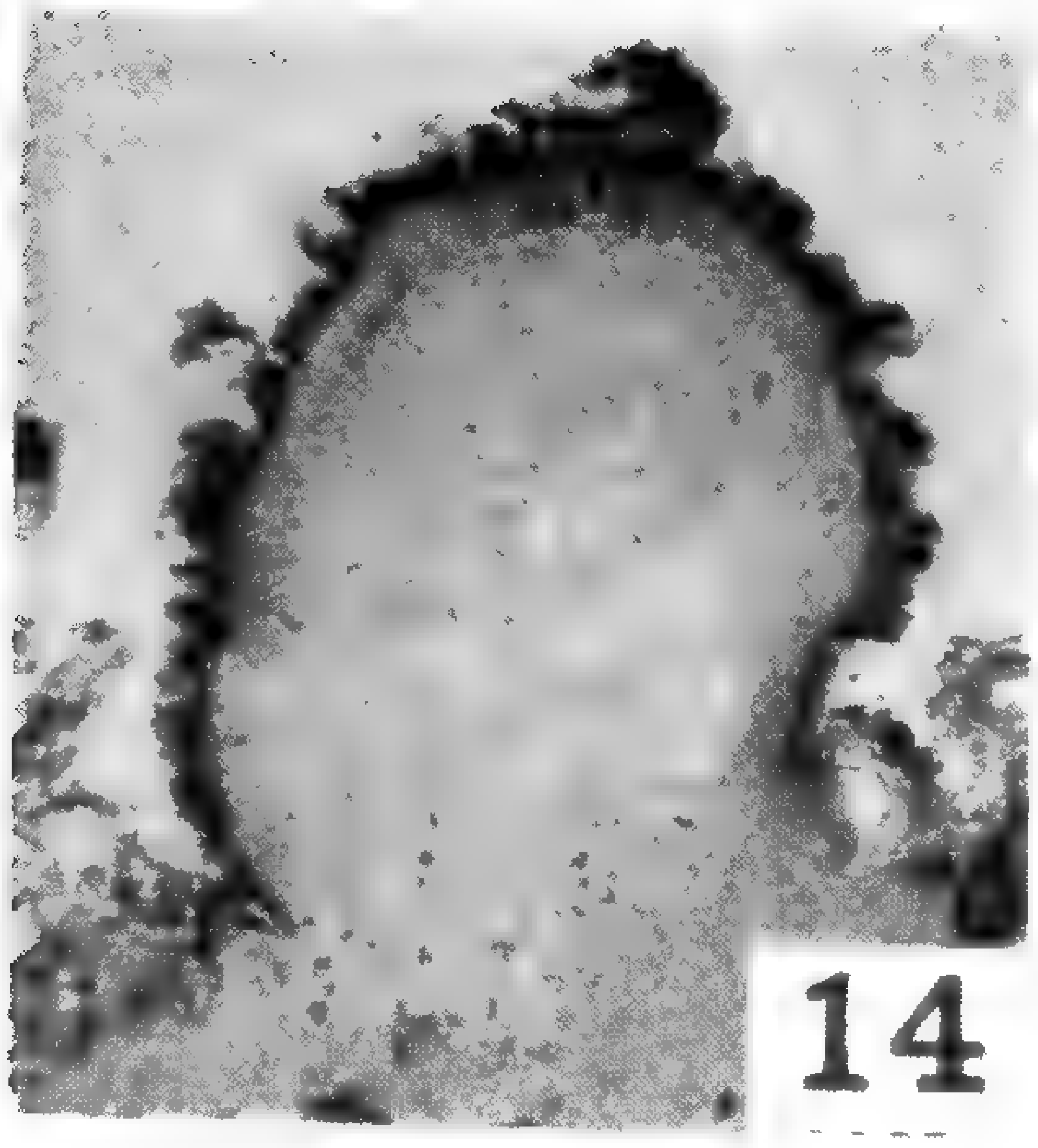
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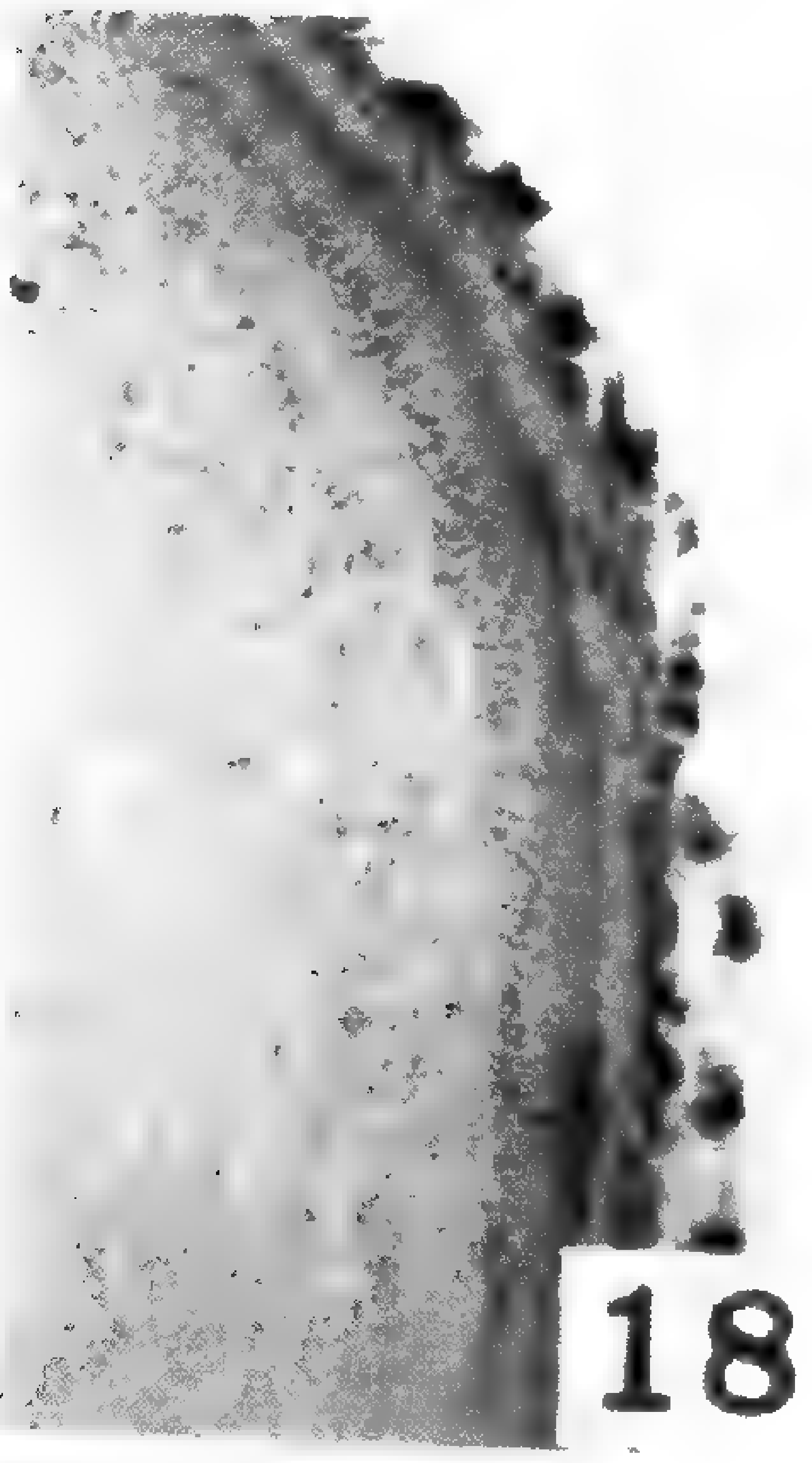
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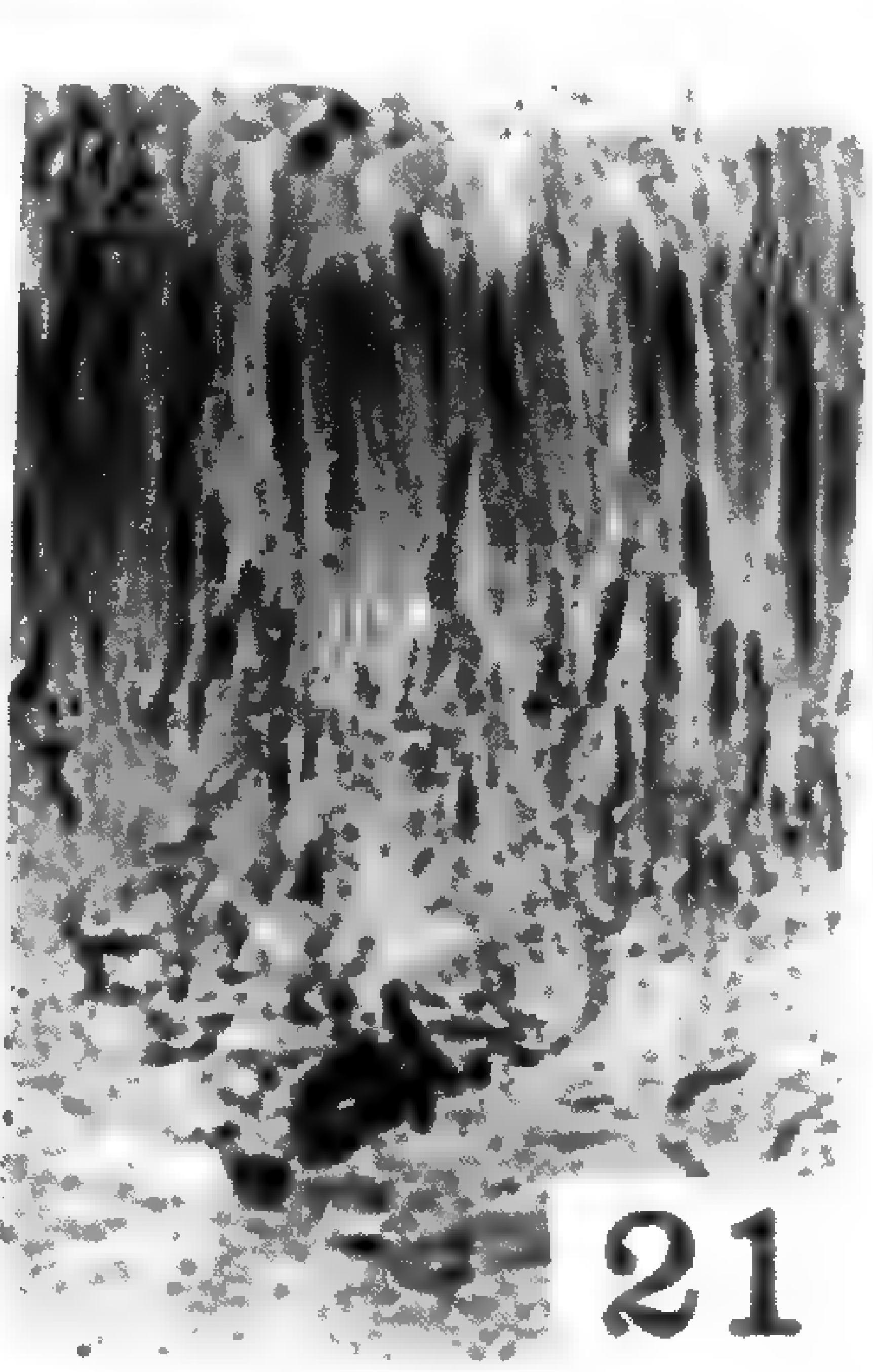
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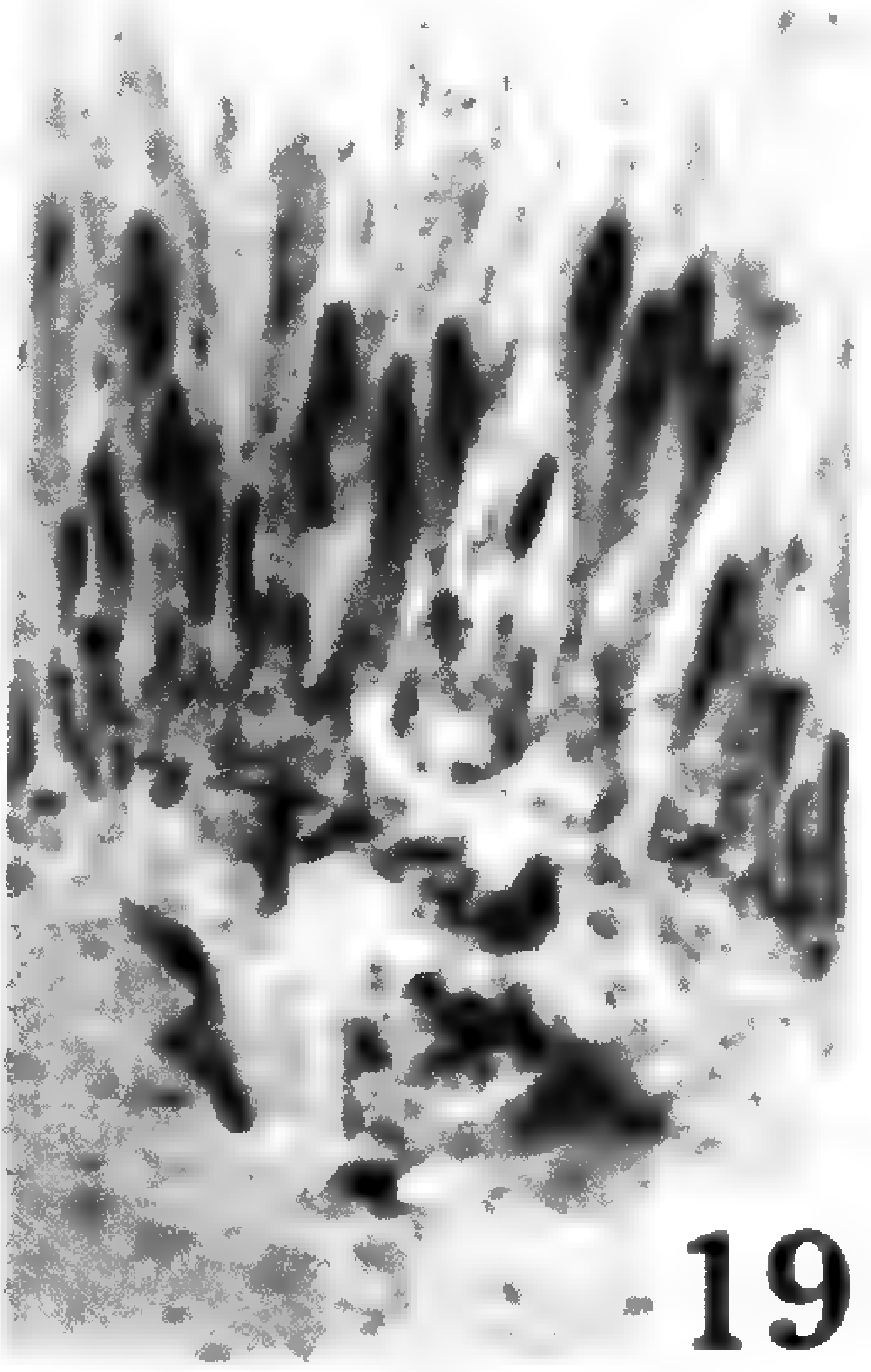
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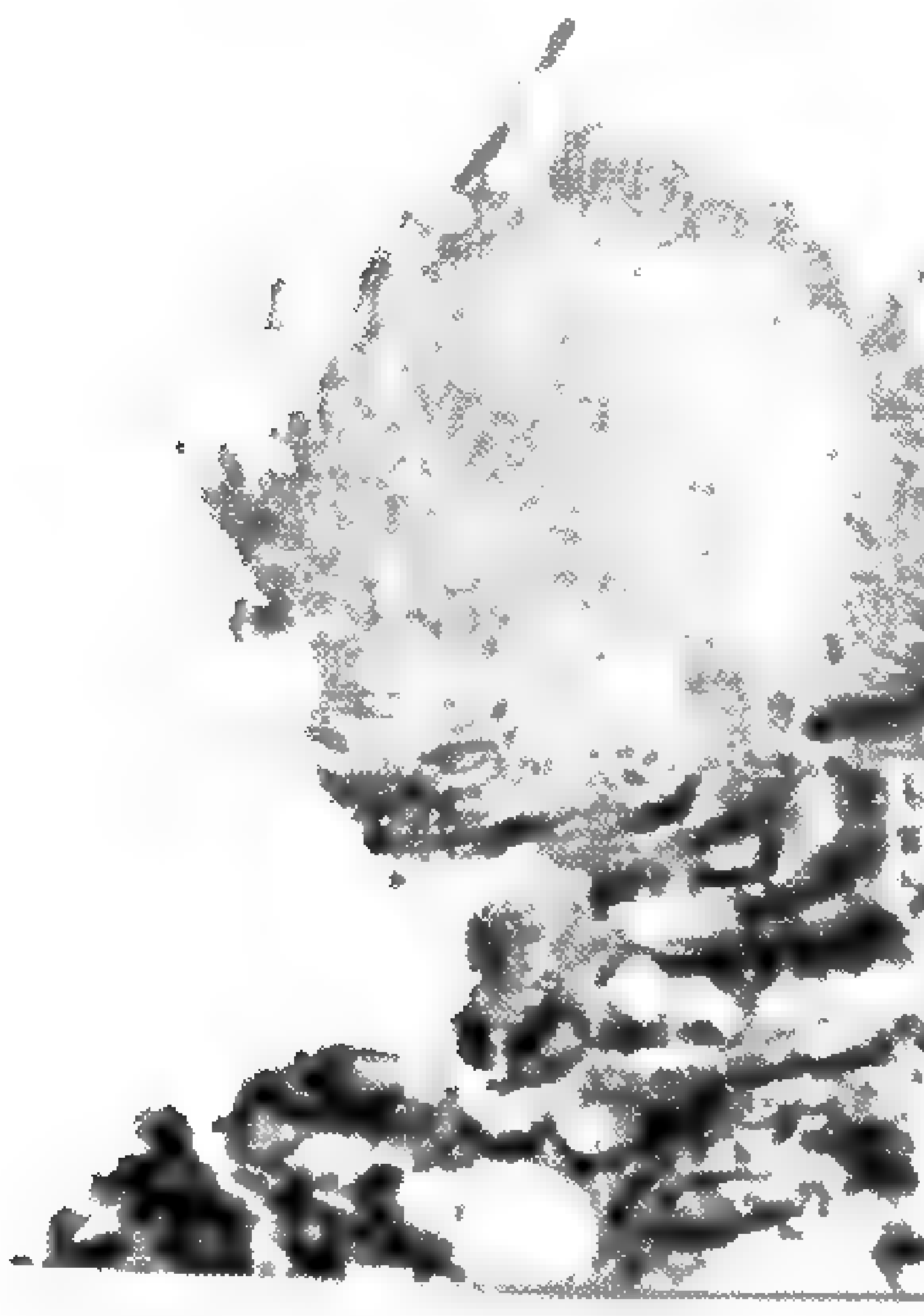
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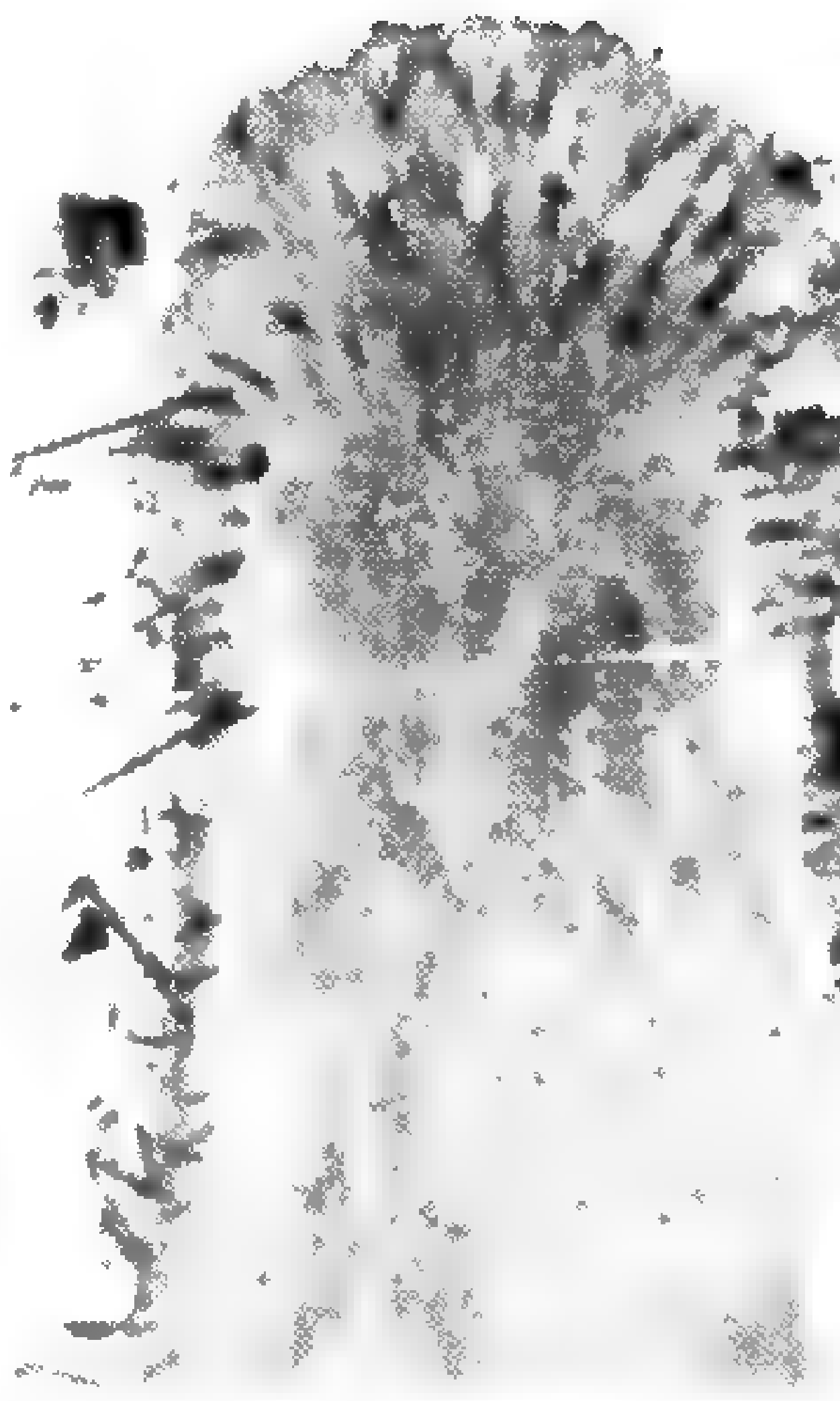
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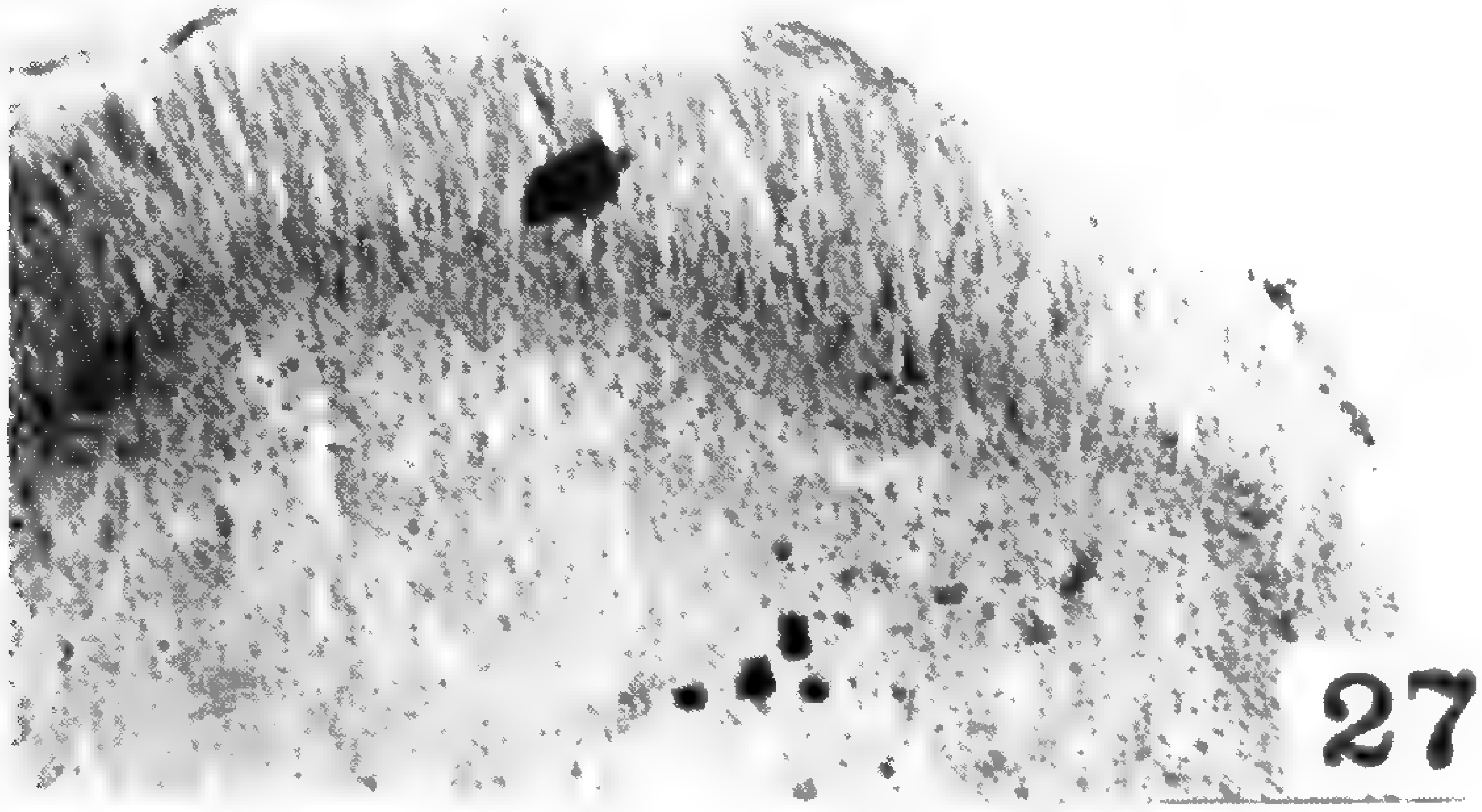
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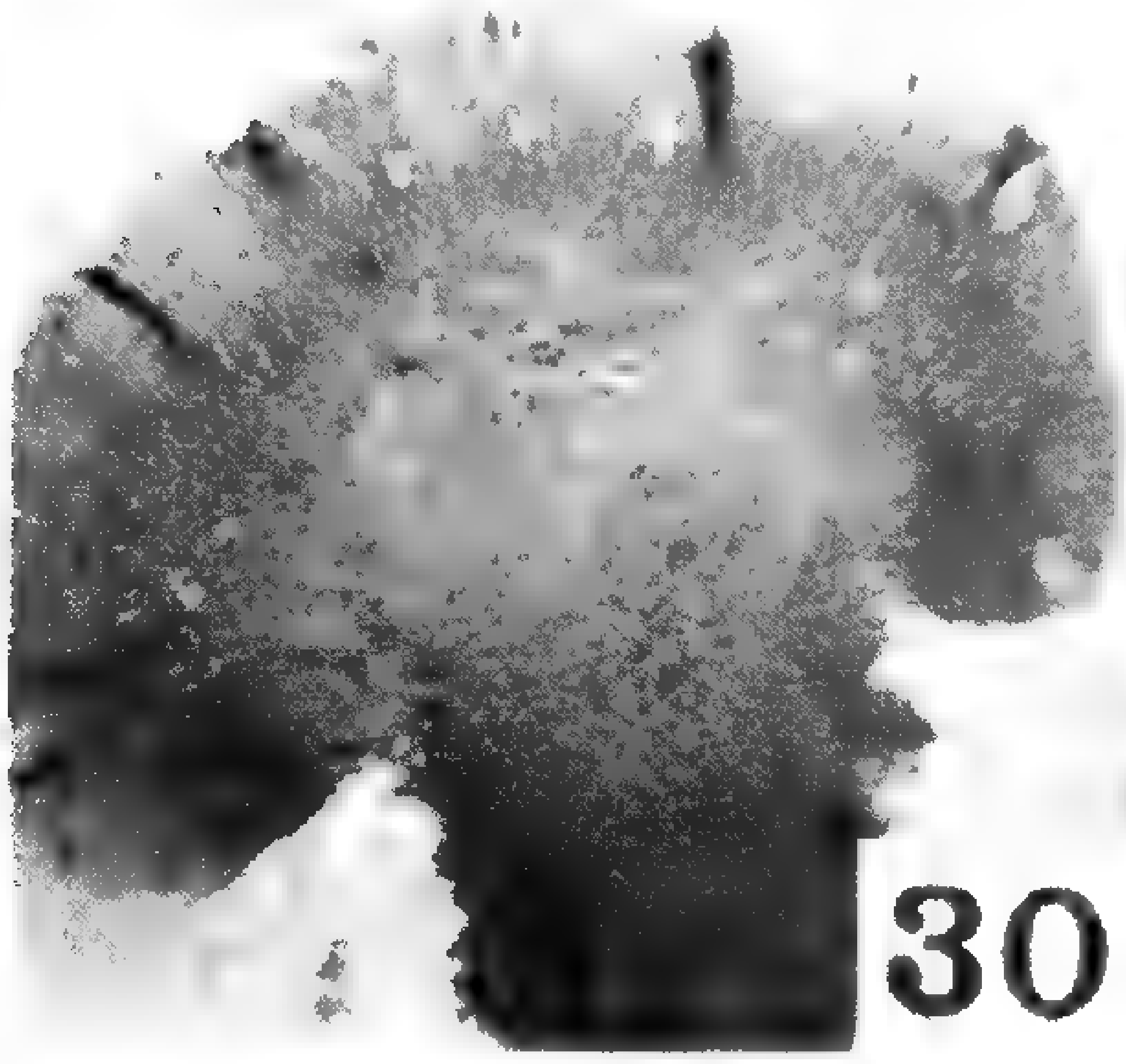
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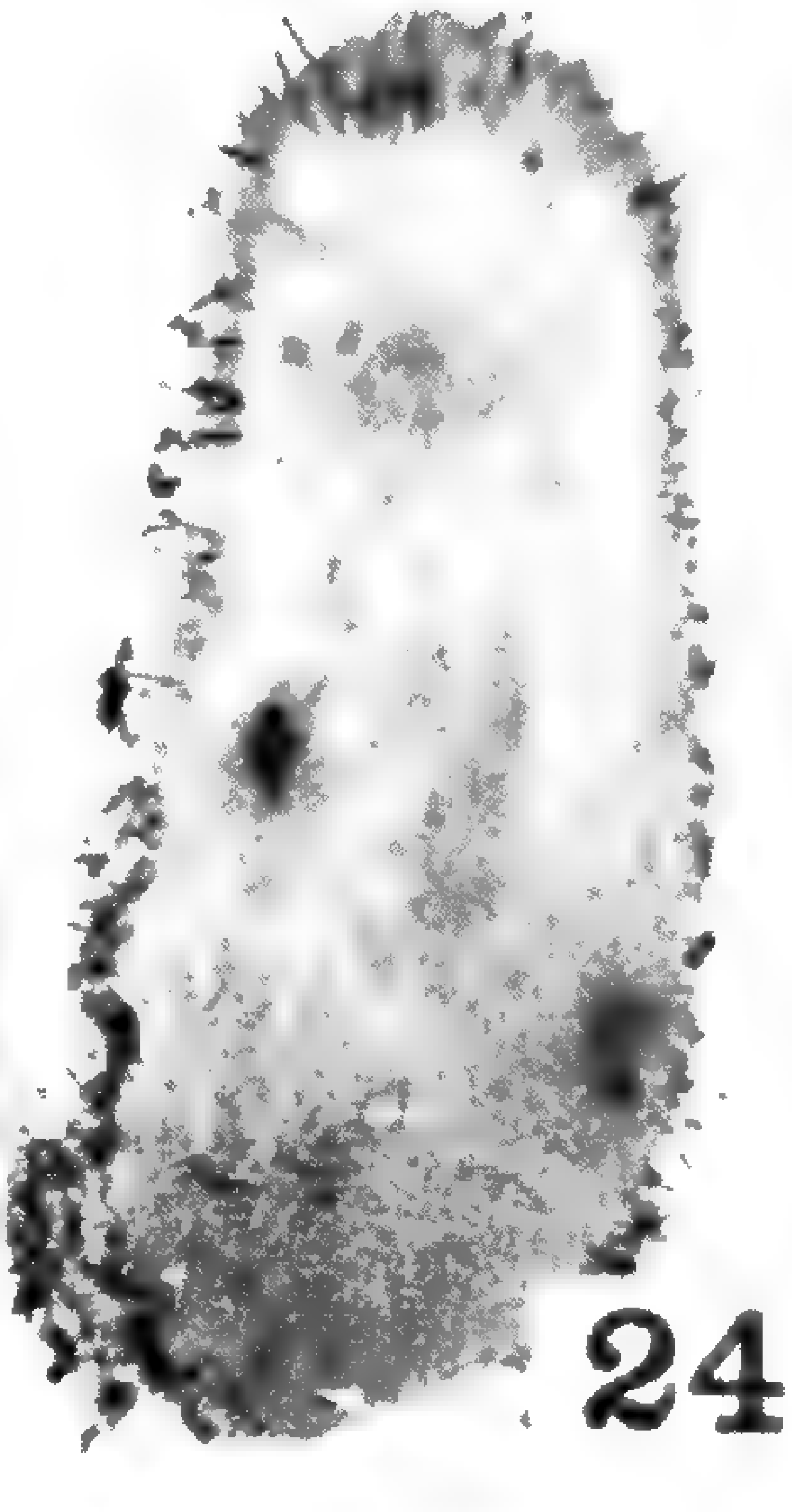
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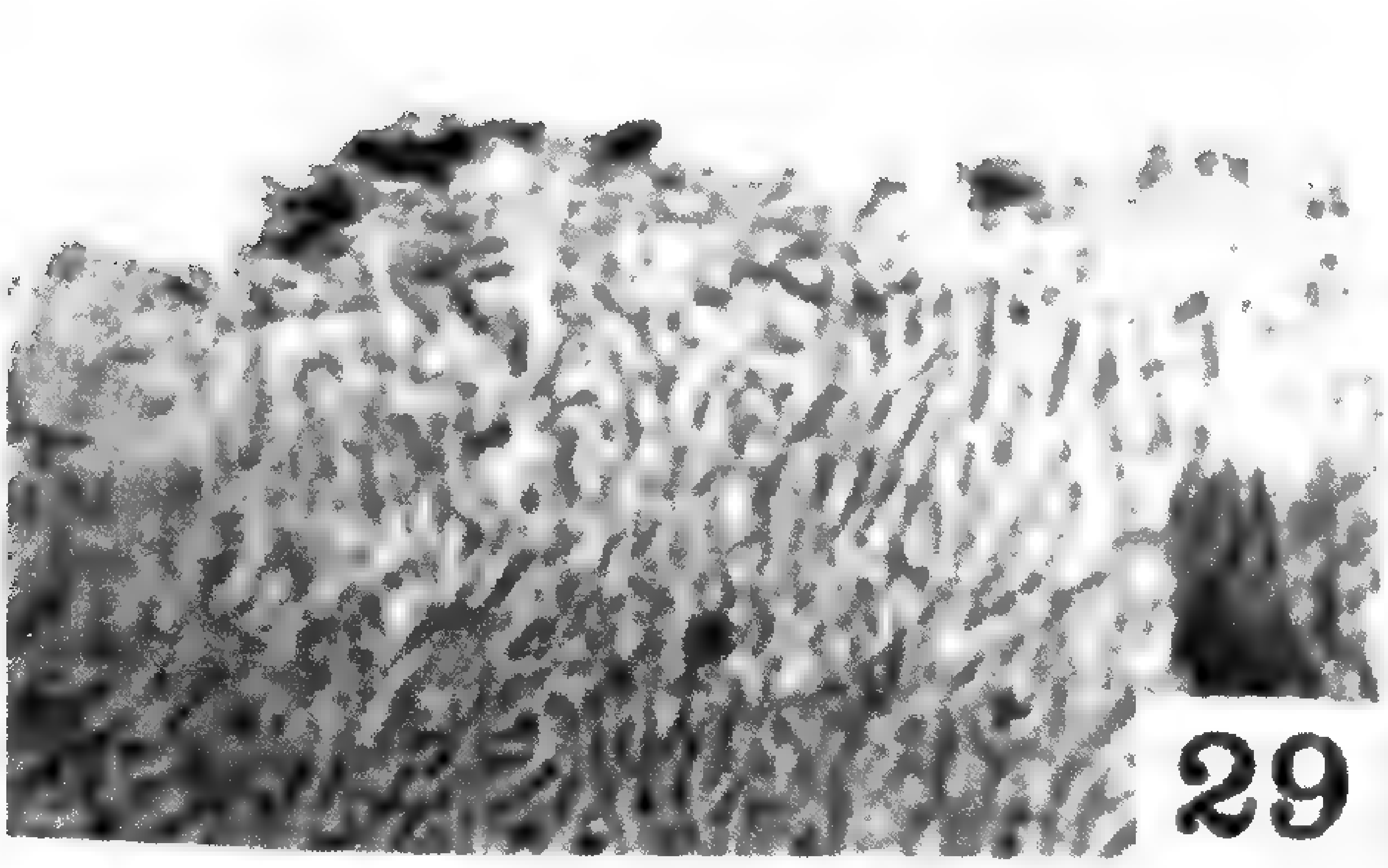
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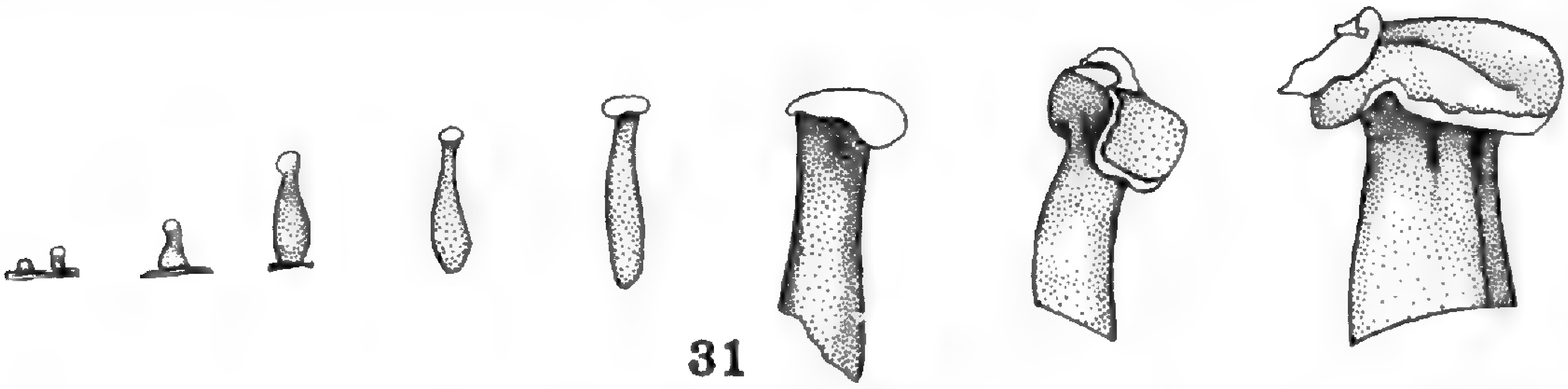
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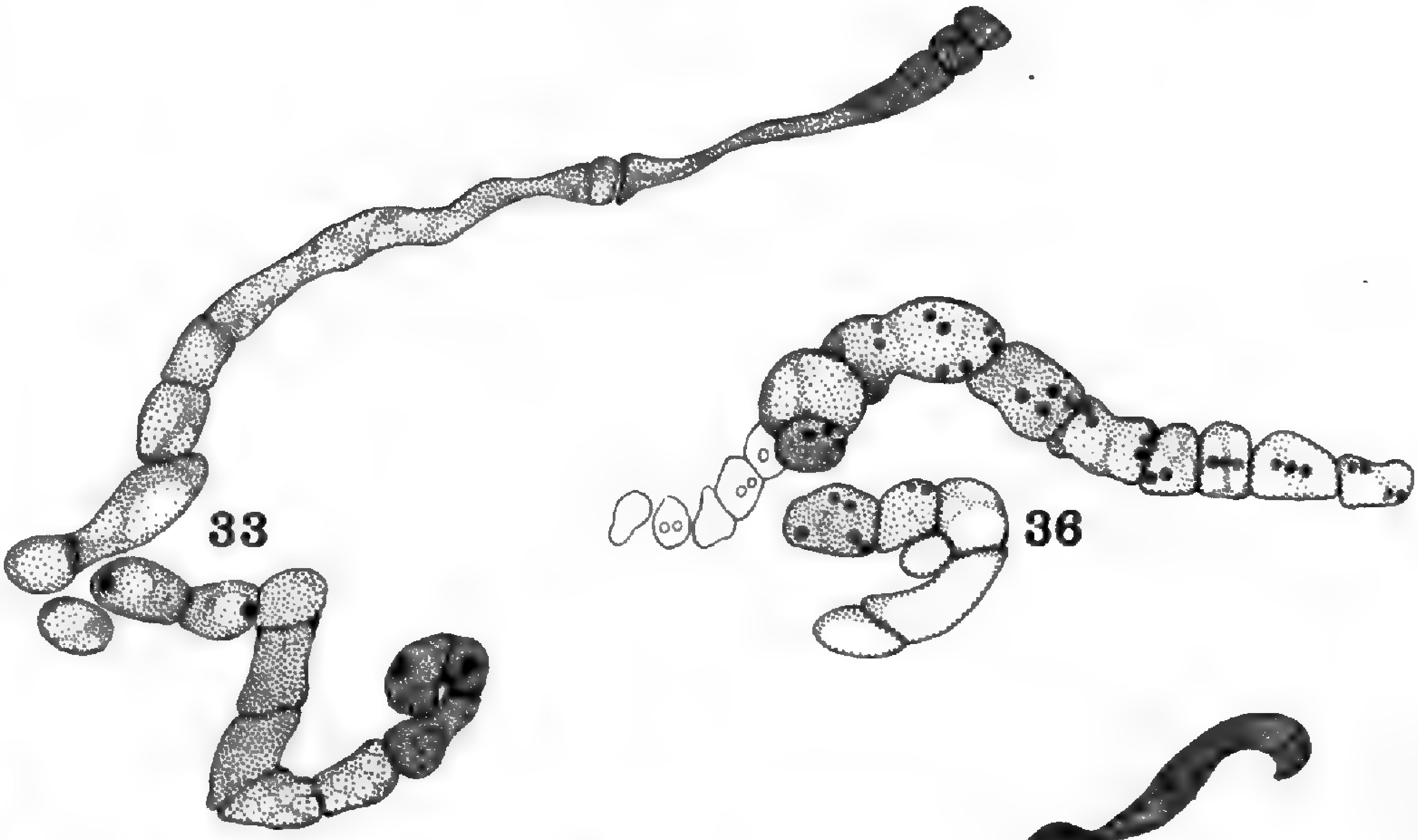
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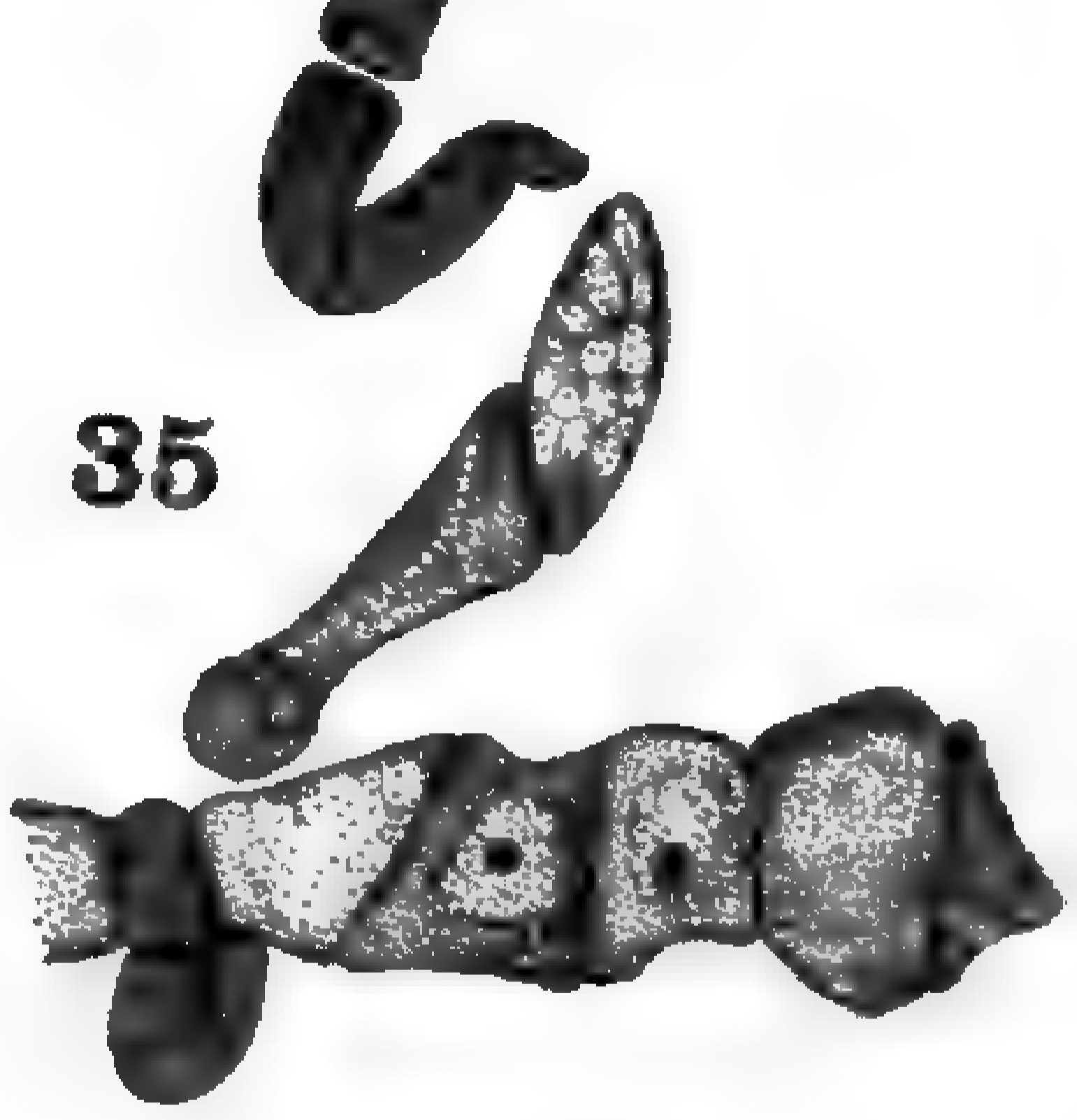
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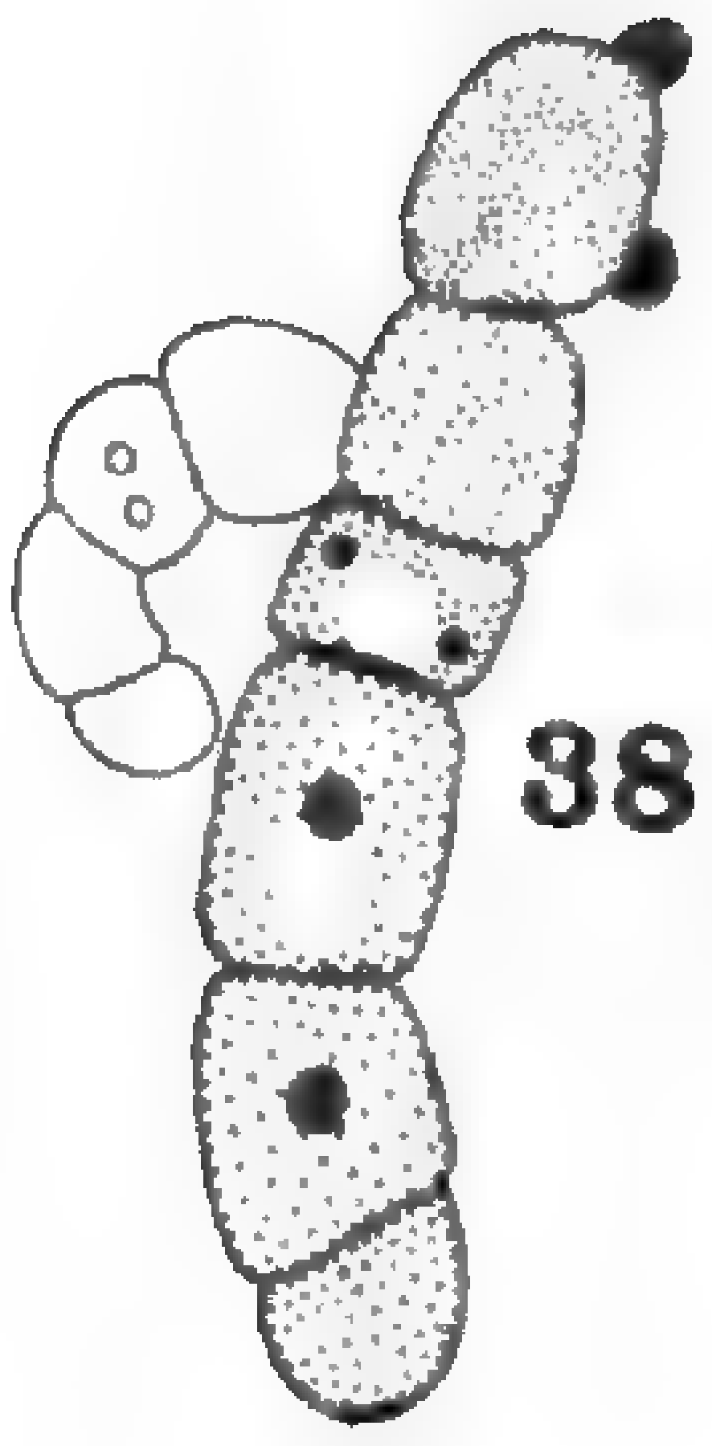
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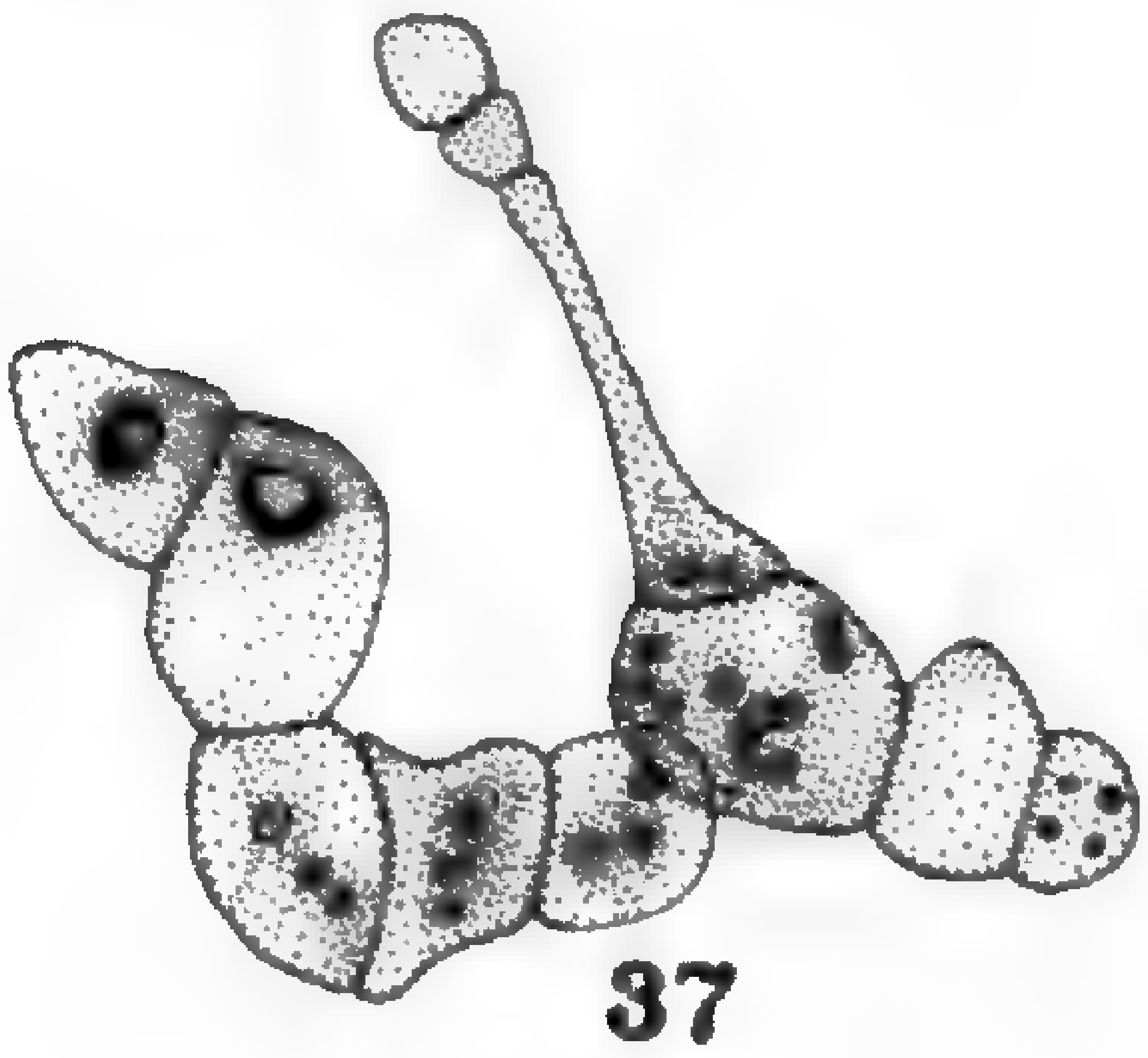
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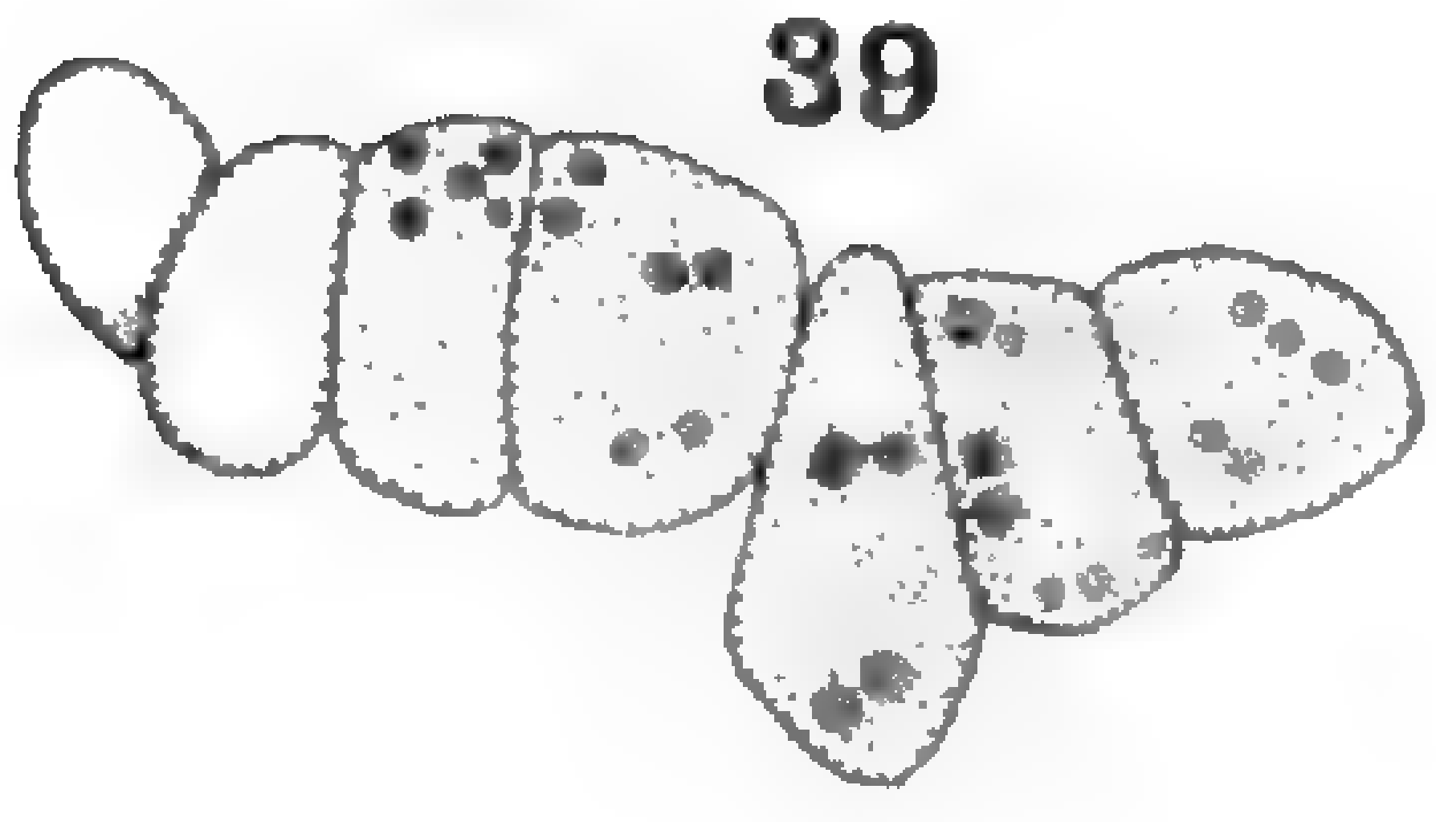
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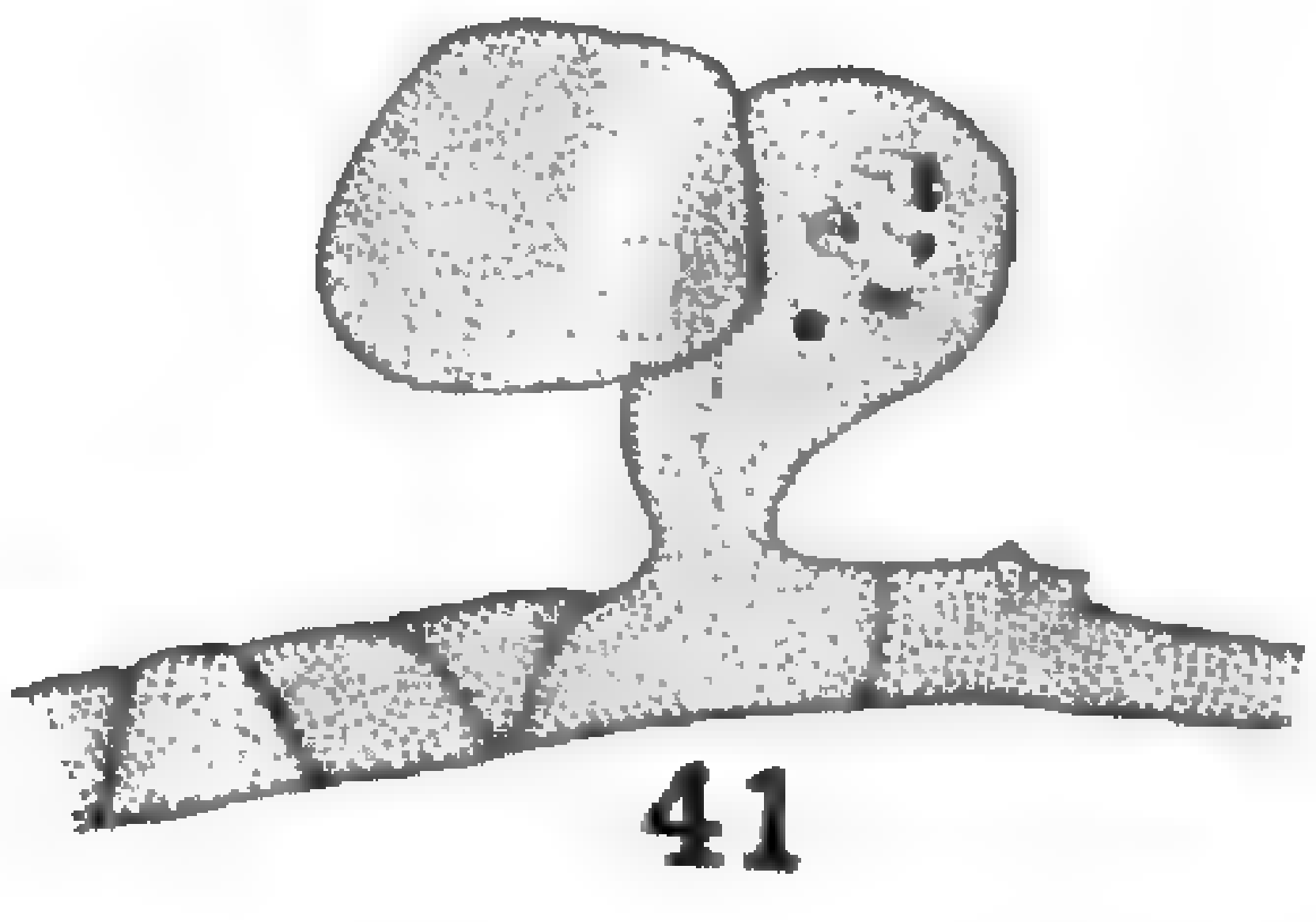
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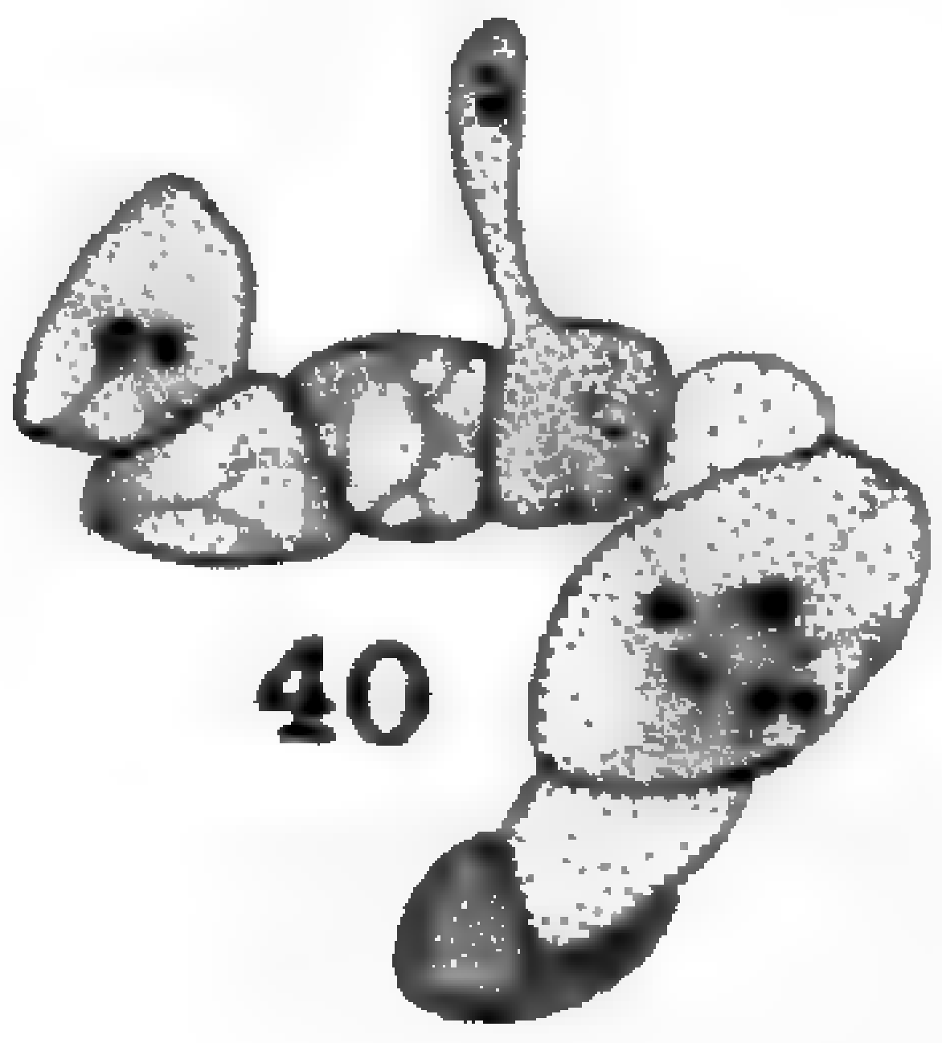
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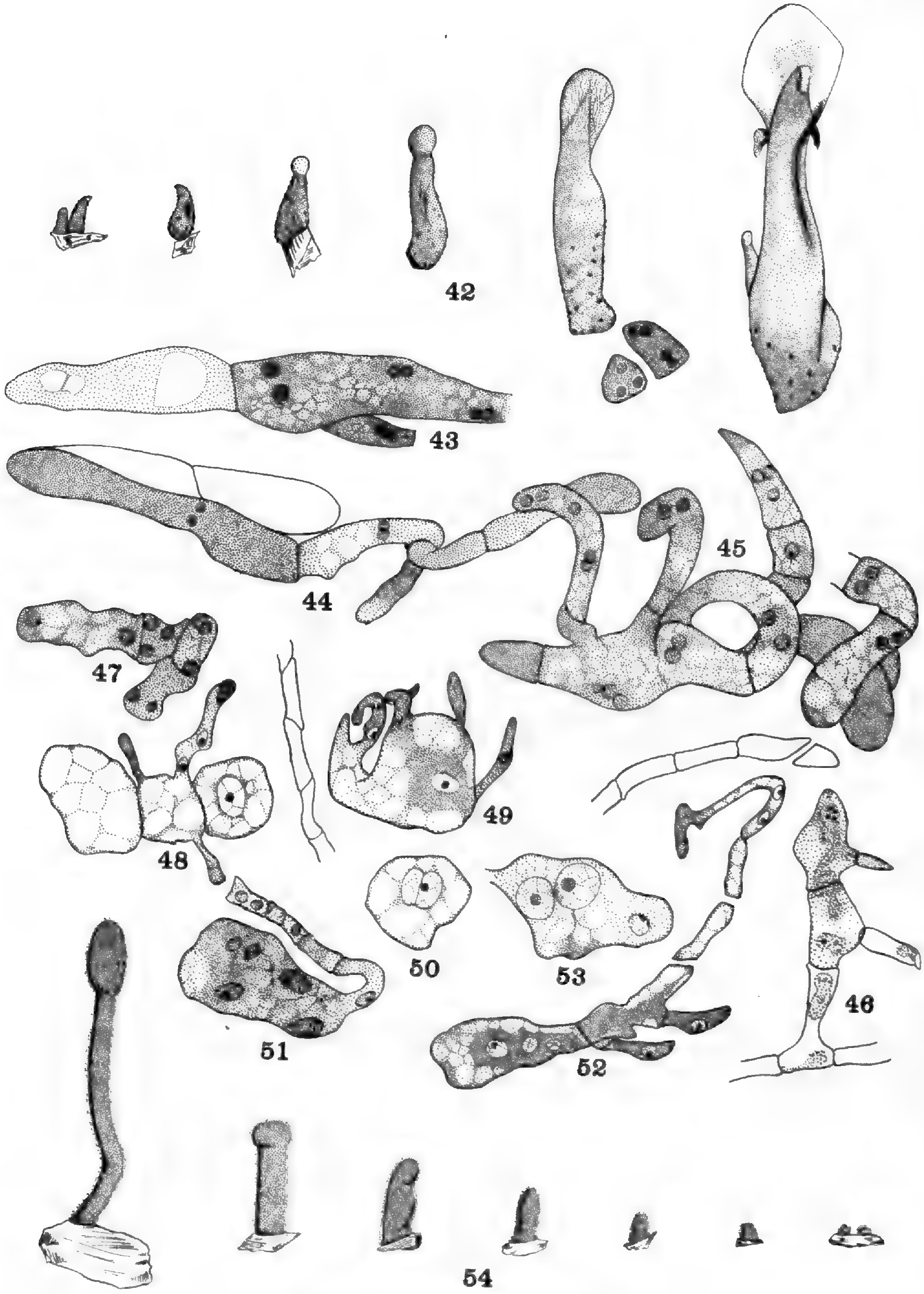

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EXPLANATION OF PLATES VIII-XII

FIGS. 1, 2.—*Icmadophila aeruginosa:* sections of young ascocarps, from NIENBURG; fig. 1, ×140; fig. 2, ×100.

FIGS. 3-5, *Cudonia lutea:* sections of young fructifications, showing generative hyphae and origin of veil; fig. 3, ×140; fig. 4, ×160; fig. 5, ×140.

FIG. 6.—*C. lutea:* section of cap of ascocarp, showing procarps and trichogynes; ×130.

FIG. 7.—*C. lutea:* section of ascocarp, showing veil inclosing young hymenium; ×70.

FIGS. 8, 9.—*C. lutea:* procarps and trichogynes enlarged; ×390.

FIG. 10.—*C. lutea:* ascogenous hyphae passing from procarp to hymenium; ×370.

FIG. 11.—*C. lutea:* lower portion of procarp with generative hyphae; ×530.

FIG. 12.—*C. lutea:* old procarp with empty cells; ×430.

FIG. 13.—*C. lutea:* first section of series through cap, showing projecting trichogyne; ×500.

FIGS. 14-17.—*Spathularia velutipes:* sections of young ascocarps; fig. 14, ×50; fig. 15, ×35; fig. 16, ×35; fig. 17, ×40.

FIG. 18.—*S. velutipes:* sections of cap, showing veil inclosing young hymenium; ×150.

FIGS. 19-21.—*S. velutipes:* procarps and ascogenous hyphae; fig. 19, ×400; fig. 20, ×250; fig. 21, ×310.

FIGS. 22-25.—*Trichoglossum hirsutum:* sections of young ascocarps; fig. 22, ×100; fig. 23, ×80; fig. 24, ×33; fig. 25, ×27.

FIG. 26.—*T. hirsutum:* hymenium; ×85.

FIGS. 27, 28.—*Leotia lubrica:* sections of young ascocarps, showing overlying fragments of tissue; fig. 27, ×140; fig. 28, ×175.

FIG. 29.—*L. lubrica:* fragment of overlying tissue enlarged; ×695.

FIG. 30.—*L. lubrica:* section of ascocarp, showing enlarged cells resembling procarps of *Spathularia;* ×70.

FIG. 31.—*Cudonia lutea:* series of ascocarps at different stages; ×3.

FIGS. 32-35.—*C. lutea:* procarps and trichogynes; ×1125.

FIGS. 36-40.—*C. lutea:* procarp cells at multinucleate stage; ×1125.

FIG. 41.—*C. lutea:* two cells of procarps with degenerated contents, showing also generative hypha; ×1125.

FIG. 42.—*Spathularia velutipes:* series of ascocarps at different stages; ×3.

FIGS. 43-45.—*S. velutipes:* procarp cells; ×2000.

FIG. 46.—*S. velutipes:* old procarp cells with depleted contents; ×1125.

FIGS. 47-53.—*Leotia lubrica:* large cells of cap, showing nucleation and in some cases ascogenous hyphae; ×1125.

FIG. 54.—*Trichoglossum hirsutum:* series of ascocarps at different stages; ×3.

A MORPHOLOGICAL STUDY OF THE UMBELLIFERAE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 298

HILARY STANISLAUS JURICA

(WITH PLATES XIII, XIV)

Introduction

The fact that the Umbelliferae are so extensive and so well distributed throughout the northern university zone has made them an object of frequent study. CÉSALPIN (9) was the first to assemble the different members of the Umbelliferae into a separate group, not only on the basis of their umbellate inflorescence, but also on the basis of a secondary character, the two-celled ovary, each cell of which gives rise to a single seed. MORISON (51) recognized the family on the basis of the same characters as CÉSALPIN, but added a number of other plants, especially some of the Valerianaceae and *Thalictrum*, which of course, were not destined to remain within this family. To these, he applied the name of imperfect Umbelliferae. According to GÉNEAU DE LAMARLIÈRE (27), however, it was left for HERMANN (1690) to establish a rational division for this family, namely, (1) species with ovate seeds; (2) species with hairy or spiny fruits; and (3) species with large and flattened fruits. Following HERMANN, MAGNOL (27), in 1709, divided the family into four groups based on the character of the surface of the fruit: (1) fruit ribbed, (2) fruit large, (3) fruit spiny, and (4) fruit long.

The classification of the Umbelliferae entered upon a new phase with LINNÉ (45), who selected or rather borrowed from his contemporary ARTHEDIUS the row of bracts of the involucre and of the involucel as a principal character, upon which he based his division of the Umbelliferae, with the already mentioned external features of the fruit as secondary characters. At this time the essential distinction between a cyme and an umbel was not considered, and accordingly very many forms were included, which later workers, notably ADAMSON (1), CRANTZ (15), SPRENGEL (63), HOFFMANN

(32, 33), KOCH (42), DE CANDOLLE (17), etc., assigned to other families. A somewhat extensive discussion of the history of the classification of Umbelliferae may be found in the work of GÉNEAU DE LAMARLIÈRE (27). For American workers, COULTER and ROSE (13) listed a condensed bibliography of all the works containing new names or new descriptions of Umbelliferae found within the North American range, from LINNAEUS' *Species Plantarum* (1753) to that of CONGDON (1900).

The first morphological work of note upon the Umbelliferae was that of TITTMANN (65), whose figures showed the germination of some species with great exactness. Influenced by the research work of their age, or at times by the somewhat peculiar nature of the plant at hand, a further study of the germination of a large number of genera and species was made by DE CANDOLLE (17), TREVIRANUS (69, 71), BERNHARDI (4), KIRSCHLEGER (40), IRMISCH (36, 37), VAN TIEGHEM (76), GÉNEAU DE LAMARLIÈRE (24, 25, 26, 27), DOMIN (18), DRUDE (20), HOLM (35), and MÖBIUS (49).

Although DE CANDOLLE (16) had already described the stem of *Ferula*, whose medullary bundles could easily lead one to mistake it for a monocotyledon stem, it was left for HOFFMANN (34) to present us with the first extensive anatomical work upon the Umbelliferae. His study of the roots of the plants of this family contains many interesting details, but it is to be regretted that he failed to distinguish the root from the rhizome, and at times even confounded it with the lower part of the aerial stem. Moreover, he paid no attention to order and very little to development.

Further anatomical studies were made by JOCHMANN (38), REICHARDT (58), DUCHARTRE (21), BEHUNECK (3) FAURE (22), GERARD (28), TRECUL (68), COURCHET (14), HOLM (35), KLAUSCH (41), GÉNEAU DE LAMARLIÈRE (27), NOENEN (55), and NESTEL (54). The work of MÖBIUS (49), however, deserves special attention, for the parallel-veined leaves of numbers of species of *Eryngium*, together with the general aspect of their gross morphology, lead many taxonomists to suspect an analogy in their anatomy to that of some of the monocotyledons. Accordingly, we have species like *Eryngium yuccifolium* Michx., *E. bromeliaefolium* Delar., *E. pandanifolium* Chan., *E. luzulaefolium* Chan., *E. junceum* Chan., and *E. scirpinium* Chan. In his extensive work covering the anatomy of the leaf, stem, rhizome, and root, MÖBIUS showed that this similarity is only apparent, and that in reality the stem of *Eryngium* is not merely a dicotyledon, but is one of an advanced type.

A rather unique and quite extensive study of the mechanical tissue in the stem and leaf was made by FUNK (**23**), as recently as 1912.

The formation of the leaves, umbels, and gross morphology next received attention from workers like JOCHMANN (**38**), BUCHENAU (**7**), MOHL (**50**), ROSSMANN (**60**), CLOS (**10, 11**), GERARD (**28**), KLAUSCH (**41**), DOMIN (**18, 19**), PETERSEN (**57**), RENNERT (**59**), TERNETZ (**66**), WRETSCHKO (**84**), and GRIESEBACH (**29**).

The oil ducts or secreting canals were studied by MEYEN (**47**), JOCHMANN (**38**), MEYER (**48**), VAN TIEGHEM (**73, 74, 75**), MÜLLER (**53**), MOYNIER DE VILLEPOIX (**52**), LANGE (**43**), and VUILLEMIN (**79**).

PAYER (**56**), TRECUL (**67**), and JOCHMANN (**38**), whose works appeared but a few months apart, were the first to attempt the organogeny of this family. In the formation and development of the leaves all three disagree, and all differ from the account given by GRIESEBACH (**29**), but the accounts of PAYER (**56**) and JOCHMANN (**38**) agree in regard to the floral development and also with the accounts of SIELER (**62**) and HANNAH (**31**). SIELER, however, interprets the "calycis primordium" of these workers as a special kind of organ, which gives rise to the calyx, and naturally he finds fault with the seemingly existing "primordialkelch," a view which I failed to receive from the reading of both PAYER'S and JOCHMANN'S works. JOCHMANN'S work especially evinces great care, and no doubt, had modern technique been available in his day, the embryogeny would have been included. Like all the earlier workers, JOCHMANN begins his study with germination, but, unlike them, he pays special attention to the root, rhizome, and stem, their anatomy, and their oil ducts. He then proceeds to discuss the development of the leaves, umbels, flower, stylopodium, style, "gynoecium," pericarp, and the seed.

Among other workers dealing with the development or histology of the fruit may be mentioned LANESSAN (**44**), BARTSCH (**2**), TREVIRANUS (70), and TANFANI (64). Quite recently an approach was made to the study of embryogeny by CAMMERLOHER (8), who studied the "Samenanlagen" of a large number of genera; and MARTEL (46) presented us with an anatomy of the flower.

Summing up all the work done, however, we find that the classification, gross morphology, anatomy, mechanical tissue, oil ducts, development of leaves and inflorescences, floral development, and development of fruit in this family are well covered, but embryogeny proper, endosperm formation, and the embryo still remain to be studied. It is with a view to filling this gap that this investigation has been entered upon.

Methods

The material used in this study was collected at different intervals in the vicinity of Lisle, Illinois. The fresh material was killed in a stock solution of chromo-acetic acid, imbedded in paraffin, and stained usually with either safranin and Delafield's haemotoxylin, or safranin, gentian violet, and orange G (omitted in a few cases).

The study centers about *Eryngium yuccifolium*, with frequent comparisons with other genera, especially *Sium cicutaefolium*.

Floral development

No attempt has been made to present a study of the development of the inflorescence or umbel, for that has already been well studied by workers listed in the introduction. Nevertheless, since the umbels of *Eryngium yuccifolium* consists of distinct compact heads, a short note in regard to the head will not be out of place.

The central or apical head of each umbel develops earlier and more rapidly than the encircling members, hence by the time the central head becomes visible to the naked eye, longitudinal sections show that the encircling heads develop in the axils of the bracts subtending the umbel, in much the same manner as described by JURICA (39) for the heads of *Dipsacus sylvestris* (fig. 1).

As already has been described by JOCHMANN (38) for *Eryngium planum*, all the flowers in the heads of *Eryngium yuccifolium* arise from the axils of bracts spirally arranged. The blossoming begins at the base and extends toward the apex (fig. 1). The individual epigynous flowers appear as an undifferentiated mass of cells, somewhat rounded at first (fig. 2), but soon broaden out, so that the individual sepal primordia forming the calyx (which is well pronounced in *Eryngium yuccifolium*) are distinctly visible (figs. 3, 4). This is soon followed by a perfect and regular acropetal succession, presenting the sequence sepals, petals, stamens, and carpels, in perfect accord with the account given by HANNAH (31) for *Sanicula marilandica;* by PAYER (56) for *Heracleum Sphondylium* (and other species of *Heracleum*), *Carum*, *Aegopodium*, *Anethum*, *Phellandrium aquaticum*, etc.; and by SIELER (62) for *Heracleum*, *Sphondylium*, *Chaerophyllum bulbosum*, *Cicuta virosa*, *Daucus Carota*, *Peucedanum cervaria* Lat., *Angelica sylvestris*, etc. (figs. 2–7). It is well to note, however, that SEILER (62) is more concerned with the study of the sequence of the appearance of individual members within a cycle than with the sequence of the cycles themselves.

JOCHMANN (38) had noted that, although the calyx primordia make their regular appearance in *Aegopodium*, etc., they fail to continue in their development ("in pristino statu remanent, et quo, magis flos accrescit, eo magis evanescunt"), and thus apparently simply remain as calyx teeth, and in many genera even these are obsolete and hardly distinguishable (figs. 9, 10).

Ovules

The carpels are distinctly two in number at first, but soon unite along their inner face, so that in cross-section they appear to be semicircular (fig. 7); or as PAYER (56), JOCHMANN (38), SEILER (62), and CAMMERLOHER (8) would have it in the forms studied by them, "semilunar" in shape. In each of the four free ends of the coalesced carpels an anatropous ovule begins to develop (figs. 11, 12), one of which soon stops, however, while the other continues in its normal development. This results in a hanging anatropous ovule, with the raphe turned inward and the micropyle outward (fig. 14). This seems to be quite general for the entire family, for it has been found in all the species studied, and it is in accord with the account of CAMMERLOHER (8), who studied the "Samenanlagen" in thirty-seven genera and forty-five species. Moreover, JOCHMANN

(38), PAYER (56), and SEILER (62) have noted it in their work. Nevertheless, it is not out of place to note that, in one exceptional case, all the flowers of one head of *Eryngium yuccifolium* developed two normal ovules in each ovary cavity (fig. 13).

Megaspore and embryo sac

By the time the ovule has reached the stage shown in fig. 17, the nucellus has become quite prominent, and the hypodermal archesporium is easily recognizable (fig. 18). This figure shows the megaspore mother cell nucleus in synapsis, which indicates that the reduction division is about to occur. The successive stages in the development of the megaspore, resulting in a linear row of four megaspores (fig. 19), as well as the destruction of the potential megaspores, present no essential deviations from the process as ordinarily described, for the embryo sac develops from the innermost megaspore, and *Sium cicutaefolium* is no exception in this case (figs. 15, 16). The nucellus has only a single layer of cells surrounding the megaspore, but the absence of tapetal cells is well compensated by the presence of a nutritive apparatus (figs. 16, 20–25) in the chalazal region. The nucellus undergoes but slight development, and then begins to break down (figs. 16, 21–25). Even in the development of the embryo sac there is nothing unusual. The megaspore nucleus divides by three successive divisions, and at first an eight-nucleate, and then a seven-nucleate embryo sac is the result. The amount of protoplasm in the developing sac is comparatively small, frequently resulting in the presence of very large vacuoles, not only in the embryo sac proper, but in the synergids and oosphere as well (figs. 23–25). The antipodal cells, three in number, can easily be seen in the embryo sac before the polar nuclei fuse, and generally are arranged in the form of a triangle (fig. 24). They soon break down, however, and only rarely can be distinguished at a later stage (fig. 16), that is, they are somewhat ephemeral, breaking down shortly before or after the fusion of the polar nuclei.

Endosperm and embryo

Shortly after double fertilization the endosperm nucleus begins to divide, forming an endosperm consisting of free nuclei (figs. 26, 27). The early divisions of the endosperm nuclei occur more rapidly than those of the embryo, and very soon cell walls make their appearance. Even after the formation of cell walls, the endosperm continues to form so rapidly that when the seed is mature the small, insignificant, yet massive embryo (figs. 28–30) is practically inclosed in rich endosperm tissue. In its development the embryo does not always divide into regular octants (figs. 28–30), but at times is quite irregular. Very frequently in its early development it looks more like a pteridophyte embryo (fig. 28), and is characterized by a long suspensor.

Relationships

The Umbelliferae are very closely related to the Araliaceae and Cornaceae, with which families they form an alliance or order known as the Umbellales, or the "Umbelliflorae" of ENGLER. Although several workers, notably HALLIER (**30**), WETTSTEIN (**83**), and WERNHAM (**82**), have elaborated various tables showing the probable phylogenetic relationships, the scheme of ENGLER is still followed by most workers. In ENGLER'S classification the dicotyledons are divided into two great divisions, the Archichlamydeae and Sympetalae, which, according to COULTER and CHAMBERLAIN (**12**), however, show no sharp distinction, for "sympetalous forms occur among the former, and polypetalous forms among the latter." The distinction is laid principally upon a single character, namely, apetaly or polypetaly for the Archichlamydeae, and sympetaly for the Sympetalae. Without doubt this is pressing a single character too far, and as a result the Umbellales "stand so stiffly apart from other Archichlamydeae as to raise the question whether they do not really belong among the higher Sympetalae" (**12**).

It will not be out of place to indicate such evidence as there is for such an assumption from the taxonomists' point of view. For this purpose the recent extensive work of VIGUIER (**78**) may be cited. In his chapter dealing with the relationship of the Araliaceae with other families, he definitely proves the close relationship of the three families forming the order Umbellales, claiming, for example, that the only character which separates the Araliaceae from the Umbelliferae is the drupaceous fruit of the former, whereas the two families have a number of general characters in common. He also states that not a single anatomical character permits an absolute separation of the two families. After describing the details of this anatomical relationship, he concludes that the Umbelliferae, with the Araliaceae, form a continued morphological series and a very natural group. VIGUIER next shows that the Cornaceae are closely related to the Araliaceae, claiming that even good systematists at times take a single genus out of one of the families and put it into another. WANGERIN (80) and WARMING (81), however, would separate the Cornaceae from the Umbellales on account of the nature of the ovule. No less apparently conclusive is the account of WETTSTEIN (83) in regard to the inter-relationship of the three families forming the Umbellales.

With this inter-relationship established, we can safely return to VIGUIER (78), who compares the Pittosporaceae with the Umbelliferae and Araliaceae on the basis of the presence of secreting canals, for which reason VAN TIEGHEM (77) has added this as a fourth family of the order Umbellales, and his studies upon the ovule of the Pittosporaceae confirm his view.

This interpretation seems plausible, for the ovary of the Pittosporaceae is likewise bicarpellate, although the ovules themselves are free and generally parietally arranged in two rows. The single integument, and the fact, as WETTSTEIN (83) has it, that the corolla is very often somewhat sympetalous ("Korolle manchmal etwas sympetal"), prove that at any rate the Pittosporaceae are out of place among the Rosales, and no doubt belong among the Sympetalae. The inclusion of the Pittosporaceae in the Umbellales by VAN TIEGHEM likewise demands the transfer of the entire Umbellales to the sympetalous dicotyledons. Accordingly they are not to be viewed as an order closing up the Archichlamydeae line, the next of kin to the Myrtales. This is in spite of the fact that the Umbellales have some characters in common with some of the Myrtales, especially with *Hippurus* of the Haloragidaceae, such as epigyny, single integument, etc., for they stand too sharply apart from the rest. The position of families forming the order Myrtales certainly deserves a reconsideration, and this has already been done in part by SCHINDLER (61), who separated *Hippurus* from the Haloragidaceae on the basis of its morphological characters, and made a new family entitled Hippuridaceae. Further rearrangement no doubt will follow later, when more morphological work has been done on the group.

Viewing the Umbelliferae from a morphological standpoint, it is clear that separate petals are the only character which they have in common with the Archichlamydeae, and they even lack sympetaly, if the Umbellales are taken as a whole on the basis of VAN TIEGHEM'S work on the Pittosporaceae; for, according to BESSEY (**5**), "one organ may be advancing while another is retrograding."

"The complete cyclic arrangement of floral members associated with definite numbers" (**12**), the single integument (fig. 17), the anatropous ovule (fig. 14), the absence of parietal tissue of the megasporangium (fig. 18), the small nucellus (fig. 18), and the complete tetrad of the megaspores (fig. 19) of the Umbelliferae, all of which are general characters of Sympetalae in contrast with those of the Archichlamydeae, from which the Umbelliferae stand so stiffly apart, prove that the Umbellales in reality belong among the Sympetalae.

The question now arises, if they are to be placed among the Sympetalae, what is their relative position? This is not difficult to answer, for the epigynous nature of their flower places them surely above the Tubiflorales, and their other floral characters put them below the Campanulales. Hence, a position in the neighborhood of the Rubiales is without question. C. E. BESSEY (**5**), E. A. BESSEY (**6**), and WETTSTEIN (**84**) consider the Umbellales as giving rise to the Rubiales. WERNHAM (**82**) notes that "within both cohorts," as he calls them, "the progress from polycarpellary to a bicarpellary gynoecium is observable; in both the ovary is only very rarely unilocular; and in both the androecium is primitively isomerous with the corolla, and the latter primitively regular." Accordingly, he recognizes the "Umbelliflorae as the representatives of a side branch from the calycifloral (rosalium) plexus, and the Rubiales as another such side branch of this stock." All this is to fit in with his scheme of a polyphyletic origin of the Sympetalae.

On the other hand, HALLIER (30) would have a common "Umbellifloren" stock reaching from the Terebinthaceae, and giving rise to Cornaceae, and through these to two branches, namely, to Umbelliferae on one side and to Rubiales on the other. From this it is evident that, although these systematists disagree as to details, they agree upon the fact that the Umbellales are related to Rubiales. Recognizing ENGLER'S scheme of classification as the one more commonly and more widely accepted, and in view of the numerous facts, both from the taxonomic and morphological field, I suggest that the Umbellales be placed among the Sympetalae parallel with the Rubiales. Even if the viewpoint of WANGERIN (80) and WARMING (81) in regard to the Cornaceae should be proved to be correct and universally accepted, this would not affect the remaining families of the Umbellales, for then the Cornaceae would be transferred to a position in close association with the Caprifoliaceae.

Summary

1. The floral development shows an acropetal succession of floral cycles, namely, sepals, petals, stamens, and carpels.

2. In the genera in which the sepals are represented by mere calyx teeth or are obsolete, the calyx primordia also make their appearance, but fail to develop any further.

3. The carpels are two in number and later fuse to form the ovary.

4. Two anatropous ovules begin to develop in each cavity, but usually the lower one reaches maturity.

5. The hanging anatropous ovule has a single integument.

6. The nucellus is very small, and the hypodermal archesporial cell is easily recognizable.

7. The megaspore mother cell produces a perfect linear tetrad, as a result of two successive divisions.

8. The embryo sac develops from the innermost megaspore, the three others aborting.

9. A regular eight-nucleate and subsequent seven-nucleate embryo sac results from three successive divisions of the megaspore, followed by the fusion of the polar nuclei.

10. The antipodals are somewhat ephemeral, breaking down either shortly before or after the fusion of the polar nuclei.

11. The endosperm nucleus is the first to divide after double fertilization, and for a while continues to produce free nuclei, but cell walls soon appear.

12. The fertilized egg is slow to divide, and undergoes no extensive development, so that the ripe seed has a small embryo imbedded in rich endosperm tissue.

13. The suspensor is somewhat long.

14. The morphological features of the Umbelliferae show that they are out of place among the Archichlamydeae, and that they belong among the Sympetalae, in spite of their separate petals, just as *Agapanthus* is a good monocotyledon in spite of its two cotyledons.

15. The epigynous nature of the flower in close affinity with that of the Rubiales warrants the placing of the Umbellales about parallel with the Rubiales, among the Sympetalae, that is, as a side line having a common origin with the Rubiales.

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LITERATURE CITED

1. ADAMSON, MICHAEL, Familles des plantes. Paris. 1763.
2. BARTSCH, E., Beiträge zur Anatomie und Entwicklung der Umbelliferenfrüchte. Diss. Breslau. 1882.
3. BEHUNECK, H., Zur Anatomie von *Oenanthe crocata*. Diss. Kiel. 1879.
4. BERNHARDI, J. J., Über die merkwürdigsten Verschiedenheiten des entwickelten Pflanzenembryo und ihren Werth für die Systematik. Linnaea **7**:561–613. 1832.
5. BESSEY, C. E., The phylogenetic taxonomy of flowering plants. Ann. Mo. Bot. Gard. **2**:109–164. 1915.
6. BESSEY, E. A., The origin of the Anthophyta. Ann. Rep. Mich. Acad. Sci. **17**:142–152. 1915.
7. BUCHENAU, F., Der Bluenthenstand und die Zweigbildung bei *Hydrocotyle vulgaris*. Bot. Zeit. **18**:365. 1860.

8. Cammerloher, H., Studien über die Samenanlagen der Umbelliferen und Araliaceen. Oest. Bot. Zeitsch. **60**:289–300, 356–360. 1910.
9. Césalpin, Andrea, Libri XVI de plantis. Florence. 1583.
10. Clos, D., La feuille et la ramification dans les Ombellifères. Toulouse Acad. Sci. Mem. **6**:195–231. 1874.
11. ———, De quelques particularités de l'inflorescence afférentes au *Bupleurum fruticosum*, etc. Assoc. Franc. C.R. **2**:460–464. 1890.
12. Coulter, J. M., and Chamberlain, Chas. J., Morphology of angiosperms. New York. 1903.
13. Coulter, J. M., and Rose, J. N., Monograph of the North American Umbelliferae. Washington. 1902.
14. Courchet, L., Étude anatomique sur les Ombellifères et sur les principales anomalies de structure que présentent leurs organes végetatifs. Ann. Nat. Bot. VI. **17**:107–129. 1884.
15. Crantz, H. J. N., Classis Umbelliferarum emendata. Lipsiae. 1767.
16. De Candolle, A. P., Organographie végétale. **1**:164. 1827.
17. ———, Mémoire sur la famille des Ombellifères. Paris. 1829.
18. Domin, Karel, Morfologická a fylogenetická studia o čeledi Umbellifer Čast I. Rozpravy České Akad. Čis. Fr. Jos. Třida II. **20**:1–20. 1908.
19. ———, Morfologická a fylogenetická studia o čeledi Umbellifer. Čast II Rozpravy Ceské Akad. Cis. Fr. Jos. Třida II. **3**:43–98. 1909.
20. Drude, Oscar, Über die systematische Anordnung der Umbelliferen. Deutsch. Natf. Verh. **2**:164–165. 1896.
21. Duchartre, P., Note sur un cas de formation de racines adventives intérieures. Bull. Soc. Bot. Fr. **16**:26–29. 1869; Note sur une particularité observée dans *Oenanthe crocata* L. *Ibid.* **16**:363. 1869.
22. Faure, Jos., L'accroissement et la marche des faisceaux dans les petioles de l'*Archangelica*. Bull. Soc. Bot. Lyon. **9**:310–311. 1882.
23. Funk, G., Beiträge zur Kenntniss der mechanischen Gewebesysteme in Stengel und Blatt der Umbelliferen. Bot. Centralb. Beih. **29**:219–295; 1912.
24. Génau de Lamarlière, L., Sur la germination de quelques Ombellifères. Assoc. Franc. C.R. **2**:480–484. 1891; and Rev. Gen. Bot. **5**:159–171. 224–229; 258–264. 1893.
25. ———, Structure comparée des racines renflées de certaines Ombellifères. Paris Acad. Sci. C.R. **112**:1020–1022. 1892.
26. ———, Sur la germination et le développement du tubercule du *Conopodium denudatum* Koch. Ass. Franc. C.R. **2**:445–449. 1892.
27. ———, Mémoire: Recherches morphologiques sur la famille des Ombellifères. Lille. 1893.
28. Gerard, R., Structures de l'axe des *Oenanthe* et considérations sur les formations anomales. Bull. Soc. Bot. **30**:299–304. 1883.
29. Griesebach, Aug. H. R., Nachtrag zu den Beobachtungen über das Wachsthum der Blätter. Wiegmann's Archiv X. **1**:345–347. 1844.

30. HALLIER, H., L'origine et le système phylétique des Angiosperms, exposés à l'aide de leur arbre généalogique. Arch. Neerland. Sci. III. **1**:146. 1912.

31. HANNAH, M., Comparative study of epigyny in certain monocotyledons and dicotyledons. Trans. Amer. Micr. Soc. **35**:207–220. 1916.

32. HOFFMANN, G. F., Genera plantarum Umbelliferarum. Mosquae. 1816.

33. ———, Syllabus plantarum Umbelliferarum. Mosquae. 1814.

34. HOFFMANN, HERMANN, Über der Wurzeln der Doldengewächse. Flora **32**:16–25; 721–728. 1848; **33**:385–389; 401–405; 657–665. 1849; **34**:513–519; 529–535. 1850; **35**:225–233; 241–256. 1851.

35. HOLM, H., *Erigenia bulbosa* Nutt., a morphological and anatomical study. Amer. Jour. Sci. **11**:63–72. 1901.

36. IRMISCH, T., *Carum bulbocastanum* und *Chaerophyllum bulbosum* nach ihrer Keimung. Abhandl. Naturf. Gesellsch. Halle **2**:47. 1854.

37. ———, Über Keimung von *Bunium creticum*. Flora **16**:33–42. 1858.

38. JOCHMANN, E. C. G. G., De Umbelliferarum structura et evolutione nonnulla. Diss. Vratislaviae. 1854.

39. JURICA, H. S., Development of the head and flower of *Dipsacus sylvestris*. BOT. GAZ. **71**:138–145. 1921.

40. KIRSCHLEGER, F., Über das Keimen des *Chaerophyllum bulbosum* L. Flora **28**:401–402. 1845.

41. KLAUSCH, P., Über die Morphologie und Anatomie der Blätter von *Bupleurum*, etc. Diss. Leipzig. 1887.

42. KOCH, G. D. J., Generum tribumque Umbelliferarum nova dispositio. Nova Acta Acad. Carol. Leop. XII. 1825.

43. LANGE, JULIUS, Über Entwickelung der Oelbehälter in den Früchten der Umbelliferen. Diss. Koenigsberg. 1884.

44. LE LANESSAN, J. L., Observations sur le developpement du fruit des Ombellifères. Bull. Soc. Linn. Paris **1**:17–18; 23–24. 1889.

45. LINNÈ, CARL, Genera plantarum. 1737 and 1742.

46. MARTEL, E., Contribuzione all' anatomia del fiore delle Ombellifère. Mem. Reale Accad. Sci. Torino **55**:271–283. 1905; Sur l'anatomie de la fleur des Ombellifères. Jour. Bot. **19**:85–87. 1905.

47. MEYEN, F. J. F., Über die Secretionsorgane der Pflanzen. Königl. Soc. Wiss. Göttingen. p. 21. Berlin. 1837.

48. MEYER, A., Über die Entstehung der Scheidenwände in dem Sekretführenden plasmofreien Intercellularraume der Vittae der Umbelliferen. Bot. Zeit. **47**:341–352; 357–366; 372–379. 1889.

49. MÖBIUS, M., Untersuchungen über die Morphologie und Anatomie der Monokotylen-ähnlichen Eryngien. Jahrb. Wiss. Bot. **14**:379–425. 1884; **17**:591–621. 1886.

50. MOHL, H. VON, Eine kurze Bemerkung über das Carpophorum des Umbelliferebfrucht. Bot. Zeit. **21**:264–266. 1863.

51. Morison, Robert, Plantarum Umbelliferarum distributio nova. Oxonii. 1672.

52. Moynier de Villepoix, R., Recherches sur les canaux sécréteurs du fruit des Ombellifères. Ann. Sci. Nat. Bot. **5**:348–365. 1878.

53. Müller, Carl, Über phloëmständige Secretkanäle der Umbelliferen und Araliaceen. Ber. Deutsch. Bot. Gessells. **6**:20–32. 1888.

54. Nestel, A., Beitrage zur Stengel und Blatt Anatomie der Umbelliferen. Diss. Zurich. 1905.

55. Noenen, F. van, Die Anatomie der Umbelliferenachse in ihrer Beziehung zum System. Diss. Erlangen. 1895.

56. Payer, J. B., Organogenie des familles: Ombellifères. Ann. Sci. Nat. Bot. III. **20**:111. 1853.

57. Petersen, E., Undersogelser over Bladnervationes hos Arter af Slaegten *Bupleurum* Tourn. Bot. Tidsskrift **26**:343–376. 1905.

58. Reichardt, H. W., Über das Centrale Gefässbündel-system einiger Umbelliferen. Sitzber. K. K. Akad. Wissensch. **21**:133–154. 1856.

59. Rennert, R. J., The phyllodes of *Oxypolis filiformis*, a swamp xerophyte. Bull. Torr. Bot. Club **30**:403–411. 1903.

60. Rossmann, G. W. J., Beitrage zur Kenntniss der Spreitenformen in des Familie der Umbelliferen. Abhdl. Naturf. Gesells. Halle. 1864.

61. Schindler, Ant. K., Die Abtrennung der Hippuridaceen von den Halorrhagaceen. Engler's Bot. Jahrb. Beibl. 77. **34**:1–77. 1904.

62. Sieler, T., Beiträge zur Entwicklungsgeschichte des Blütenstandes und der Blüte bei den Umbelliferen. Bot. Zeit. **28**:360–369; 376–382. 1870.

63. Sprengel, Curtius, Plantarum Umbelliferarum denuo disponendarum Prodromus. N. Schr. Nat. Gesells. II. Halae. 1819; Umbelliferarum genera quaedam melius definita. Berlin Ges. Nat. Freunde Mag. **6**:255–261. 1814.

64. Tanfani, E., Morfologia e istologia del frutto e del seme. Nuovo Giornale Bot. Ital. **23**:451–469. 1891.

65. Tittmann, J. A., Die keimung der Pflanzen. Dresden. 1821. (pp. 103 seq. and pp. 111 seq., *pls*. 14–16).

66. Ternetz, C., Morphologie und Anatomie der *Azorella Selago*. Bot. Zeit. **60**:1–20. 1902.

67. Trecul, Aug., Mémoire sur la formation des feuilles. Ann. Sci. Bot. III. **20**:235 seq. 1853.

68. ———, Des vaisseaux propres dans les Ombellifères. Comptes Rend. **63**:154 and 201. 1866; Des vaisseaux propres dans les Araliacees. Comptes Rend. **64**:886 and 990. 1867.

69. Treviranus, L. C., Symbolarum phytologicarum quibus res herbaria illustratur. **1**:1–92. 1831.

70. ———, Über Fruchtbau und einige Gattungen der Doldengewächse. Bot. Zeit. **19**:9–14. 1861.

71. Treviranus, L. C., and Reinhold, G., Vermischte Schriften anatomischen und physiologischen inhalts. **4**:187. Bremen. 1821.

72. Van Tieghem, Ph., Recherches sur la symétrie de structure des plantes vasculaires. Ann. Sci. Nat. Bot. V. **13**:5–314. 1870.

73. ———, Sur les canaux oleo-resineux des Ombellifères et des Araliacées. Bull. Soc. Bot. Fr. **19**:113–129. 1872.

74. ———, Sur les canaux sécréteurs du pericycle dans la tige et la feuille des Ombellifères et des Araliacées. Bull. Soc. Bot. Fr. **31**:29–32. 1884.

75. ———, Mémoire sur les canaux oleo resineux. Ann. Sci. Nat. Bot. **16**:96–201. 1872.

76. ———, Sur la germination du *Bupleurum aureum*. Bull. Soc. Bot. Fr. **38**:402–404. 1891.

77. ———, Structure de quelques ovules et parti qu'on en peus tirer pour améliorer la classification. Jour. Bot. **12**:197–220. 1898.

78. Viguier, Rene, Recherches anatomiques sur la classification des Araliacées. Ann. Sci. Nat. Bot. IX. **4**:1–207. 1906.

79. Vuillemin, P., Sur l'anomalie du système sécréteur des *Hydrocotyle*. Bull. Soc. Bot. Fr. **32**:1–14. 1885.

80. Wangerin, W., Die Umgrenzung und Gliederung der Familie der Cornaceae. Engler's Bot. Jahrb. Beibl. 85. **38**:1–88. 1906.

81. Warming, E., Observations sur la valeur systématique de l'ovule. Mindeskrift Japetus Steenstrup. Copenhagen. 1913.

82. Wernham, H. F., Floral evolution with particular reference to the sympetalous dicotyledons. New Phytol. **10**:109–120; 145–159; 217–261; 293–305. 1911; **11**:145–166; 217–235; 290–305; 373–397. 1912.

83. Wettstein, R., Handbuch der Systematischen Botanik. Leipzig and Wein. 1911.

84. Wretschko, M., Zur Entwickelungsgeschichte des Umbelliferenblattes. Bot. Zeit. **22**:305–309; 313–315. 1864.

EXPLANATION OF PLATES XIII, XIV

Abbreviations: *fh*, floral head; *ib*, involucral bract; *fb*, floral bract; *fp*, floral papilla; *s*, sepal; *p*, petal; *st*, stamen; *c*, carpel; *ct*, calyx tooth; *o*, ovule; *es*, embryo sac; *mm*, megaspore mother cell; *m*, megaspore; *rn*, remains of nucellus; *na*, nutritive apparatus; *pn*, polar nucleus; *a*, antipodals; *ra*, remains of antipodals; *fn*, fusion nucleus; *e*, egg; *syn*, synergids.

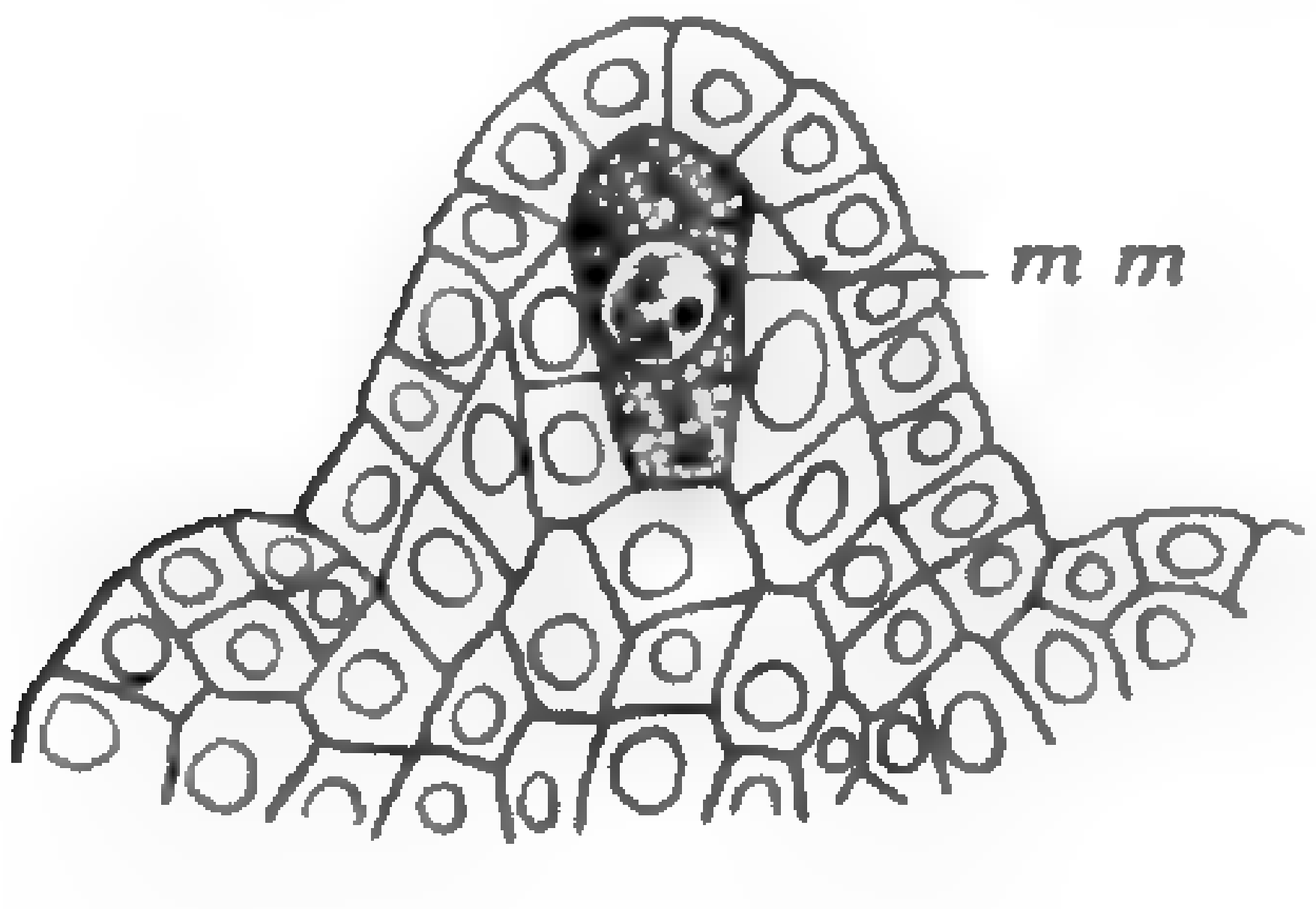
Fig. 1.—A central head of *Eryngium yuccifolium*, showing origin of lateral heads composing umbel.

Figs. 2–7.—Stages in floral development of *E. yuccifolium*.

Fig. 8.—Single mature flower of *E. yuccifolium* in cross-section, showing arrangement of floral cycles.

Figs. 9, 10.—Stages in floral development of *Zizia aurea*, especially showing calyx teeth.

JURICA on UMBELLIFERAE



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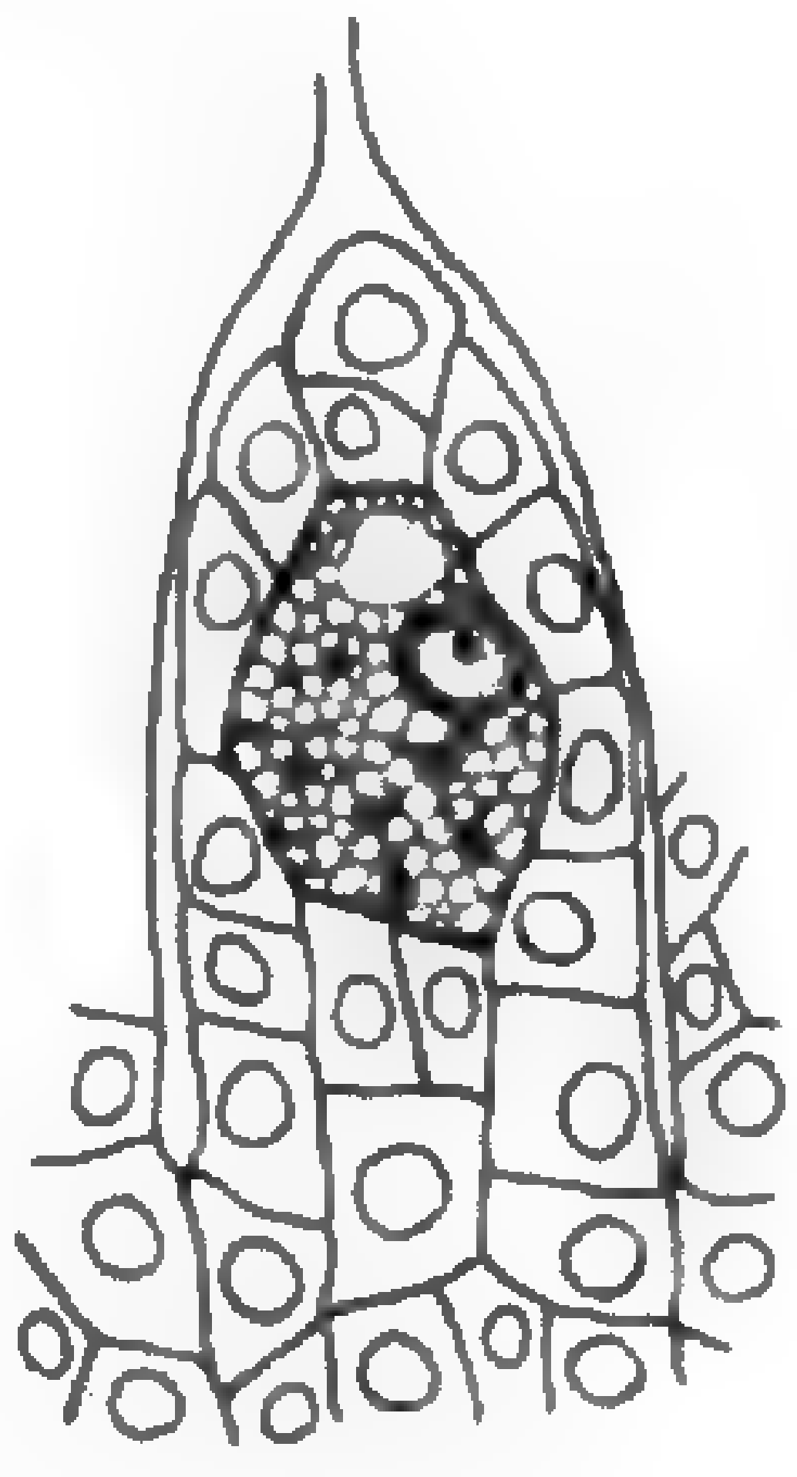

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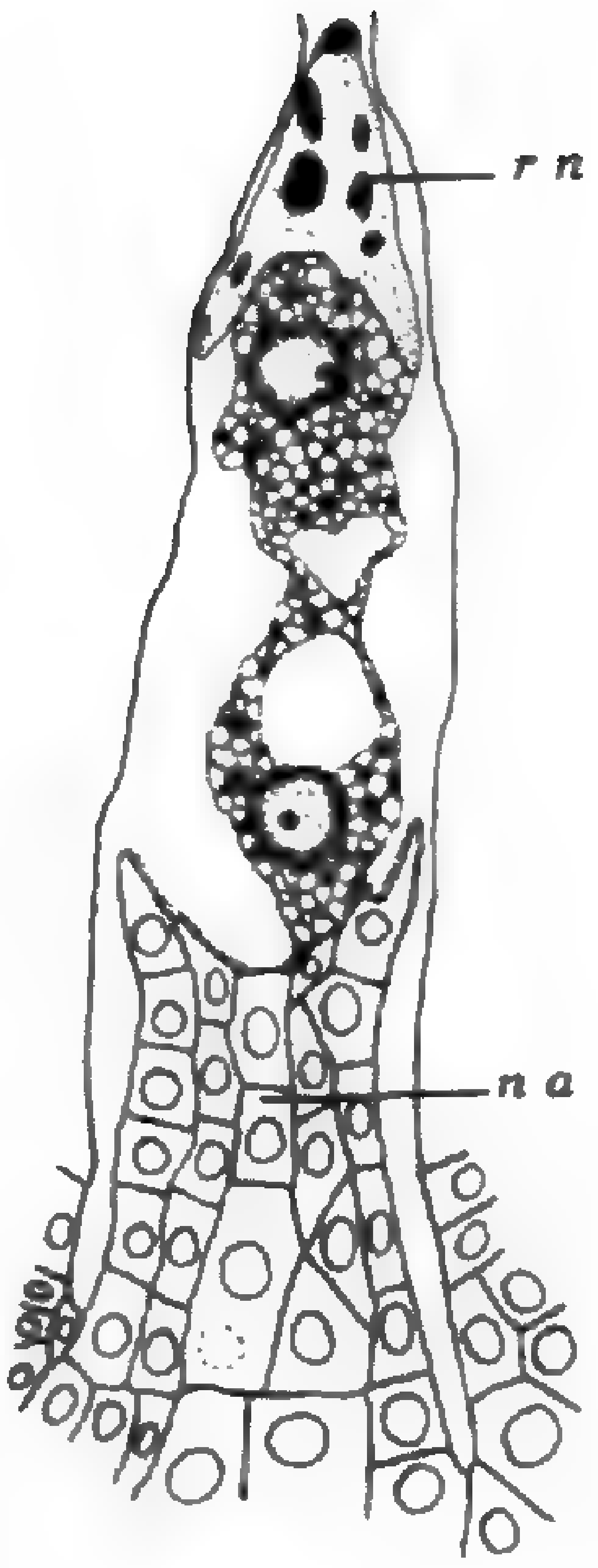

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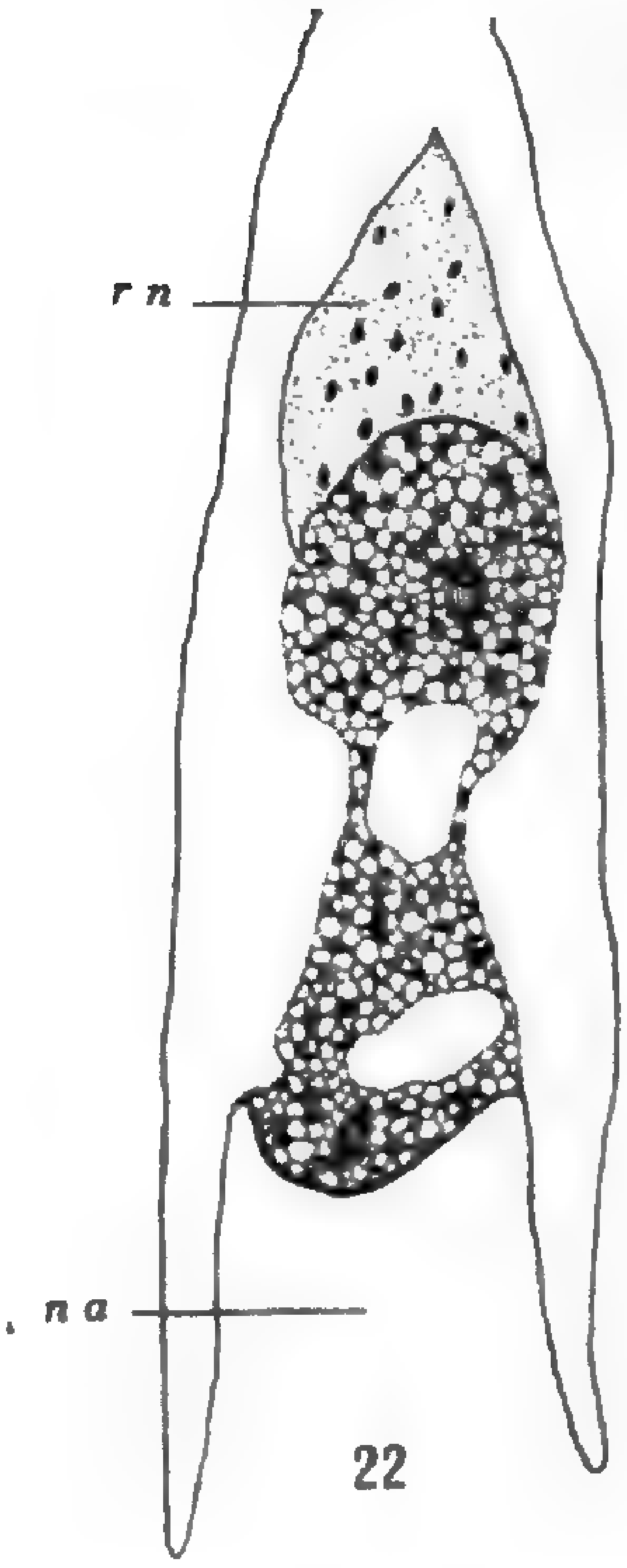

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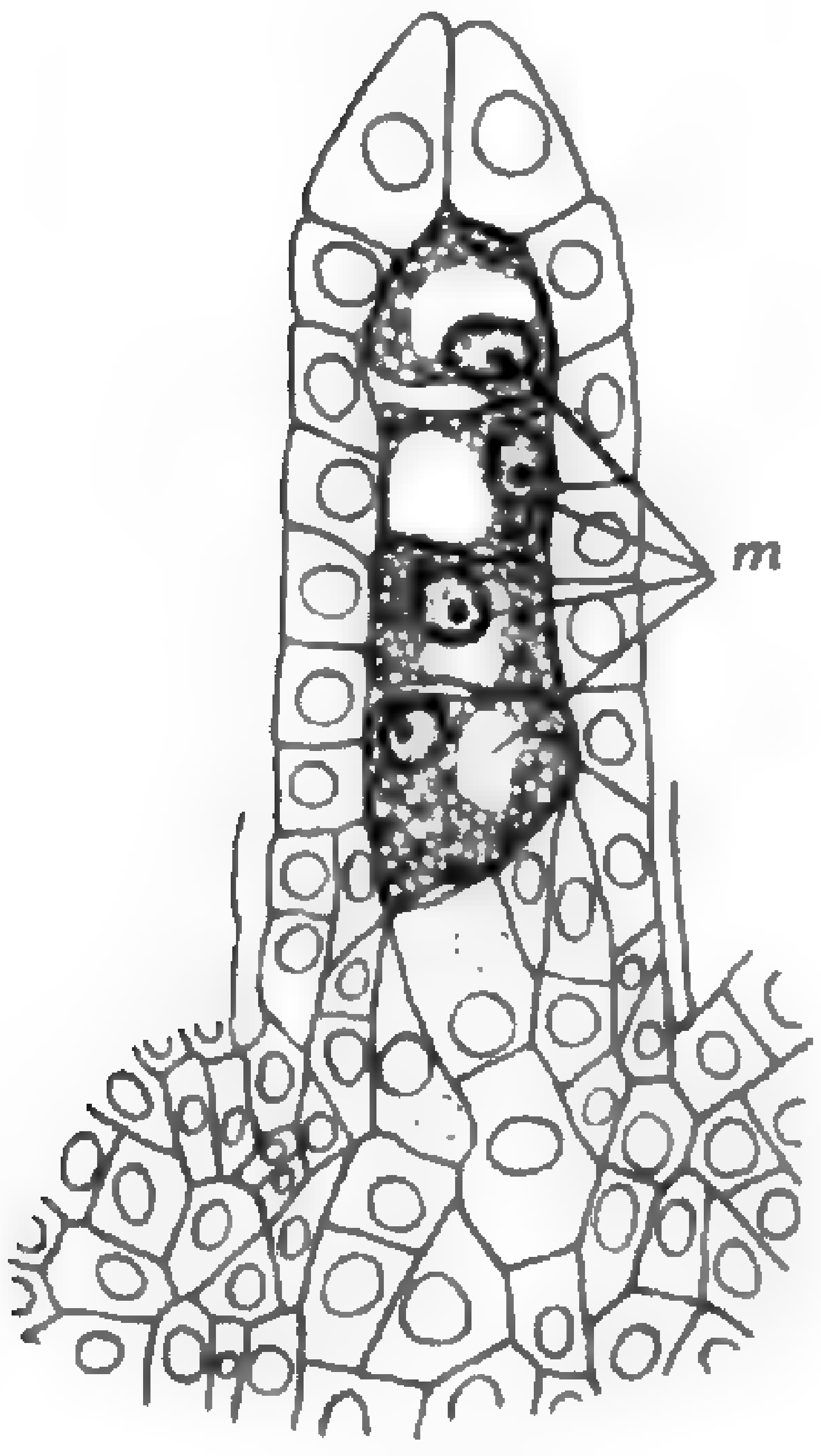

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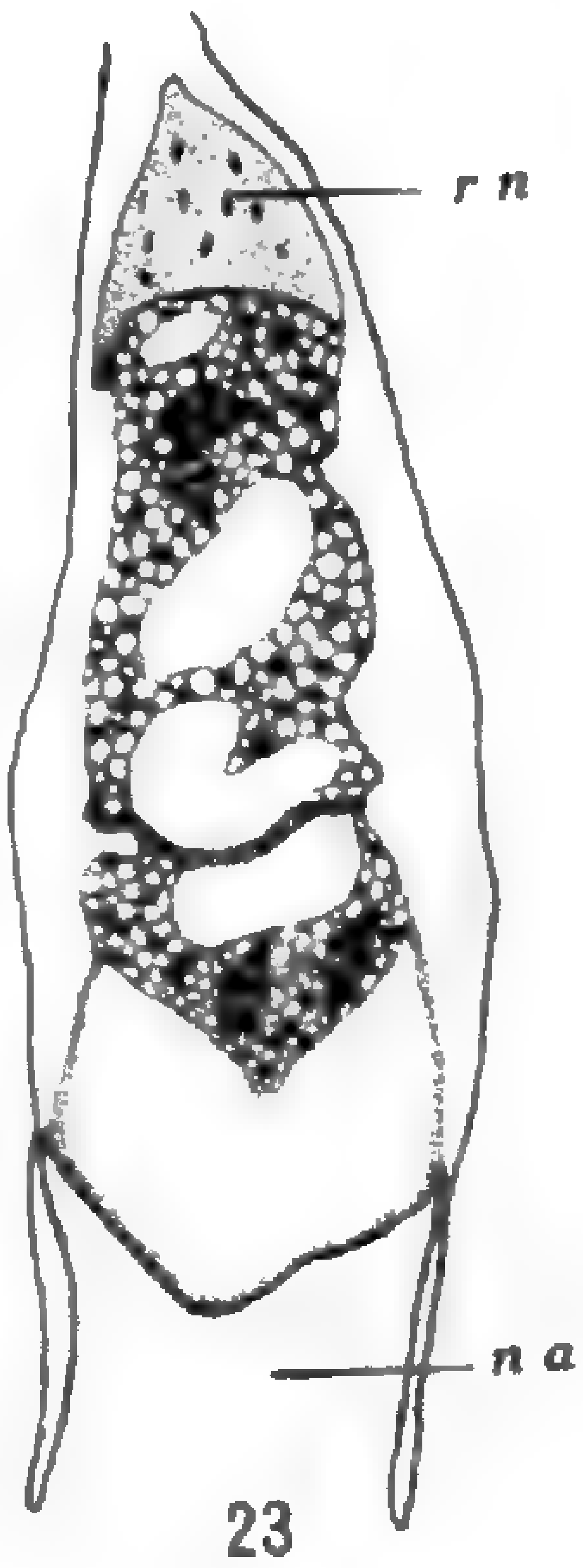

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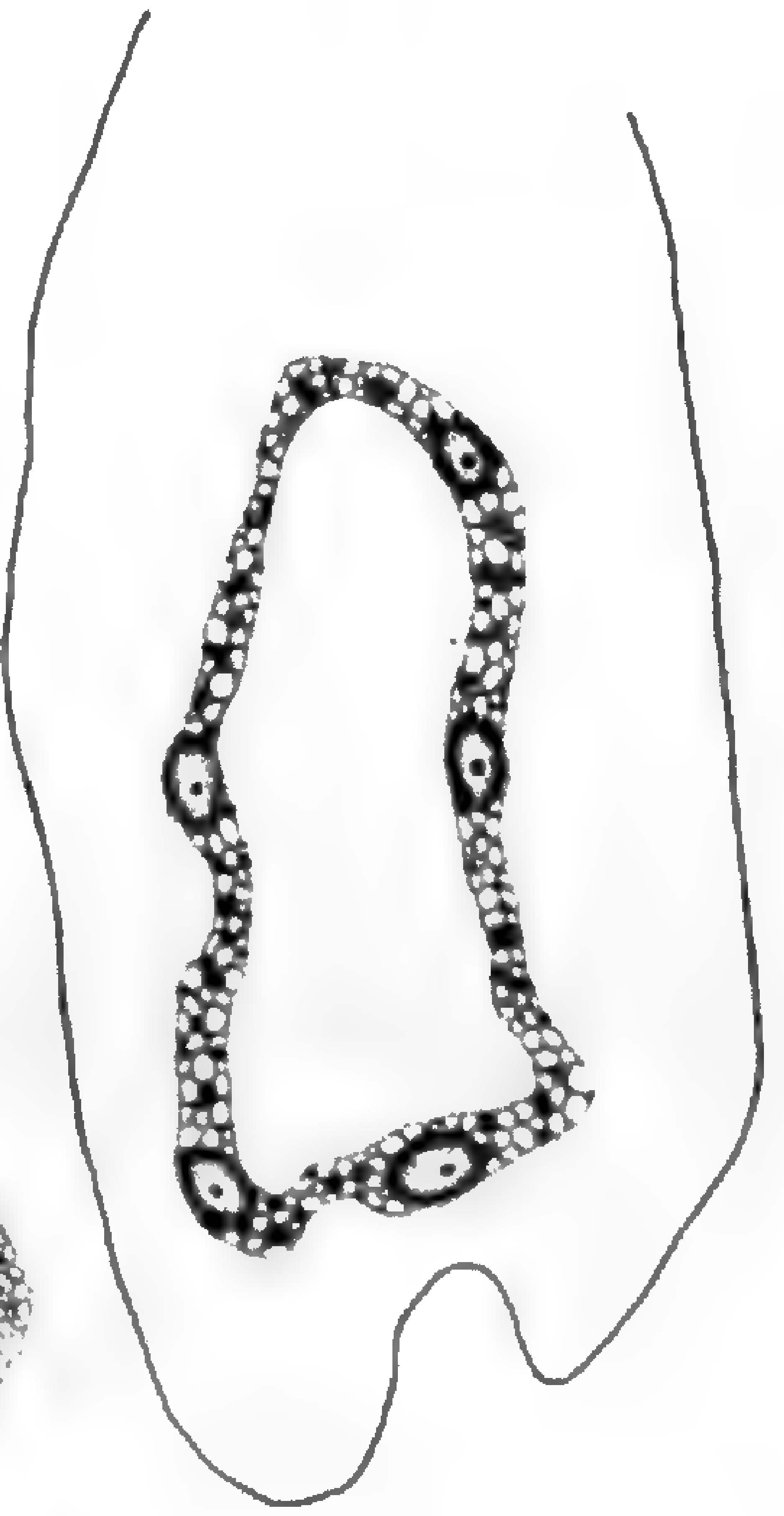

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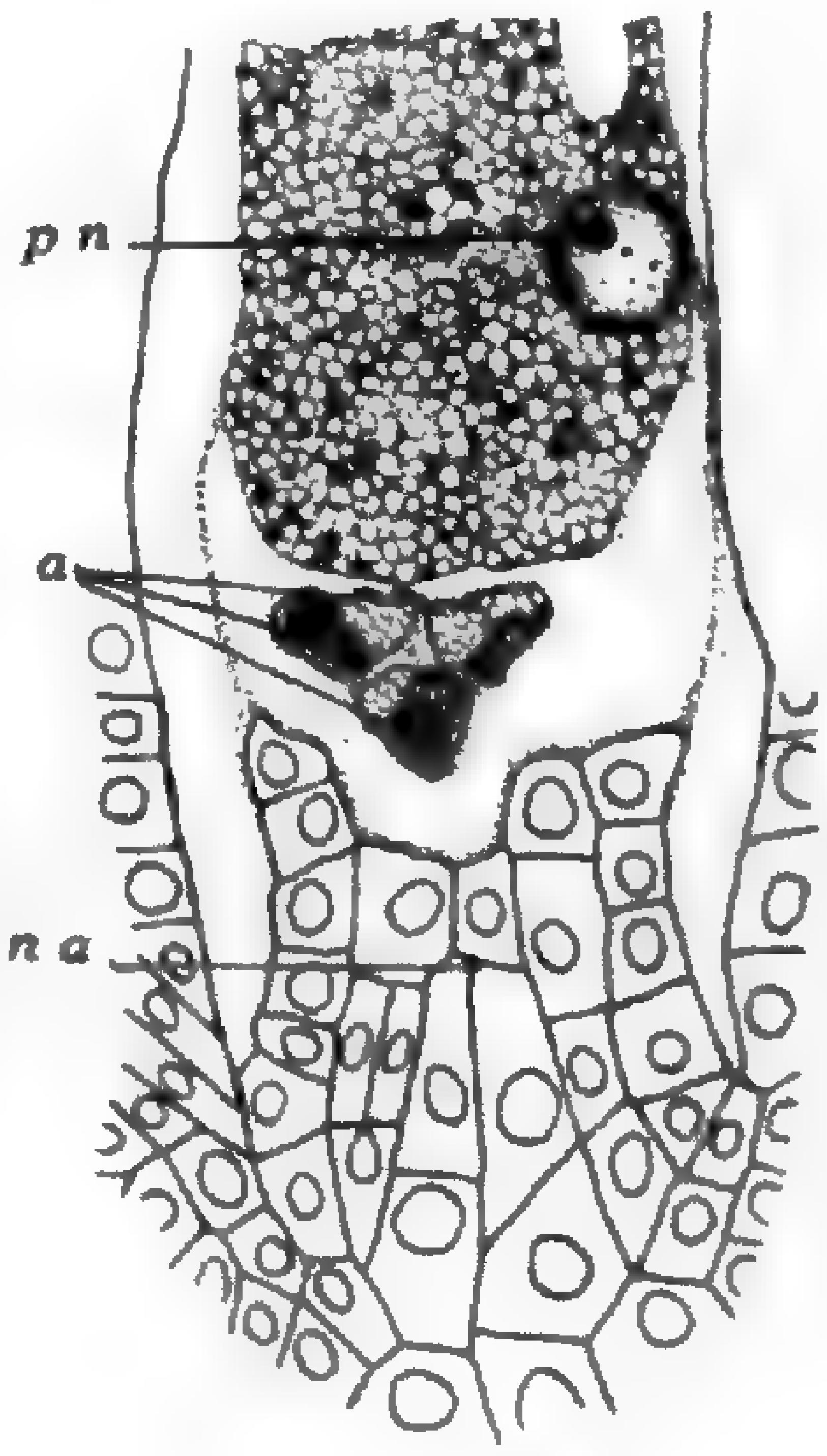

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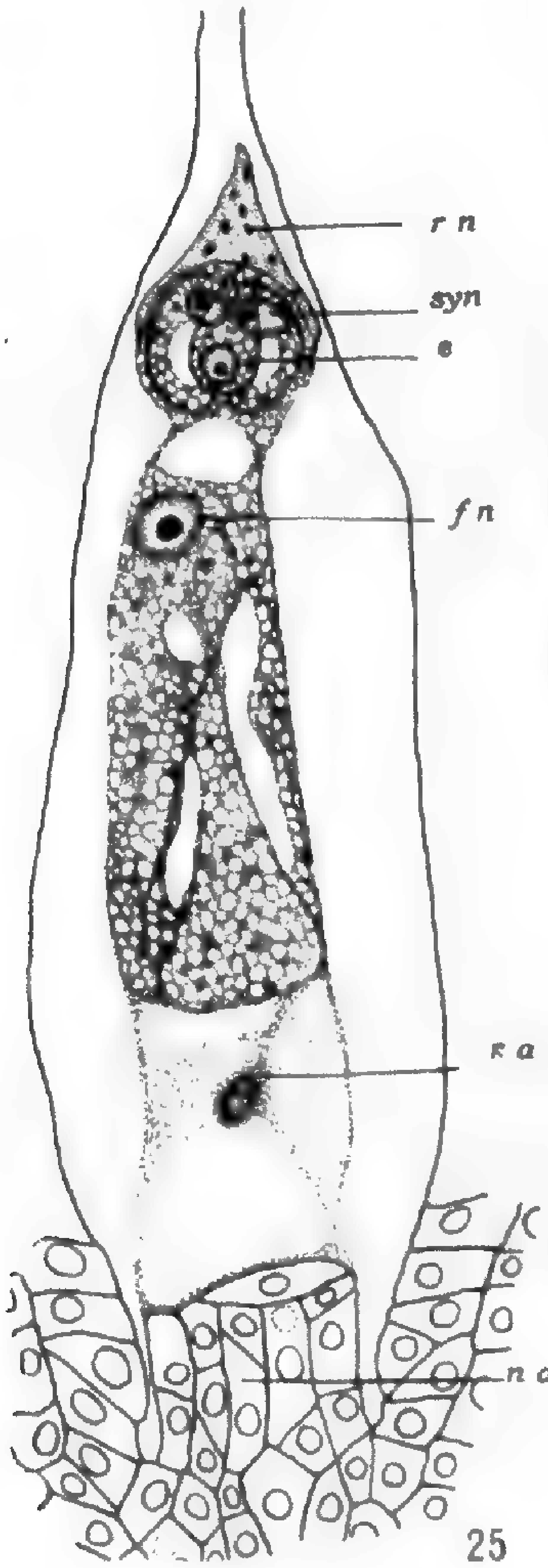

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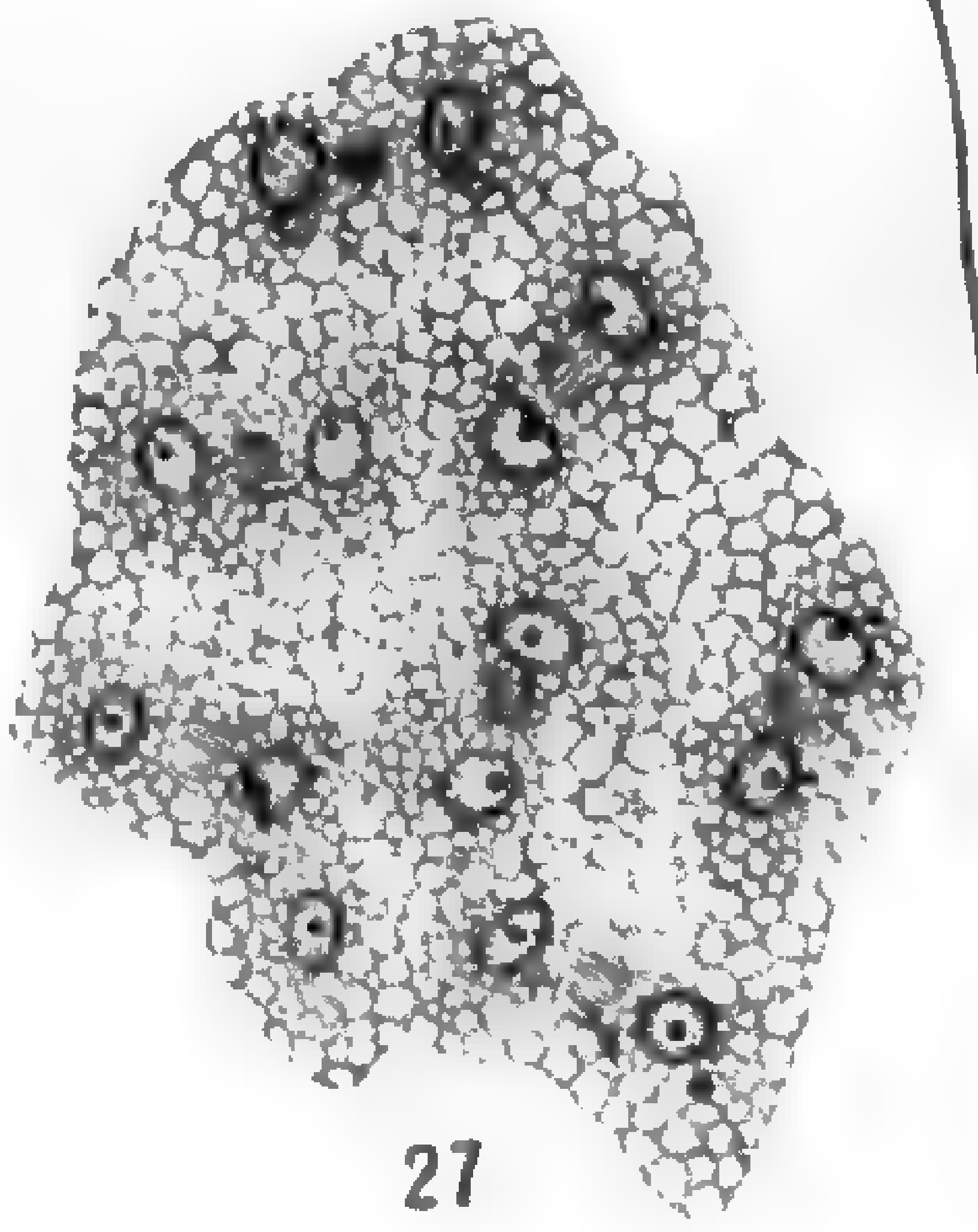

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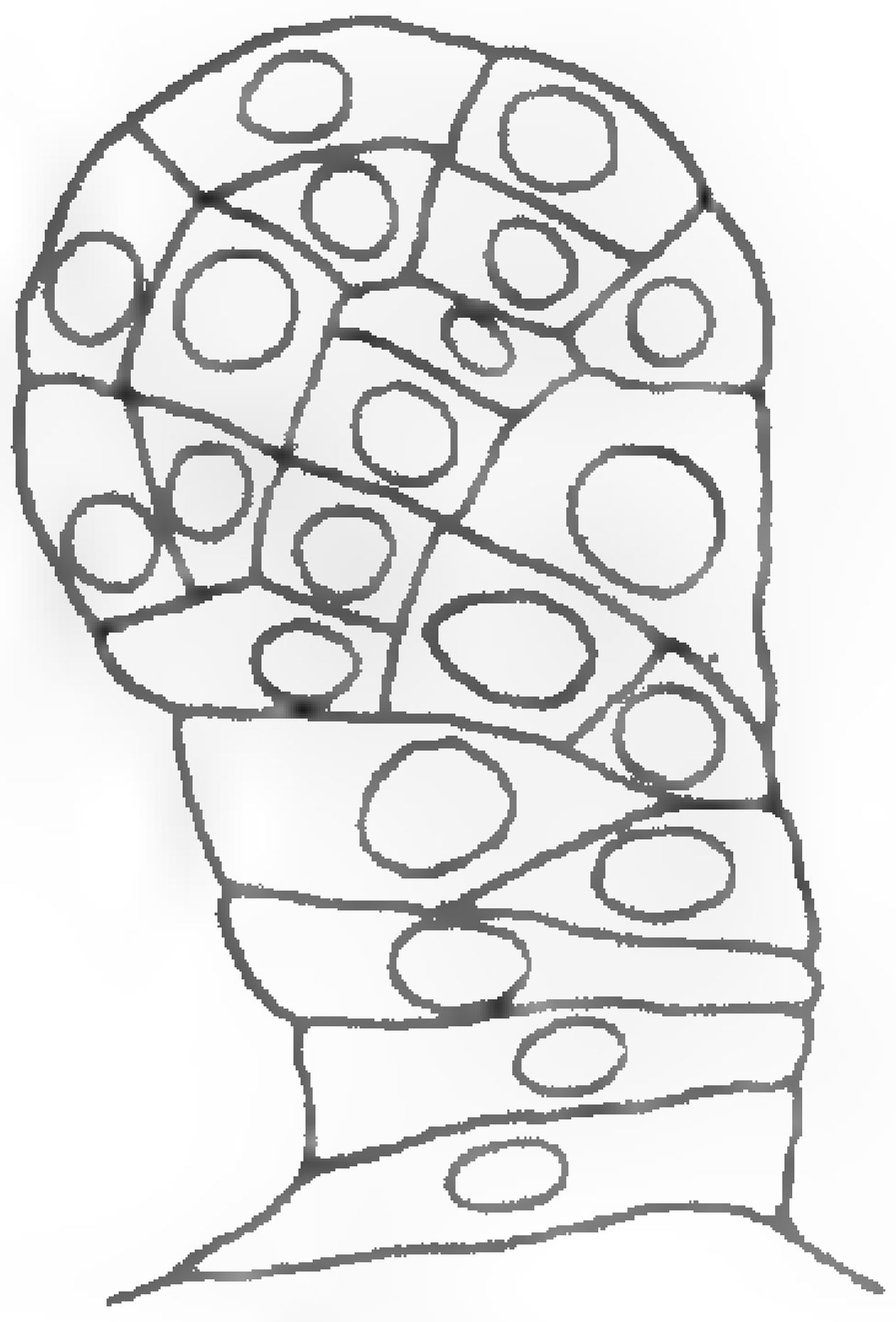

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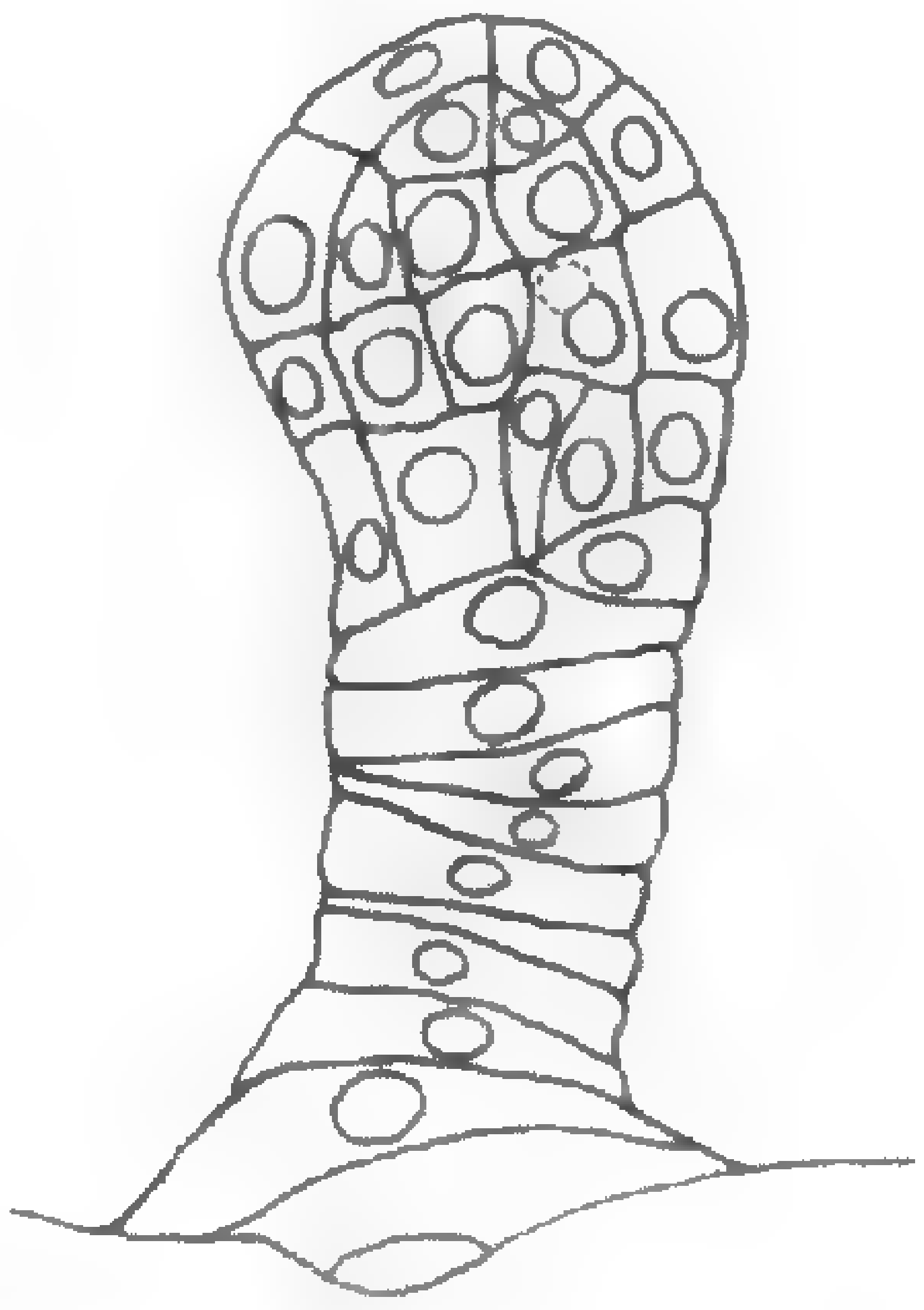

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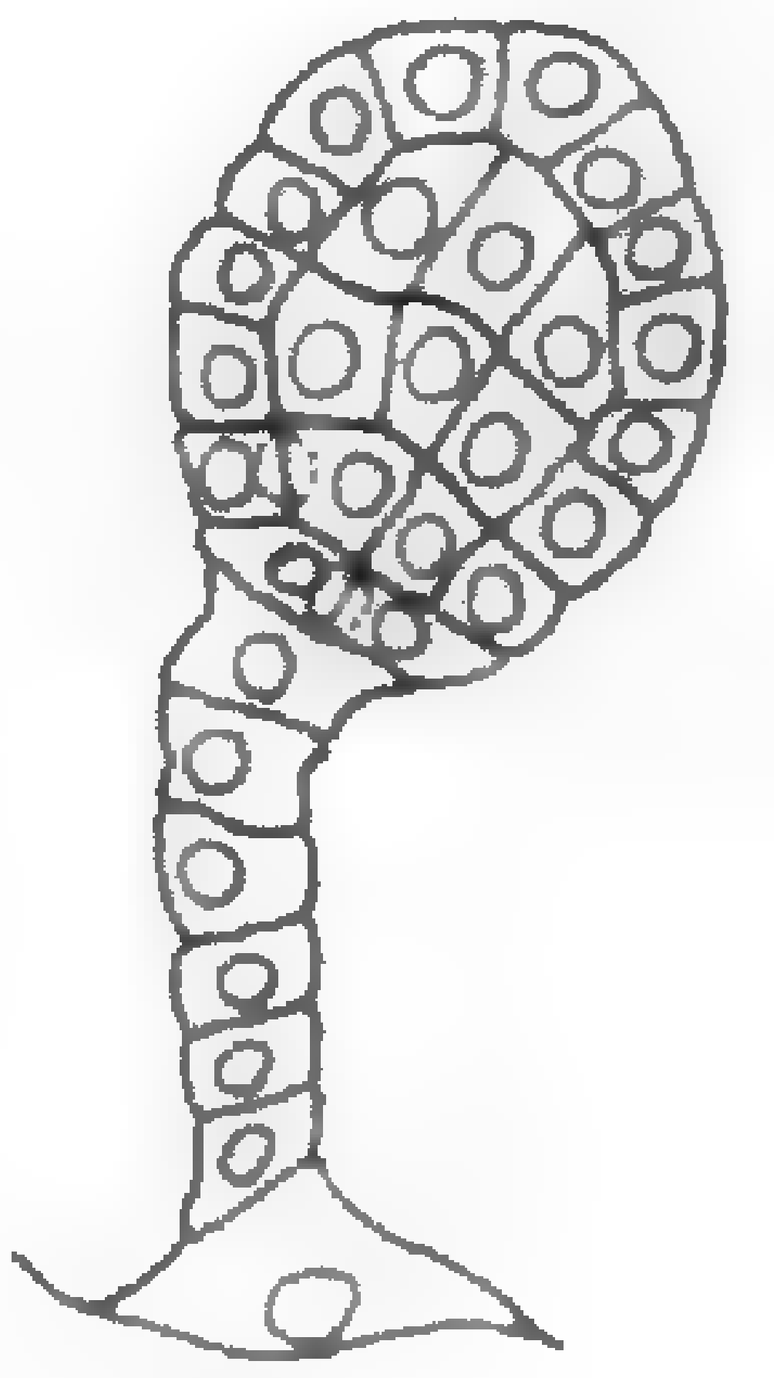

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JURICA on UMBELLIFERAE

FIG. 11.—Longitudinal section of ovary of *Sium cicutaefolium*, showing the two ovules beginning development in one of ovary cavities.

FIG. 12.—Section of *E. yuccifolium* ovary, showing the two ovules in single ovary cavity; only lower ovule generally develops.

FIG. 13.—Longitudinal section of ovary of *E. yuccifolium*, showing an unusual condition, the developing of two ovules in single ovary cavity.

FIG. 14.—Anatropous ovule of *E. yuccifolium.*

FIG. 15.—Nucellus of *Sium cicutaefolium*, showing two cells, result of first division of megaspore mother cell.

FIG. 16.—Mature embryo sac of *S. cicutaefolium*, showing breaking down of nucellus, egg, one of synergids, fusion nucleus, three antipodals, and nutritive apparatus.

FIGS. 17–30.—*Eryngium yuccifolium.*

FIG. 17.—Ovule showing single integument and nucellus.

FIG. 18.—Nucellus showing megaspore mother cell in synapsis.

FIG. 19.—Nucellus showing four megaspores.

FIG. 20.—Single megaspore from which embryo sac will develop; nucellus beginning to break down.

FIG. 21.—Two-nucleate embryo sac, showing nutritive apparatus.

FIGS. 22, 23.—Two and four-nucleate embryo sac, showing nuclear divisions.

FIG. 24.—Lower end of embryo sac, showing one of polar nuclei, three antipodals, and nutritive apparatus.

FIG. 25.—Mature embryo sac, showing remains of broken down nucellus, egg, synergids, fusion nucleus, remains of antipodals, and nutritive apparatus.

FIG. 26.—Embryo sac in cross-section, showing endosperm formation by free nuclei.

FIG. 27.—Free nuclei of endosperm of embryo sac in longitudinal section.

FIGS. 28–30.—Embryo and suspensor.

DETERMINATION OF MOISTURE CONTENT OF EXPRESSED PLANT TISSUE FLUIDS[1]

ROSS AIKEN GORTNER AND WALTER F. HOFFMAN

The fact that the physico-chemical properties of plant tissue fluids reflect in many instances the ecological environment of the plant, and that the ability of a plant to exist under widely different environmental conditions appears to depend largely upon its ability to adjust the physico-chemical properties of its tissue fluids to the new environment has only recently been recognized. HARRIS (3) has pointed out that any thorough ecological study should include physico-chemical studies of the plant saps. GORTNER and HARRIS (1) have indicated some of the precautions which must be taken in order to secure an accurate measure of the osmotic pressure of expressed plant saps by the cryoscopic method, and in subsequent papers HARRIS and his co-workers (4, 5, 8–14) have investigated the physico-chemical properties of plant saps in a variety of habitats. The physico-chemical determinations which have been used have necessarily been limited to those which are adapted to field laboratory facilities, and which do not require excessive amounts of either time, apparatus, or plant materials. The determinations have therefore been confined exclusively to the measurement of osmotic pressure by the cryoscopic method, the electrical conductivity by the conventional wheatstone bridge, and more recently hydrogen ion concentration (unpublished data).

Throughout all this work it has been recognized that a knowledge of the moisture content of the expressed plant saps would be most desirable. For example, a knowledge of the total solids combined with the depression of the freezing point would permit the calculation of the "average molecular weight" of the dissolved solutes. An increase in "average molecular weight" in a different environment might logically be interpreted as indicating a response

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[1] Contribution from the Division of Agricultural Biochemistry, University of Minnesota; published with the approval of the Director as Paper no. 322, Journal Series, Minnesota Agricultural Experiment Station.

to the changed environment by the elaboration of more colloidal materials. In some of the earlier papers (**2, 6, 7**) it was possible to secure these data by the rather laborious procedure of drying weighed portions of the saps in a water oven at 100° and weighing the residue. This method, of course, is wholly unsuited to field studies where hundreds of samples are involved, and is likewise inaccurate, inasmuch as caramelization of the sugars always takes place when plant saps are dried by means of heat. Marked caramelization can only be prevented by drying at room temperature *in vacuo* over sulphuric acid, or by drying in a vacuum oven at not to exceed 50° C. When such methods are employed constant weight is not reached until several days have elapsed.

Another objection to any drying process for field laboratory work lies in the fact that there may be suspended cell débris in expressed plant sap. With abundant laboratory facilities at hand it is comparatively easy to remove such materials by means of a high speed centrifuge or rapid filtration, but in a field laboratory not equipped with a powerful centrifuge, the removal of such débris may be so incomplete as to seriously affect values of dry matter determinations obtained by a drying process.

It recently occurred to one of the writers that it might be possible to determine the moisture content by making use of the refractive index of the plant sap. This method has been employed by sugar manufacturers for many years, and refractometers may be purchased which have a special "sugar scale" from which the percentage of a sugar in a syrup may be read directly.

Tables of refractive indices were consulted and they confirmed this theory, for the refractive indices of solutions of inorganic salts and proteins *in the concentrations normally present in plant saps* appeared to be sufficiently near the values for solutions of carbohydrates so that no excessive errors should result. Accordingly a high grade Abbé refractometer was secured, provided with a special sugar scale, and carefully standardized by the Bureau of Standards, and determinations were made on a series of plant saps with the results shown in table I. This table does not represent selected determinations, but instead every determination which was completed is included, with the exception of two or three where accidents

TABLE I

Leaves of	Refractive index 20° C.	Δ	Moisture by refractometer (percentage)	Moisture over H_2SO_4 *in vacuo*, room temperature (percentage)	Moisture dried 12 hours more at 100° *in vacuo* (percentage)	Moisture dried 6 hours more at 100° *in vacuo** (percentage)	Average molecular weight of solutes
1. Datura Stramonium	1.3433	0.987	92.75	...	...	94.18	147
2. Sambucus canadensis	1.3508	1.212	88.00	...	...	90.60	210
3. Bryophyllum calycinum	1.3415	0.508	94.10	...	...	94.98	230
4. Panicum sp	1.3420	0.984	93.90	...	...	94.64	123
5. Zebrina pendula	1.3372	0.370	97.00	98.33	98.42	...	155
6. Cyperus alternifolia	1.3411	0.956	94.50	95.19	95.55	...	113
7. Ricinus communis	1.3472	0.747	90.30	90.81	92.39	...	267
8. Salix sp	1.3571	1.576	84.00	84.34	87.01	...	227
9. Triticum vulgare var. Marquis	1.3410	0.897	94.50	94.80	95.48	...	121
10. Triticum vulgare var. Marquis	1.3404	0.857	94.85	94.79	95.51	...	118
11. Triticum vulgare var. Marquis	1.3409	1.396	94.60	95.09	95.80	...	77
12. Triticum vulgare var. Marquis	1.3401	0.889	95.00	94.63	95.60	...	110
13. Triticum vulgare var. Turkey	1.3533	1.276	86.50	86.85	...	88.94	230
14. Triticum vulgare var. Buffum	1.3561	1.445	84.60	84.67	...	87.33	236
15. Bryophyllum calycinum	1.3414	0.474	94.10	94.40	...	95.01	246
16. Cereus sp	1.3400	0.505	95.10	95.27	...	...	190
17. Triticum vulgare var. Buffum	1.3600	1.719	82.20	82.85	...	85.59	234
18. Triticum vulgare var. Minhardi	1.3455	1.147	91.50	91.80	...	92.62	150
19. Triticum vulgare var. Super	1.3434	1.000	92.90	93.15	...	93.88	142
20. Triticum vulgare var. Super	1.3474	1.085	90.30	91.53	...	...	184
21. Triticum vulgare var. Super	1.3346	...	98.85	98.86	...	...	...

* Nos. 1, 2, 3, 4 were dried directly in vacuum oven.

happened to the duplicate set being dried either over sulphuric acid or in the vacuum oven.

The tissue fluids were obtained by means of a specially constructed press bowl, and a hydraulic press, after the tissue had been rendered permeable by a preliminary freezing of the tissue for at least eight hours, following the procedure recommended by GORTNER and HARRIS (1) and used in all of the previous work. All saps were centrifuged perfectly clear from suspended débris. We have included in the table values for Δ, the depression of the freezing point (corrected for under cooling), and the "average molecular weight"[1] of the solutes.

Certain of the samples (nos. 9–14 and 17–21) were collected for another purpose by Mr. ROBERT NEWTON, and the significance of the various values will be discussed by him in a later paper. It is sufficient for our purpose to point out the difference between nos. 11 and 14. We have here two wheat saps differing approximately 3 per cent in the freezing point depression (and consequently in osmotic pressure), and at the same time differing by nearly 300 per cent in total solids. This difference can only be due to a difference in colloidal content, a fact that has been proved by NEWTON, using several other methods. Had we been concerned only with determinations of osmotic pressure, electrical conductivity, and hydrogen ion concentration, we might have concluded that these saps possessed practically identical physico-chemical properties, whereas such a conclusion is far from the truth.

Sample no. 21 is a wheat sap dialyzed completely free of sugar and electrolytes, and represents the non-dialyzable colloidal material. It will be noted that the refractometric method measures this colloidal material quantitatively. NEWTON recently had occasion to prepare, in this laboratory, gum acacia sols containing 1, 2, 3, 5, 7, and 10 per cent of highly purified gum acacia, and refractometric readings for total solids on the resulting solutions gave values corresponding with those of the weighed gum acacia which had been added to make the sols.

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[1] Calculated by aid of published tables. Cf. HARRIS, J. A., and GORTNER, R. A., Tables of the relative depression of the freezing point, 1860/Δ, to facilitate the calculation of molecular weights. Biochem. Bull. **3**:259–263. 1914.

One great advantage of the proposed method is that only two or three drops of sap are required for the determination. A film of sap is placed upon the prism, the prism is closed, and as soon as the thermometer inclosed within the prism has reached 20° C. the reading of the moisture content can be made. The entire procedure need not take more than two minutes.

The column "Moisture by refractometer" is read direct from the scale of the instrument, the next column was obtained by weighing 10–20 gm. of the sap into a glass weighing bottle, and drying to constant weight at room temperature in a vacuum desiccator over sulphuric acid. The dried residue varied in color from a clear green to light brown, and still retained the characteristic odor of plant sap.

The next two columns were obtained by heating the residues from the sulphuric acid vacuum treatment in a Freas vacuum oven at 100° under a vacuum of 28 inches for the stated period of time, and again weighing. It will be noted that in each instance there is a higher moisture content indicated by the further drying in the vacuum oven. We believe this to be almost entirely due to the decomposition of carbohydrates, since there was always marked browning or blackening of the residue, and a pronounced burned sugar odor. In no instance was the residue again wholly water soluble, and in every instance the water extracts of the residue were dark opaque brown, with a strong caramel odor. Further proof that this loss of weight is due to decomposition of carbohydrates is afforded by the fact that the loss in weight continues for a long time, and constant weight is in many instances not reached even after seventy-two hours' drying in the vacuum oven at 100° C.

It will likewise be noted that in most instances the refractometer indicates slightly more total solids (that is, less water) than does the drying over sulphuric acid *in vacuo*. In every instance the sulphuric acid in the vacuum desiccator darkened as the drying progressed, indicating that volatile organic compounds were dissolving in the sulphuric acid. It is self evident that esters, alcohols, ethers, and volatile oils are present in all plant saps, and none of these would be estimated by any drying method. We believe, therefore, that at least a part of the excess total solids indicated by the new method are due to such volatile compounds, and that the refractometer reading more nearly expresses the true value of the moisture content than can be obtained by any known method.

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LITERATURE CITED

1. Gortner, R. A., and Harris, J. A., Notes on the technique of the determination of the depression of the freezing point of vegetable saps. Plant World **17**:49–53. 1914.
2. ———, On a possible relationship between the structural peculiarities of normal and teratological fruits of *Passiflora gracilis* and some physico-chemical properties of their expressed juices. Bull. Torr. Bot. Club **40**: 27–34. 1913.
3. Harris, J. A., Physical chemistry in the service of phytogeography. Science N.S. **46**:25–30. 1917.
4. ———, On the osmotic concentration of the tissue fluids of phanerogamic epiphytes. Amer. Jour. Bot. **5**:490–506. 1918.
5. ———, On the osmotic concentration of the tissue fluids of desert Loranthaceae. Mem. Torr. Bot. Club **17**:307–315. 1918.
6. Harris, J. A., and Gortner, R. A., Physico-chemical properties of vegetable saps. II. A comparison of the physico-chemical constants of the juice of apples and pears of varying size and fertility. Biochem. Bull. **3**:196–201. 1914.
7. Harris, J. A., Gortner, R. A., and Lawrence, J. V., Physico-chemical properties of vegetable saps. III. A comparison of the physico-chemical constants of the juices expressed from the wall with those from the included capillary whorl in proliferous fruits of *Passiflora gracilis*. Biochem. Bull. **4**:52–78. 1915.
8. ———, The relationship between osmotic concentration of leaf sap and the height of leaf insertion in trees. Bull. Torr. Bot. Club **44**:267–286. 1917.
9. ———, The osmotic concentration and electrical conductivity of the tissue fluids of ligneous and herbaceous plants. Jour. Phys. Chem. **25**:122–146. 1921.
10. Harris, J. A., and Lawrence, J. V., Cryoscopic determinations on tissue fluids of plants of Jamaican coastal deserts. Bot. Gaz. **64**:285–305. 1917.
11. ———, The osmotic concentration of the sap of the leaves of mangrove trees. Biol. Bull. **32**:202–211. 1917.
12. ———, The osmotic concentration of tissue fluids of Jamaican montane rain forest vegetation. Amer. Jour. Bot. **4**:268–298. 1917.
13. ———, On the osmotic pressure of the tissue fluids of Jamaican Loranthaceae parasitic on various hosts. Amer. Jour. Bot. **3**:438–455. 1916.
14. Harris, J. A., Lawrence, J. V., and Gortner, R. A., The cryoscopic constants of expressed vegetable saps as related to local environmental conditions in the Arizona deserts. Physiol. Res. **2**:1–49. 1916.

MOISTURE CONTENT OF PEACH BUDS IN RELATION TO TEMPERATURE EVALUATIONS

EARL S. JOHNSTON

(WITH TWO FIGURES)

The moisture content of peach fruit buds shows a marked increase in late winter and early spring. Experiments performed at the Maryland Agricultural Experiment Station in 1919 show that moisture values based on the dry weight of buds increased from an average ratio of 0.69 on January 7 to 3.65 on March 28. The following year the average ratio increased from 0.73 on January 29 to 3.51 on April 1. Such marked increases come after the end of the rest period. CHANDLER[1] has shown for several varieties of peach that the rest period ends during the latter part of December or the first part of January. The work of AUCHTER in 1919 (unpublished), at the Maryland Experiment Station, shows that the rest period for fruit buds of six peach varieties was completed by December 25. It is true that there is a fluctuation in the moisture content of dormant buds, but such variations are slight in comparison with those coming at the time the buds start their growth. Concerning buds of the plum, STRAUSBAUGH[2] states:

> During the period of dormancy the moisture content of the semihardy varieties fluctuates with the temperature. Periods of low temperatures are accompanied by a loss of moisture from the leaf and fruit buds, and higher winter temperatures, which are seldom above freezing in Minnesota, by an increase in moisture content.

It is interesting to note that the moisture content of his hardy variety, Assiniboine, remained fairly constant during dormancy.

Since the increase in moisture content following dormancy seems to be related to growth of the bud, an examination has been made of the available moisture data of peach fruit buds and of

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[1] CHANDLER, W. H., Winter killing of peach buds as influenced by previous treatment. Mo. Agric. Exp. Sta. Bull. 74. 1907.

[2] STRAUSBAUGH, P. D., Dormancy and hardiness in the plum. BOT. GAZ. 61: 337–357. 1921.

temperature data obtained during the last three years (1919–1921) at the Maryland Station. The 1919 data include those previously reported by JOHNSTON.[3] Fifteen Elberta and fifteen Greensboro trees are averaged for this year's data. The 1920 data have not yet been reported, but are the averages of twenty-seven trees, representing eighteen varieties. The buds of this year were collected from an orchard that had been severely pruned the year previous. The 1921 data are very meager, having been obtained from two trees only, one an Elberta, the other a Greensboro. Moisture determinations, however, were made more frequently during this year.

Temperature evaluations

Three kinds of temperature indices have been applied to these moisture data. In making use of the remainder indices, it is assumed that 43° F. (6.1° C.) is the "zero" for growth and other physiological processes, and that daily mean temperatures above

TABLE I

MOISTURE CONTENT OF PEACH BUDS WITH CORRESPONDING TEMPERATURE INDICES SUMMED FROM JANUARY 1 OF EACH YEAR

DATE	MOISTURE	TEMPERATURE INDICES SUMMED FROM JANUARY 1		
	Ratio of water content to dry weight of buds	Daily mean above 43° F.	Physiological index	Exponential index
1919				
January 7	0.69	14	12.6	2.94
February 7	0.85	22	37.3	16.16
March 7	1.61	55	86.2	31.31
March 28	3.65	173	218.8	59.83
1920				
January 29	0.73	0	2.5	1.00
March 2	0.77	0	10.3	5.16
March 22	1.49	48	66.5	18.86
April 1	3.51	147	177.0	36.12
1921				
January 26	0.86	32	36.4	11.47
February 12	0.99	37	51.0	18.14
February 18	1.13	65	85.0	24.19
February 25	1.16	65	85.3	24.19
March 5	1.46	96	120.7	32.56
March 11	2.27	185	252.3	45.10
March 14	2.72	223	294.0	50.67

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[3] JOHNSTON, EARL S., An index of hardiness in peach buds. Amer. Jour. Bot. 6:373–379. 1919.

43° are "effective" units. These daily mean temperatures (average of daily maximum and minimum) above 43° have been summed from January 1 to each date on which the moisture determinations were made. The physiological summation indices, as derived by LIVINGSTON,[4] were obtained from the daily mean temperatures, and are likewise summed from January 1 to each particular date. The chemical efficiency indices proposed by LIVINGSTON and LIVINGSTON[5] were summed from the indices corresponding to the daily mean temperatures. These three kinds of temperature indices are presented, together with the moisture data, in table I.

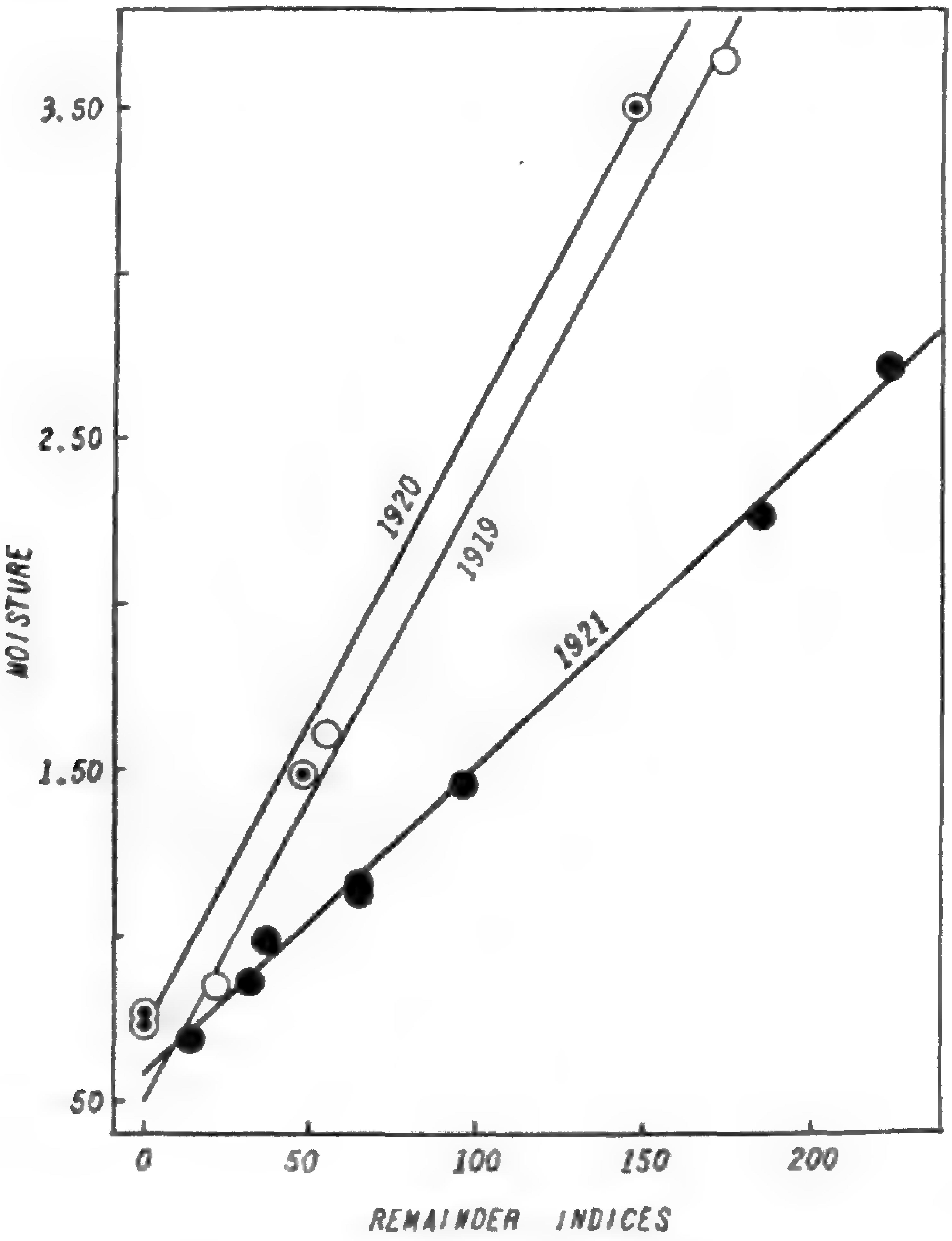

FIG. 1.—Moisture content of peach buds plotted against remainder indices summed from January 1 for years 1919–1921.

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

[5] LIVINGSTON, B. E., and LIVINGSTON, G. J., Temperature coefficients in plant geography and climatology. BOT. GAZ. 56:349–375. 1913.

A general survey of the temperature indices in table I at once shows that the 1920 season was colder and less advanced by April 1 than those of 1919 and 1921 on March 28 and March 11, respectively. If the sum of the daily mean temperatures above 43° be used as the criterion, it is seen that the season of 1921 was more advanced on February 18 than the 1919 season on March 7, and the season of 1920 on March 22. The moisture content of the fruit buds on January 26, 1921, was practically the same as that on February 7, 1919, and somewhat greater than that on March 2, 1920. Such early development of buds increases the danger of fruit loss by spring freezes.

TABLE II

MOISTURE CONTENT OF PEACH BUDS WITH CORRESPONDING TEMPERATURE INDICES SUMMED FROM JANUARY 1 OF EACH YEAR, EXPRESSED AS NUMBERS RELATIVE TO EACH SEASONAL RANGE, FIRST AND LAST VALUES BEING 0 AND 1.00 RESPECTIVELY

Date	Moisture	Temperature indices summed from January 1		
	Ratio of water content to dry weight of buds	Daily mean above 43°F.	Physiological index	Exponential index
1919				
January 7	0	0	0	0
February 7	0.05	0.05	0.12	0.23
March 7	0.31	0.26	0.36	0.50
March 28	1.00	1.00	1.00	1.00
1920				
January 29	0	0	0	0
March 2	0.01	0	0.04	0.12
March 22	0.27	0.33	0.37	0.51
April 1	1.00	1.00	1.00	1.00
1921				
January 26	0	0	0	0
February 12	0.07	0.03	0.06	0.17
February 18	0.15	0.17	0.19	0.32
February 25	0.16	0.17	0.19	0.32
March 5	0.32	0.34	0.33	0.54
March 11	0.76	0.80	0.84	0.86
March 14	1.00	1.00	1.00	1.00

The moisture values given in table I have been plotted against the summation values of the daily mean temperatures above 43° F., and are presented in fig. 1. The slopes of the 1919 and 1920 curves are practically the same, while that of 1921 is quite different. There are apparently some conditioning influences at work before January 1 that determine the slopes of these curves.

After the slopes have once been "predetermined," the increase seems to depend mainly on temperature, either directly or indirectly. It is thus possible to predict the moisture content of the buds for any date after the first of January to the time the pink petals of the buds begin to show. The daily mean temperatures and the origin of the slope of the curve must be known. If the origin be considered the first moisture value with its corresponding temperature summation after January 1, or after the end of the rest period, then the slope can be determined from one other later moisture determination.

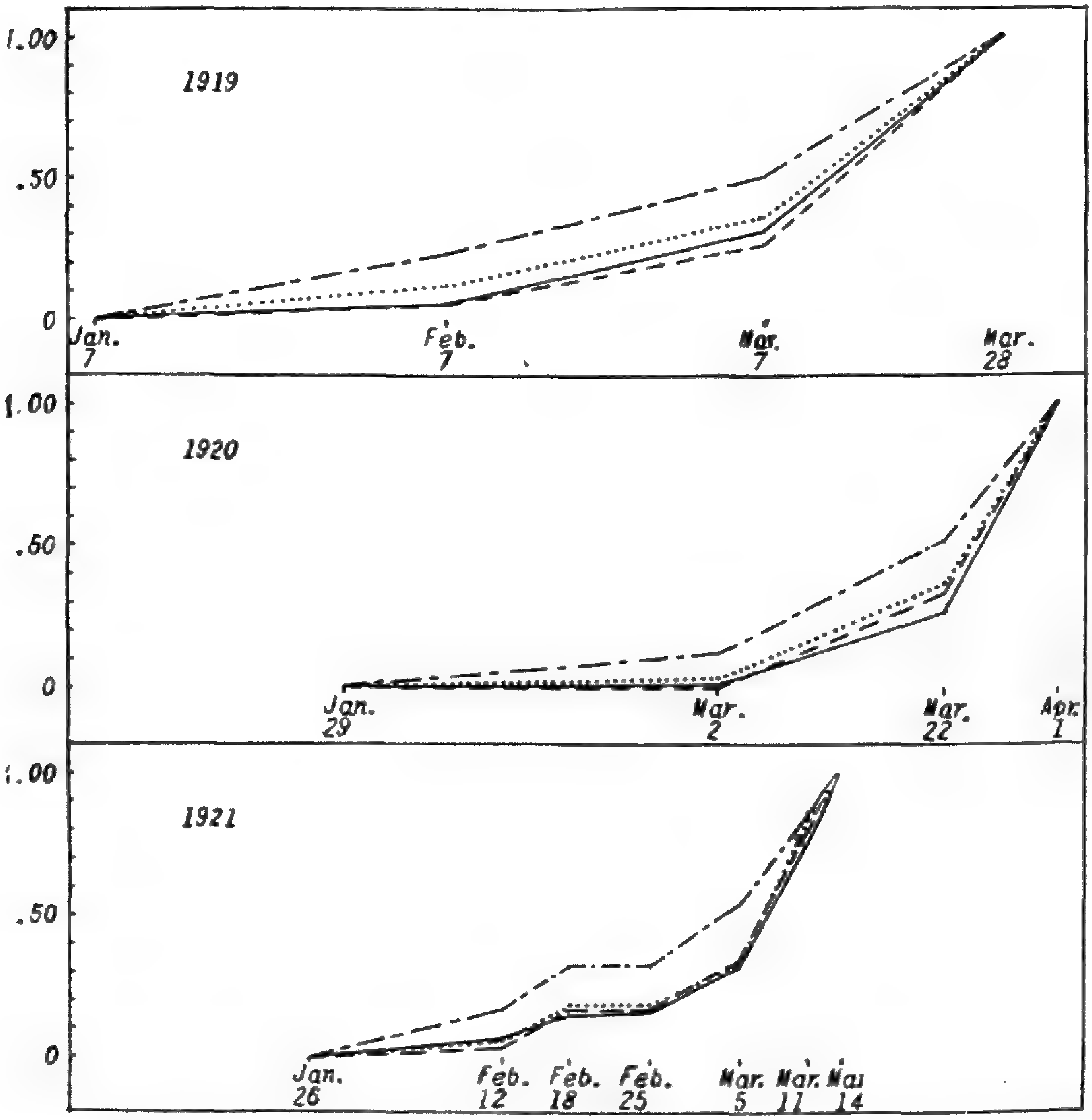

FIG. 2.—Proportionate increase in moisture content of peach buds (continuous lines) and corresponding increases in remainder indices (dash lines), physiological indices (dot lines), and exponential indices (dash-dot lines) for years 1919–1921.

The other temperature data in table I were also plotted against the corresponding moisture values, but are not presented in graphic form. The curves obtained by using the physiological indices were practically straight lines, and quite similar to those obtained from the daily mean temperatures above 43° F. The relationship between the moisture and the exponential indices, however, was not linear.

The data of table I have been rearranged so that the first reading of each measurement for each year is zero and the last unity. Within this range it is somewhat easier to compare the proportionate amount of change in each measurement. These data are presented in table II, and in the form of graphs in fig. 2. The close agreement between the moisture and the temperature indices representing the summation of daily mean temperatures above 43° F. is readily seen.

Conclusions

It is realized that the observations discussed in this paper are somewhat limited in number, but nevertheless they suggest a definite relationship between air temperature and the rate of increase in the moisture content of peach fruit buds. There can be little doubt, however, that other conditioning influences are operative before January 1, that determine the manner in which these buds responded to temperature.

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ALTERNARIA FROM CALIFORNIA

D. G. MILBRAITH

(WITH TWO FIGURES)

Frequent occurrence of a distinct spot on the leaves of cabbage and cauliflower[1] in certain sections of California led to an investigation of its causes and distribution. In culture media, infected leaf tissue taken from the spot yielded constantly a form of *Alternaria* which was found to be a species hitherto undescribed. Macroscopically the infected area appeared smooth, although somewhat sunken, but lacked the profuse growth of conidia and the zonation which characterize the lesions produced on leaves of cabbage by *Alternaria brassicae* (Berk.) Sacc. The disease was found to be restricted to that district of the state lying directly south of San Francisco, where high relative humidity and a uniform temperature prevail. In this district there have been planted annually about 2000 acres of cabbage and cauliflower for shipment to eastern markets, and in view of the frequency of the occurrence of the disease it may be the cause of a pathological problem in transit as well as in the field.

The first symptom of the disease is the appearance of small black specks about 1 mm. in diameter. Often countless specks appear simultaneously in the laminae. Infection occurs almost entirely on apparently vigorous leaves, young as well as old; and under conditions of high relative humidity the initial lesion may develop into a spot ranging from 0.5 to 1 mm. in diameter in fourteen days. The spots are circular, somewhat sunken, black with a purplish cast, the center being darker than the margin (fig. 1). The color depends largely upon the color of the leaf, for upon pale green leaves, as found on Winningstadt and Cannon-ball cabbage, lighter spots are produced than on the dark green varieties of cabbage. Sporulation of the fungus is sparse on still vigorous leaves, but becomes profuse on yellow and detached leaves.

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[1] The term cauliflower includes both cauliflower and broccoli.

The causal fungus grew luxuriantly on all common culture media. Sporulation was abundant, and the mycelium was greatly suppressed. *En masse*, the color of the colony is light olivaceous on starchy media and dark olivaceous on nitrogenous media.

FIG. 1.—Cabbage leaf showing abundant spots caused by *Alternaria oleracea;* note absence of zonation characteristic of many *Alternaria* spots.

Zonation in the colony was not visible. A description of the fungus is as follows:

Alternaria oleracea, n. sp.

Hyphae short, greatly subordinated, straight, sparsely branched, occasionally septate, very light olivaceous to hyaline, average 4.5 μ in diameter; conidiophores short, light olivaceous, short branched, branches almost even in length, about 35 μ; conidia olivaceous to brown, echinulation none in culture four months old, tapering very slightly to apical cell, tapering frequently absent, apical cell frequently hyaline, conidia catenulate, as many as eight in one chain, catenulation both on host and on culture media, longitudinal septation very rare, considerable constriction of the walls at septation, size of the conidia taken from artificially inoculated cabbage leaf 29.7–61.2 μ by 8.7–12.3 μ, average size 43.7 by 10.5 μ. Conidia developed on standard lima bean agar measured on the seventh day as follows: 1-septate, 13.4–16 μ by 6.5–7.5 μ; 2-septate, 17.5–18.7 μ by 8.7 μ; 3-septate, 20–26.5 μ by 8–12 μ; 4-septate, 34–36 μ by 12.5–14 μ; 5-septate, 30–38 μ by 9.5–14 μ; 9-septate, 64.7–70 μ by 10–14 μ (fig. 2).

Inoculation experiments with pure cultures were made on growing cabbage and cauliflower plants in the field and in pots under control in glass cages, at the field station of the Office of Cotton, Truck, and Forage Crop Disease Investigations, located at Alhambra, California. The varieties of cabbage used in these experiments were Winningstadt, Late Drumhead, Early Jersey Wakefield, and Fat Dutch, and the varieties of cauliflower were two selections commonly grown in the San Francisco district. In Alhambra the relative humidity of the atmosphere is considerably lower than in the San Francisco district, where the disease is prevalent. On account of this variation, open field inoculation did not progress above a small speck. When the inoculated leaf was covered with a wax paper sack in which there had been suspended a water-soaked mass of gauze, lesions of normal size were produced readily. Successful infections were produced by spraying conidia, suspended in water, on uninjured leaves, and by wounding and inserting conidia. Infections were obtained more rapidly in glass cages where high relative humidity could be maintained. Within seven days lesions ranging in diameter from 1 cm. to 1.5 cm. were produced. Similar inoculation experiments were made with *Alternaria brassicae* obtained from old cauliflower and cabbage leaves, and with *Alternaria* sp. from spots on tomato fruit. The former produced spots with definite zonations, while the latter failed to grow on cabbage and cauliflower.

Black leaf spot of cabbage has been attributed to the fungus *Alternaria brassicae* for a long time. HARTER and JONES[2] state that the black spot fungus may attack the cabbage plant at any stage of its growth, but it is not common except on the older leaves in the field or on heads in storage. In the field it appears on the lower or outer leaves of the maturing plant as distinct, roundish, black spots, commonly marked with concentric brown zones. SACCARDO[3] describes the fungus *A. brassicae* (in translation) as follows:

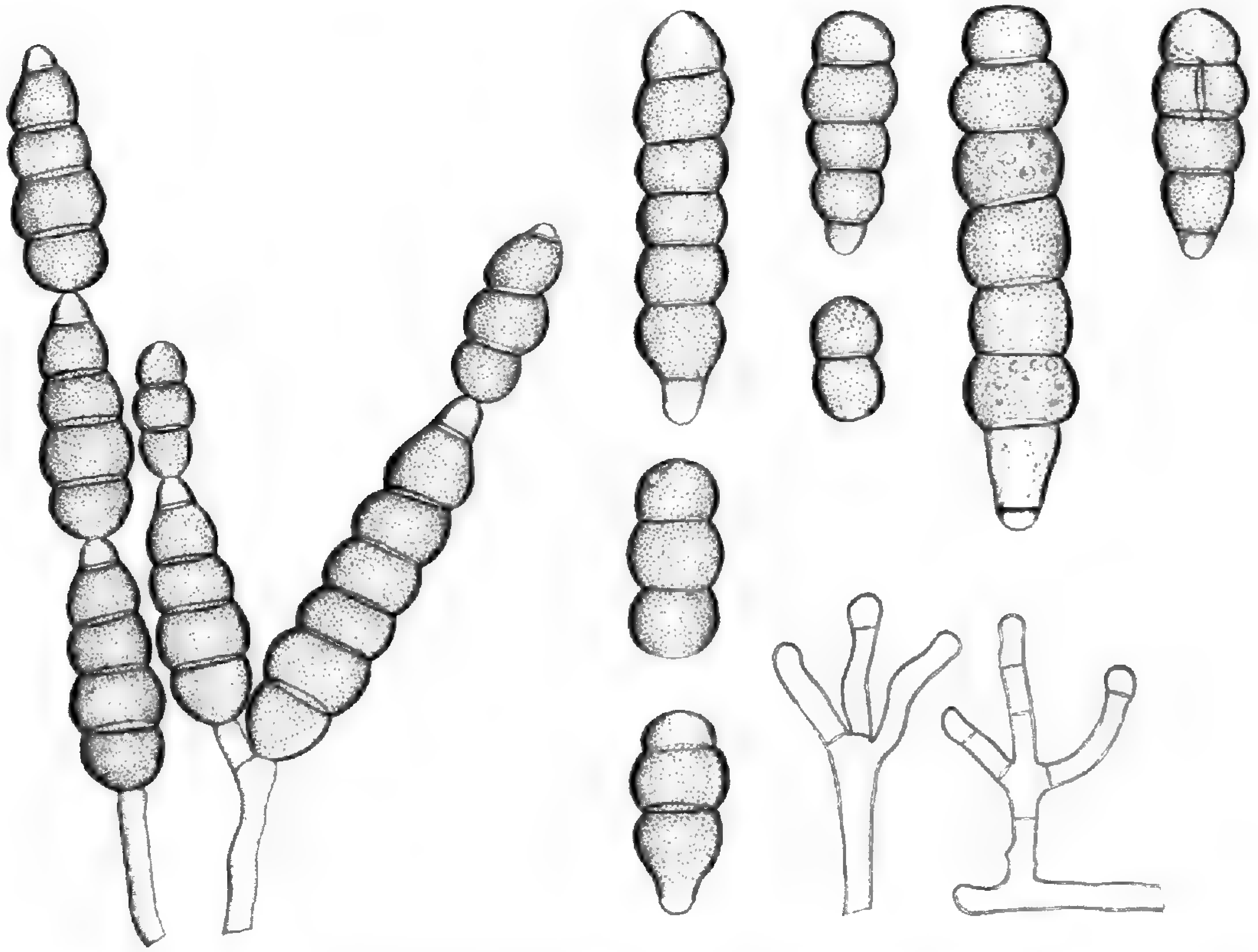

FIG. 2.—Types of conidia and conidiophores of *Alternaria oleracea* from cabbage leaf.

Hyphae short, compact, little branched, even growth, in tufts; conidia deciduous, elongate, fusoid, clavate, size 60–80 μ by 14–18 μ, 6–8 septate, muriform, olivaceous; fungus found on decaying and old leaves of *Brassica oleracea*.

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[2] HARTER, L. L., and JONES, L. R., Cabbage diseases. U.S. Dept. Agric. Farmers' Bull. 925. *figs. 13.* 1918.

[3] SACCARDO, P. A., Sylloge Fungorum 4:546. 1886.

MASSEE and COOKE give similar descriptions, except that COOKE employs the term *Macrosporium brassicae* Berk., and describes the conidia as antennaeform, rather longer than the peduncle. ELLIOTT[4] concluded that *A. brassicae* is morphologically similar to *A. solani* (E. and M.) Jones and Grout, and placed both in the same group, which he based upon having long, narrow, regular, tapering spores with few longitudinal septae. It appears that the form of *Alternaria* which the writer dealt with differs from *A. brassicae* both morphologically and in the form of spot produced on cabbage. In view of this difference, it is highly probable that a new species is involved in the production of the leaf spot found in the San Francisco district, and it is suggested that it be named *Alternaria oleracea.*

DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

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[4] ELLIOTT, JOHN A., Taxonomic characters of the genera *Alternaria* and *Macrosporium*. Amer. Jour. Bot. 4:439–476. *pls. 19–20.* 1917.

BASISPORIUM GALLARUM MOLL., A PARASITE OF THE TOMATO[1]

G. B. RAMSEY

(WITH ELEVEN FIGURES)

While studying the various diseases of the tomato which are found under transit and market conditions, the writer became interested in an unusual fungus which was isolated from the California crop of November 1919. Observations have been made in the Chicago market during the past three seasons to see whether the fungus recurred on California tomatoes, or whether it could be found on those from Florida, Cuba, Mexico, and other tomato shipping districts, but this particular fungus has not been isolated again. The potential seriousness of this fungus as a wound parasite of the tomato, however, and the fact that it has not been reported as a plant pathogen, seemed to make it desirable to publish this note.

The original isolation was made from a soft, red, blister-like lesion near the blossom end of a ripe tomato. A luxuriant growth developed upon the nutrient agar plate, and a great number of spores were formed within a few days. The characteristic smooth, black, subspherical spores borne singly upon their club-shaped sporophores made it comparatively easy to place the fungus in the genus *Basisporium*.

Basisporium gallarum was established as a new genus and species in 1902 by MOLLIARD,[2] in order to properly locate and describe a hyphomycete which he had found upon dead larvae of *Lipara lucens* Meigen, within galls which this insect produces on *Phragmites communis* Trin. He does not mention having found this fungus before, or of later finding it upon any other host. So far as the

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[1] Contribution from Research Laboratory on Market Diseases of Vegetables and Fruits; Department of Botany of University of Chicago and United States Department of Agriculture cooperating.

[2] MOLLIARD, M., Bull. Soc. Myc. France, p. 167. 1902.

writer has been able to ascertain, no mention has ever been made in the literature of the parasitism of *Basisporium* upon plants.[1]

Numerous inoculation experiments have shown conclusively that the fungus is strongly pathogenic to the fruits of the tomato. Pure culture re-isolations of *Basisporium* have been obtained many times from the advancing edge of characteristic lesions produced upon fruits inoculated with a transfer from the original culture. Single spore cultures have been made and used in all experiments described, in order to avoid all possibilities of contaminating organisms.

All attempted inoculations upon unwounded surfaces of both ripe and green tomatoes have failed. Inoculations in wounded surfaces of ripe fruits have always produced the characteristic decay described. Soft, red lesions, two inches in diameter, have been produced on ripe fruits held in a moist chamber at room temperature, within four days. An abundance of pale, smoke colored mycelium is developed in a humid atmosphere. Ripe fruits inoculated in such wounds do not decay at a temperature of 9°–10° C. Specimens have been held two weeks without showing signs of decay when kept at this temperature.

Inoculation experiments with mature green tomatoes have proved positive. It is quite evident, however, that the fungus would grow much more rapidly and produce rot more quickly in ripe fruits. Green tomatoes examined after being inoculated and kept in moist chambers at room temperature for five days showed only slight surface discoloration at the wound. The locule underneath, which was turning pink, showed discoloration and decay to a depth of one-half inch.

The spores of *Basisporium* germinate readily in nutrient solutions. In freshly expressed juice of green tomatoes, as well as that of the ripe fruits, 90–100 per cent germination is obtained within twenty-four hours at 20°–21° C. At 34°–35° C. practically all spores germinate within twenty-four hours; while at 9°–10° C. only about one-half germinate in the green juice, and 80–90 per cent in

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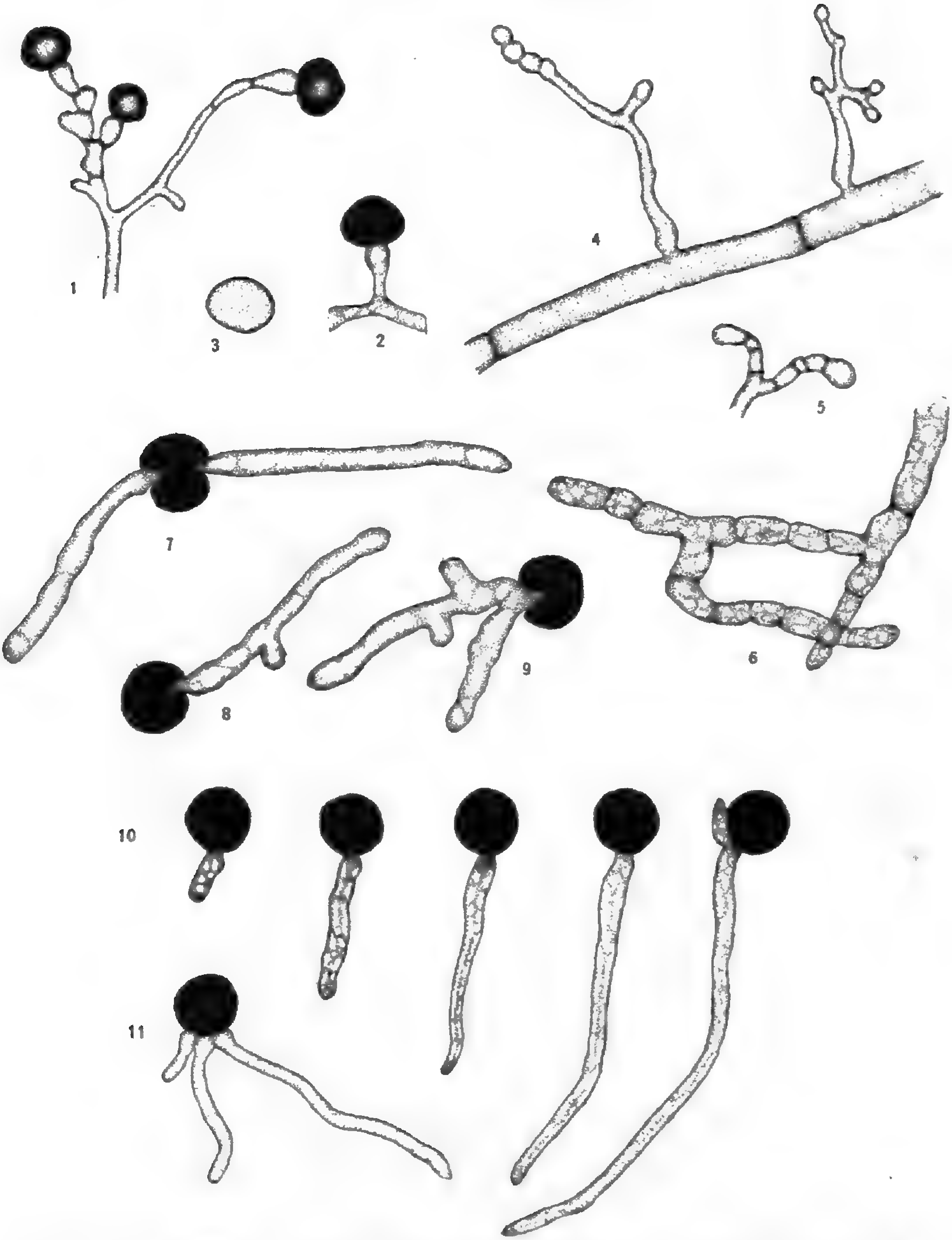
[1] In a letter, Miss A. E. JENKINS of the Office of Pathological Collections, Washington, D.C., reports *Basisporium* as having been found on cultures from corn, wheat, and dewberries.



FIGS. 1–11.—Fig. 1, sporophore bearing three slightly immature spores; fig. 2, mature spore; fig. 3, microtome section through mature spore showing thickness of opaque wall; fig. 4, large hypha on surface of agar plate bearing two young fertile hyphae; fig. 5, showing method of branching and spore formation at tips of fertile aerial hyphae; fig. 6, large sterile mycelium taken from pulp of infected tomato; figs. 7–9, spores germinated at room temperature in freshly expressed juice of ripe tomatoes; fig. 10, showing hourly growth of germinating spores in Standard Nutrient Salt Solution, at room temperature; fig. 11, spore germinated in sterile distilled water at room temperature. (All drawings made with the aid of a camera lucida.)

the ripe juice. It is interesting to note in this connection that although the spores germinate readily in fresh tomato juice at 9°–10° C., decay is practically if not totally prevented in both ripe and green tomatoes at this temperature.

In sterile distilled water at 10° C. only a very few spores have ever been observed to germinate. After three days, usually less than 1 per cent have germinated. At room temperature about 10 per cent germinate within twenty-four hours, and 20–30 per cent in forty-eight hours. The spores seem to absorb water rapidly, and burst before having a chance to send forth a germ tube when placed in sterile distilled water at 34°–35° C.

Basisporium grows well on most nutrient agars. Potato agar plus 2 per cent dextrose has been used with excellent results in culture studies of this fungus. The mycelium is white at first, moderately thick and cottony, later turning pale smoke color as sporulation takes place. Fine growth has been obtained at a temperature as high as 35° C., but sporulation seems to be inhibited somewhat at that temperature. Plate cultures held at 9°–10° C. for seven days developed only a very thin, flat growth whose radius was 3 mm. Taking the other temperature experiments into consideration, it would seem that 10° C. is approximately the minimum at which *Basisporium* will grow.

Spores are borne abundantly on the terminal as well as the lateral branches of the small fertile hyphae. The spores are black, subspherical, smooth, and have a tendency to be slightly conical on top and flattened underneath. Average measurement through the axis is 11.4 μ, and through the transverse diameter 15.3 μ. The fertile aerial hyphae are 3.5–5 μ in diameter. Large sterile hyphae within decaying tissues of tomatoes and on surface of agar in plate cultures are 12–17 μ in diameter. These measurements show a slight variation from those given by MOLLIARD. The original drawings also show a narrower constriction of the sporophore at its junction with the spore. These differences are felt to be of minor importance, however, and, although a new host is involved, do not seem to justify the making of a new species.

BUREAU OF PLANT INDUSTRY
WASHINGTON, D.C.

BRIEFER ARTICLES

SAGENOPTERIS, A MESOZOIC REPRESENTATIVE OF THE HYDROPTERACEAE

(WITH ONE FIGURE)

The question of the botanical relationship of the genus *Sagenopteris* has been a debated one for a great many years. The genus was founded by PRESL in 1838 for a Rhaetic (upper Triassic) form from south Germany, and since that time a considerable number of species have been described from all over the world, all of which come from rocks of Mesozoic age. The genus has been discussed by SCHIMPER, NATHORST, BERRY, and SEWARD. Nearly all students have recognized its pteridophytic affinities, and many have suggested a relationship with the so-called water ferns (Hydropteraceae). NATHORST was the most emphatic advocate of the latter relationship, which was based upon habit and venation of the vegetative parts. He fortified it by his discovery in the Rhaetic beds near Palsjö in Sweden of fruitlike bodies, which, aside from their resemblance to the sporocarps of the modern *Marsilea*, did not represent the fruits of any known Coniferophyte or Cycadophyte present in the Rhaetic flora of Sweden, and therefore were considered to be the sporocarps of *Sagenopteris*, being found in association with the fronds of *S. undulata* Nathorst.

Somewhat similar remains, considered to represent the sporocarps of *Sagenopteris*, have been recorded by ZIGNO from the Jurassic of Italy, and by HEER from the Upper Cretaceous of western Greenland (HEER referred the latter to *Marsilea*). In describing the Lower Cretaceous of Maryland in 1911, I considered the evidence sufficiently good to warrant referring the Potomac species of *Sagenopteris* to the Hydropteraceae. The most abundant of these Potomac species, and the only one at all well characterized, namely *S. elliptica* Fontaine, has been found to have a considerable geographic range. It is not only present in the Patuxent formation (the oldest member of the Potomac group), but occurs also in the Patapsco formation, which there is good evidence to consider to be of Albian age. This species has also been recorded from the Knoxville and Horsetown beds of California, the Kootenay formation of Montana, and the Lower Cretaceous of Queen Charlotte Islands.

The occasion for the present note is the presence of this species (*S. elliptica* Fontaine) in the lower part of the Blairmore formation of western Canada, in association with characteristic sporocarps which are almost identical with those described by NATHORST from much older beds on the other side of the world. The supposed sporocarps from the Blairmore formation (fig. 1) probably belong to the same botanical species as *S. elliptica*, but in view of the fact that this has not been demonstrated, and in accordance with paleobotanical usage, they may be named **Sagenopteris canadensis**, sp. nov. It may be remarked parenthetically that not all of the recognized species of *Sagenopteris* based upon foliar remains are clear cut, the European Jurassic *S. Phillipsii* being not obviously different from *S. rhoifolia*, or from the European Lower Cretaceous *S. Mantelli*. The last is scarcely, if at all, to be distinguished from, and has often been confused with, the American *S. elliptica*.

Fig. 1.—Sporocarps of *Sagenopteris canadensis*; ×4.

The sporocarp, which it is believed belongs to the latter, may be described as follows: Sporocarp hard and resistant, stalked, bean-shaped, gibbous, slightly flattened at the sides, more recurved and slightly more narrowly rounded at one end, about 5 mm. in length, and about 3 mm. in height, with fifteen or sixteen transverse encircling veins which are impressed, and appear as sulcae in the material, retaining more carbonaceous matter because thicker, and appearing blacker than the remainder of the wall; bands between these impressed veins lighter in color, and with a thin central line more or less developed.

SEWARD in his latest work refers *Sagenopteris* to the Hydropteraceae tentatively, summing up his remarks with the statement that "decisive evidence as to its position in the plant kingdom is at present lacking; the inclusion of the genus as a possible member of the Hydropterideae has still to be justified."

It would seem that the discovery of bodies that have all the megascopic features of sporocarps, that cannot be referred to any other known elements of the associated flora, in association with foliage, which in habit, form, and venation independently suggests comparisons with the genus *Marsilea*, at two such remote localities as Sweden and western Canada, is strong presumptive proof of relationship. Moreover, these two occurrences are very different in age, thus showing no obvious change in the sporocarps during the time that elapsed between the Rhaetic and the mid-Cretaceous, a time interval of at least several million years, and comparable in magnitude with the time that has elapsed from the mid-Cretaceous to the present. If these sporocarps preserve their appearance during the older interval, this conservative feature becomes an argument of validity in comparing their latest occurrence with the *Marsilea* sporocarps of the present.

The evidence, then, that *Sagenopteris* is related to the recent Hydropteraceae is about as conclusive as we can hope to secure in the absence of structural material, which is present in about 0.0001 per cent of the cases with which the paleobotanist has to deal.—EDWARD W. BERRY, *Johns Hopkins University, Baltimore, Md.*

A BISPORANGIATE SPOROPHYLL OF LYCOPODIUM LUCIDULUM

(WITH ONE FIGURE)

The occurrence of more than a single sporangium on a sporophyll in *Lycopodium* is so unusual that it is believed the following account will be of interest.

BOWER[1] records a case in which a sporophyll of *L. rigidum*, from a specimen in the Glasgow University Herbarium, bears "two sporangia of slightly unequal size placed side by side. They are individually smaller than the average sporangia in the near neighborhood on the same axis." BOWER's statement would hold equally true for a similar case in *L. lucidulum* recently found in the writer's laboratory. As will be noted from fig. 1, the larger of the sporangia shows the normal kidney shape typical of the sporangium of *Lycopodium*, while the smaller has more the form of a football. The relative thicknesses of the two stalks correspond closely to the size of the sporangia. Both stalks are slightly lateral to the

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[1] BOWER, F. O., Note on abnormal plurality of sporangia in *Lycopodium rigidum* Gmel. Ann. Botany **17**:278–280. *figs. 18.* 1903.

midrib of the leaf, the smaller being the farther removed. Normally the stalk of the single sporangium is immediately above the midrib of the leaf.

The origin of such a situation can only be conjectured. Since the sporangium of *Lycopodium* arises from a transverse row of initials, it is probable in this case that there was a sterilization of archesporial tissue within this row, separating the two ends of the row, each of which developed to maturity in a practically normal manner, producing the two distinct and separate sporangia.

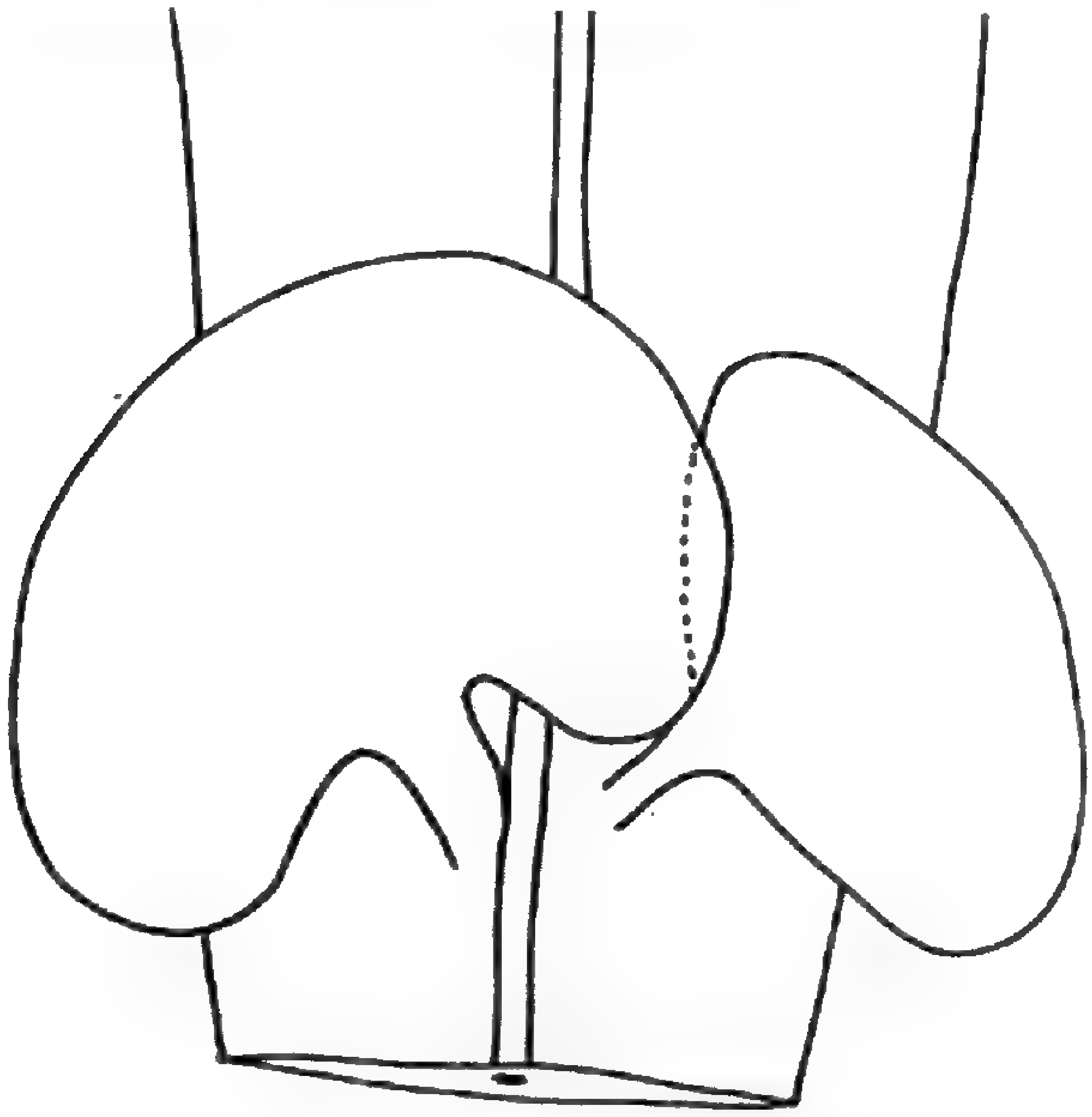

FIG. 1

The formation of the "subarchesporial pad" in the sporangium of *Lycopodium* may be regarded as a sterilization of potentially sporogenous tissue, which, while not producing complete septation of the sporangium, is suggestive as a prelude to the complete septation which occurs in other forms, such as *Psilotum*, as a result of the sterilization of complete plates of tissue. In this case the sterilization may be thought of as having occurred so early as to result in two completely distinct sporangia. Whether the polysporangiate condition in Pteridophytes may have arisen in a similar way is of theoretic interest, the synangium being regarded as intermediary between the monosporangiate and the polysporangiate conditions. On the other hand, the two sporangia may have arisen from two distinct groups of initials.

BOWER, concluding his note on *L. rigidum*, states that "it shows how even the most rigid facts of morphological experience are liable to exception, and that this applies equally to spore-bearing members, in cases where their forms seem most stereotyped."

To summarize, (1) bisporangiate sporophylls in *Lycopodium* are very rare, a single case of each being known in *L. rigidum* and *L. lucidulum;* (2) the two sporangia may have arisen as a result of very early sterilization of archesporial tissue, or from two distinct groups of initials.—A. W. DUPLER, *Juniata College, Huntingdon, Pa.*

A SIMPLE APPARATUS FOR CONTROLLING TEMPERATURES

(WITH ONE FIGURE)

In developing the control equipment for a humidity chamber in which it was desired to control temperatures over long periods of time, it was found essential to use a temperature control apparatus activated by an electric current taken from the ordinary lighting circuit. Troublesome failures with dry cells and storage batteries led to the construction

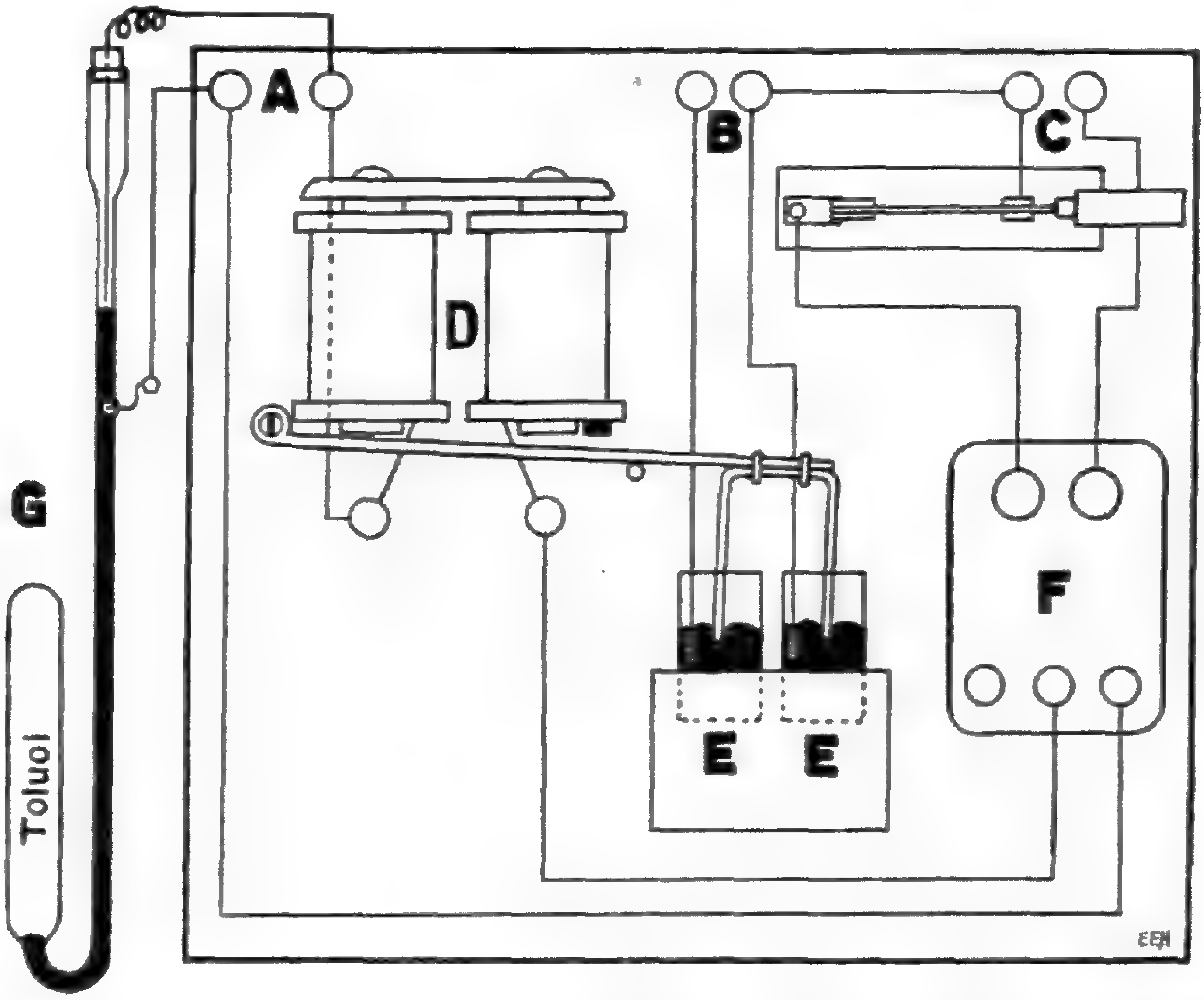

FIG. 1.—*A*, binding posts to which wires from thermostat tube (*G*) are attached; to left-hand binding post (*B*) is attached one of the wires leading from heating unit; *C*, binding posts to which wires from main lighting circuit are attached (one of the wires leading from heating unit is attached to right-hand post); *D*, "transformer bell" electro-magnetic coils wound for alternating current with bell and clapper removed (to extreme right of armature bar is attached a piece of U-shaped heavy copper wire); attached to lower part of right-hand magnet is a small piece of rubber cut from a rubber stopper, which projects far enough beyond magnet bar to eliminate most of the "chatter" or "buzz" when coils are operating; *E*, two glass vials (0.75×1.5 inches) partly filled with mercury, into which points of the U-shaped copper wire are immersed; wires leading from *B* are in contact with the mercury in vials; *F*, small, 3 volt, bell-ringing, alternating current transformer which furnishes 6, 8, or 14 volts as desired (here connected to furnish 8 volts, alternating current); *H*, small single-blade knife switch used to test action of coils at *D*; the asbestos-covered board upon which units *A* to *F* are mounted measures 9.5×11.5 inches, and is designed for wall attachment; use of transite board or other insulating material and mounting to reduce fire risks is recommended.

of the apparatus shown in fig. 1. This is a modification of a somewhat similar instrument secured through Dr. K. F. KELLERMAN, and used for some time at the Laboratory of Forest Pathology at Madison, Wisconsin, in controlling the temperature of a Bausch and Lomb incubator.

The main advantage of the apparatus here described lies in the fact that the same current (110 volts, 60 cycle, alternating) which passes through the heating units is used, after reducing the voltage to 6, 8, or 14 volts, to operate the relay (fig. 1*D*). The apparatus is simple, comparatively cheap, and when once set up and adjusted needs little attention. It will stand continuous service for long periods of time, and when used in connection with a toluol and mercury filled tube (fig. 1*G*) controls temperatures within a range of a quarter of a degree Fahrenheit.

When the mercury column in the tube (*G*) rises and contacts with the fine wire held by the cork, the coils at *D* draw the armature bar up to the poles of the magnet, the U-shaped wire is pulled free from the mercury at *E*, and the current supplying the heating unit is broken, thus shutting off the heat. A reversal of this action turns the heat on again.

At *E* the make and break of the current supplying the heat units is very positive in action. There is no arcing of the current between the mercury and the wire points, so long as the U-shaped wire is raised sufficiently above the mercury surface. The small voltage of the current passing through the mercury column in the tube (*G*) reduces the spark to a minimum, and for this reason little trouble is encountered with arcing, vaporizing of mercury, or clouding of the tube where contact is made between the fine wire and the mercury. A metal cap with adjusting screw carrying a fine platinum wire may be substituted for the cork and wire as shown.—ERNEST E. HUBERT, *Laboratory of Forest Pathology, Bureau of Plant Industry, in cooperation with the Forest Products Laboratory, Madison, Wisconsin.*

CURRENT LITERATURE

BOOK REVIEWS

Yeasts

A few years ago a group of French savants arranged for a new *Encyclopêdie Scientifique.* Under the direction of TOULOUSE, some of the most distinguished scientists are attempting to prepare a series of complete and readable monographs covering the whole range of science. The plan calls for about a thousand volumes, classified under forty main sections of knowledge.

If GUILLIERMOND'S[1] volume on yeasts is a fair sample, this encyclopedia will be indispensable to every school of science. This volume includes an ample bibliography, index, and analytic table of contents. The introduction defines the yeasts, morphologically and physiologically, and places them as "a family of the Ascomycetes, known by the name of Saccharomycetes." A brief historical résumé concludes that three names will always be connected with the study of yeasts, PASTEUR, HANSEN, and BUCHNER.

The first chapter deals with the morphology of vegetation and reproduction. The second one, on cytology, discusses the nucleus, metachromatic bodies, and other particles, both in vegetation and reproduction. The metachromatic bodies are stated to be composed probably of a compound of nucleic acid. They are identical with A. MEYER'S volutin grains. Two chapters on physiology are rich in material. The subjects of the following chapters are phylogeny, methods of study and culture, identification of species, variation of species, and classification. The second part is a systematic description of the known genera and species. Constant reference throughout all the chapters to the little known Schizomyces and Zygosaccharomyces adds much to the interest of the work. Every page of this admirable volume is full of clear, terse statements of observation, and carefully balanced discussion. It is rare for as keen an investigator to have such a lucid and logical style.

The translation of GUILLIERMOND by TANNER[2] is really a new edition. In bringing the work up to date, GUILLIERMOND has been responsible for the morphology, phylogeny, and classification, and TANNER for the physiology. Most of the text, however, is carried over without change. Unfortunately, the translation has been poorly done. Almost every page of the English

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[1] GUILLIERMOND, A. T., Les Levures. II. pp. 565. Paris: Octave Doin et fils. **1912.**

[2] GUILLIERMOND, A. T., The yeasts. Translated and thoroughly revised in collaboration with the original author by F. W. TANNER. pp. xix+424. New York: John Wiley and Sons. **1920.**

edition furnishes distressing mistranslations. When the French idiom *ne . . . . que* is translated as a flat negative, the author is made to say the exact reverse of what he means, and the rest of the paragraph becomes absurd. The French use of the article has been carried over literally, even where it fits the English idiom! Again, where the author says, for example, a fluid *may reach* a certain concentration, the faithful translator puts it, *is able to reach.* "Matières hydrocarbonnes" becomes "hydrocarbon materials," in heavy-faced type; and the paragraph discusses sugar, cellulose, and glycogen! Perhaps the consistent reference to WAGER as WAGNER in the text is an error of proofreading, for the name is correct in the bibliography and in the French text. The American book is very well printed, with large, open, legible type, but no one can safely use it without having a copy of the original at hand, or at least without having enough knowledge of French to read the original author's meaning between the lines.—H. S. CONARD.

NOTES FOR STUDENTS

Electrons in photosynthesis.—An attempt has been made by DIXON and POOLE[3] to interpret photosynthesis in terms of the electronic theory. On the basis of photo-electric phenomena in sensitizers of photographic films, and the absorption spectra of chlorophyll, they believe that the first action of light is to disturb electrons in the chlorophyll molecule. Experiments were made to determine whether the electrons were actually ejected by the incident radiation, or whether the disturbances were too weak to do more than displace the electrons within groups of atoms, or from molecule to molecule of chlorophyll.

By delicate electrometer measurements they were able to establish the occurrence of a slight photo-electric effect in chlorophyll under illumination, but this effect is apparently produced by ultra violet radiations, not by those of visible frequencies and synthetic activity, for they find that the effect is magnified about 2000 times by use of a light rich in ultra violet rays. Quantitative use of the data showed that possibly 75 electrons per square centimeter per second might be ejected from a layer of chlorophyll by light from a 500 watt lamp. In terms of energy, this effect is utterly negligible, for the actual synthesis of food in plants goes on at a rate which would require about nine trillion times as much energy as these ejected electrons could supply. It is necessary to conclude, therefore, that ejection of electrons is not significant in photosynthesis, and that the chlorophyll cannot ionize substances external to itself by electronic bombardment. If this be true, then the synthetic reactions must concern the chlorophyll molecule itself, and the electrons are merely shifted from atom to atom, or molecule to molecule, as in ordinary chemical reactions. These shiftings, of course, will alter linkages, and change the chemical character of atomic groups, probably rendering inactive groups of atoms reactive. Such

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[3] DIXON, HENRY H., and POOLE, HORACE H., Photosynthesis and the electronic theory. Sci. Proc. Roy. Dublin Soc. **16**:63–77. 1920.

a conception favors the chemical theories of photosynthesis which assume that chlorophyll itself enters into the reactions, rather than those which assume that the synthetic reactions are performed externally to the chlorophyll by means of energy absorbed and transformed by the pigments.

That the light actually displaces electrons seems to be proved by DIXON and BALL[4], who show that the chlorophyll acts as a sensitizer of photographic films at the temperature of liquid air, a temperature believed to be too low for chemical reactions other than electronic displacement. They suggest that chlorophyll α and β might have an important connection in the synthetic process, as indicated by the following equations:

$$\underset{\text{(chlorophyll }\alpha\text{)}}{C_{55}H_{72}O_5N_4Mg}+CO_2\rightarrow\underset{\text{(chlorophyll }\beta\text{)}}{C_{55}H_{70}O_6N_4Mg}+HCHO$$

$$\underset{\text{(chlorophyll }\beta\text{)}}{C_{55}H_{70}O_6N_4Mg}+H_2O\rightarrow\underset{\text{(chlorophyll }\alpha\text{)}}{C_{55}H_{72}O_5N_4Mg}+O_2$$

The fact that *in vitro* experiments with chlorophyll α and CO_2 do not yield formaldehyde could be explained by accepting WILLSTÄTTER's assumption that before the CO_2 will react with the chlorophyll it must first be combined into a carbamino acid, which can then be decomposed by the reactive group in the chlorophyll, which group was rendered reactive by the electronic shifting due to light.

Regardless of whether the discovered facts are sufficient to establish the relations between electronic displacements and synthesis of carbohydrates, the attempt made by the authors to carry over into physiological interpretations the newer conceptions of electron chemistry is praiseworthy, and will be followed with great interest by physiologists. Ultimately all the chemical processes of life must be interpreted along similar lines.—C. A. SHULL.

The mycoplasm theory.—In two brief notes in English and an extended discussion in German, ERIKSSON[5] makes a spirited defense of his mycoplasm theory. Only incidental reference is made in these papers to the work on the grain rust, on which the theory was established, but the previously published conclusions on the downy mildew of spinach, the late blight of potato, and the hollyhock rust are reaffirmed, critics are replied to, not without acerbity, and in the case of the hollyhock rust new observations and experiments are adduced which, the author believes, still further support his hypothesis, of which he is not only the originator, but has been, to date, almost the sole protagonist. In

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[4] DIXON, HENRY H., and BALL, NIGEL G., Photosynthesis and the electronic theory. II. Notes Bot. School, Trinity Coll., Dublin **3**:199–205. 1922.

[5] ERIKSSON, JAKOB, The mycoplasm theory, is it dispensable or not? Phytopath. **11**:385–388. 1921.

———, The life of *Puccinia malvacearum* Mont. within the host plant and on its surface. Phytopath. **11**:459–463. 1921.

———, Das Leben des Malvenrostpilzes (*Puccinia malvacearum* Mont.) in und auf der Nährpflanze. Handl. Kungl. Svensk. Vetensk.-Akad. **62**^{5}:1–190. *figs. 31.* 1921.

the first paper he lists additional pathogens which he believes have a mycoplasm stage in their life histories, making a total of fifteen rusts, four downy mildews, three powdery mildews, one slime mould, and the causal agent of tobacco mosaic for which this curious combination of host and fungous protoplasm is claimed as proved or suspected.

Additional data are presented which lead the author to reaffirm, with some modifications, his previously expressed belief in the physiological dimorphism of the teleutospores, as evidenced by their mode of germination; one sort, in moist air, giving rise to long hyphae terminating in chains of conidia; the other kind, under the same conditions, germinating in ordinary teleutospore fashion, producing basidiospores. The latter, on penetrating the host tissue, give rise at once to an intercellular mycelium from which new sori develop in a few days, and are therefore the agencies by means of which the rapid spread from plant to plant is effected. On the other hand, the conidia discharge their contents into a host cell, with the contents of which they form an intimate intracellular protoplasmic union, that is to say, a mycoplasm, which multiplies within the host, even entering the embryo, thus forming the hibernating stage of the fungus. In spring, with the renewal of growth in a dormant plant or the germination of a seed, the fungus element separates out from the mycoplasm, organizes an intercellular mycelium, and eventually produces sori. Numerous observations are reported tending to show that the rapid spread of the disease from plant to plant occurs only late in the year, when basidiospores are being produced, the summer (conidial) infections not appearing until the following season. A series of experiments is reported in which the host plants were watered with weak solutions of copper sulphate. This resulted in a perceptible diminution in the number of summer pustules which appeared, these being due, according to the view outlined, to the conidial infections of the previous year, the fungus wintering over within the host. The copper solution was of no avail against the late summer basidiospore infection. Endeavors to inject the copper solution into the leaves and stems were unsuccessful. Without attempting to summarize the voluminous data, it may be admitted that the evidence seems to favor the view that the fungus may winter over within the host tissues. This is by no means a proof, however, that it is in a state of mycoplasmic symbiosis with the host. Furthermore, it must be regarded as a serious oversight that no notice whatever is taken of the carefully planned experiments of BAILEY,[6] which seem to point very decidedly to conclusions quite opposed to those of ERIKSSON. Certainly the facts in the case must be explained, and the mycoplasm theory admittedly offers a theoretical explanation. The widespread opposition to it is based on the feeling that the facts may ultimately be accounted for satisfactorily without the theory. If this shall prove to be impossible, the mycoplasm theory offers itself as a working hypothesis, which, however, must be confirmed by far less ambiguous cytological evidence than has yet been presented before it can be regarded as established fact.—G. W. MARTIN.

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[6] Ann. Botany **34**: 173–200. 1920.

White pine blister rust.—The present state of our knowledge concerning this most important tree rust is ably summarized by SPAULDING[7] in a recent contribution from the Bureau of Plant Industry. The extensive scope of the treatment is partially indicated by the bibliography of 180 titles. The opinion is expressed that *Cronartium ribicola* is of Asiatic origin, that it spread throughout Europe during the nineteenth century, and was introduced into North America on young trees of *Pinus Strobus* after 1900. The life history of the fungus and its relations with its various hosts are exhaustively discussed. It is regarded as established that the overwintering is chiefly by the mycelium in the bark of living pines, and that it is in this stage that the long migrations have taken place, but it is also recognized that the fungus may overwinter on *Ribes*. The attempts which have been made to control the disease are reviewed, and the conclusion is reached that its eradication is impossible except in the case of small isolated advance infections, but that the systematic removal of all *Ribes* in white pine forest areas will keep the disease in check, and that this method is both practicable and profitable.

ERIKSSON[8] renews his contention that the Peridermiums on *Pinus Strobus*, *P. silvestris*, and *P. Cembra* are biological races of a single species in which the specialization is not yet definitely fixed. He believes that he has evidence that the white pine blister rust is transmitted by diseased seed and also from pine to pine. The direct evidence presented is not of the sort that can be regarded as final. To supplement it, so far as the matter of the spread from pine to pine is concerned, great emphasis is laid upon the undoubted autoecism of closely related species, with particular stress upon the results of HAACK. These, it is interesting to note, are characterized as worthless by SPAULDING. The latter admits, however, that the work of MEINECKE and of HEDGCOCK on *Peridermium cerebrum*, and that of KLEBAHN on *Peridermium pini* "throw doubt on the strict heteroecism of the aeciospores of all stem-inhabiting pine Peridermiums." This is very far from proving the autoecism of the aecidiospores of *Peridermium strobi*, as ERIKSSON seems to assume. The point is obviously one of fundamental importance, and it is to be hoped that the experiments now under way in this country will shortly throw more light upon it. ERIKSSON finds in recent American and European experiences of the overwintering of the fungus on *Ribes* a complete justification of his own early expressed belief concerning this phase of the problem. That such overwintering occurs cannot longer be doubted. That it is of any great importance in the perpetuation of the disease is still very questionable.—G. W. MARTIN.

Isoetes.—OSBORN[9] has obtained some very interesting results from an investigation of *Isoetes Drummondii*, a species widely distributed in certain

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[7] SPAULDING, PERLEY, Investigations of the white pine blister rust. Bull. 957. U.S. Dept. Agric. pp. 100. *pls. 6. figs. 13.* 1922.

[8] ERIKSSON, JAKOB, The connection between *Peridermium strobi* Kleb. and *Cronartium ribicola* Dietr., is it obligate or not? A critical review. Arkiv Botanik **18**^{1}: 1–40. *figs. 6.* 1922.

[9] OSBORN, T. G. B., Some observations on *Isoetes Drummondii* A. Br. Ann. Botany **36**: 41–54. *figs. 15.* 1922.

parts of South Australia. "It grows terrestrially in seasonal swamps during the period of winter rainfall. During the dry summer it aestivates, as do the other geophytes with which it is associated." The stock is buried, and during the vegetative season only a small rosette of linear leaves is visible above the soil. On approach of the dry season, the leaves dry up and become detached, leaving their tough bases and sporangia upon the stock, wholly buried and invisible.

This species seems to be unique among Pteridophytes in its method of spore liberation. There is a special mechanism for freeing the spores which depends for its action upon saturation with water, not upon dryness (as in other Pteridophytes). Other peculiar features of the species are in the nature of preparation for this remarkable method of spore dispersal. In his summary, OSBORN describes the performance as follows: "Early in the rainy season, the hardened bases of the sporophylls are forced above the surface of the soil in a projectile-like mass, carrying with them the sporangia, by the expansion of certain pads of mucilage cells formed at the close of the previous vegetative season on the extreme bases of the sporophylls and from the superficial cells of the leaf-bearing cortex. About the same time the leaves of the new vegetative season begin to appear. The imbricate mass of sporophyll bases breaks up upon the surface of the soil, and the spores are set free by a tearing away of the sporangium wall from its attachment to the sporophyll when sodden. This is due to a difference between the tension of the inner and outer surfaces of the sporangium wall when saturated, and results in an eversion of the wall."

TAKAMINE[10] has investigated the gametophytes of *Isoetes japonica* and *I. asiatica*, with some interesting results. The female gametophyte of *I. japonica* usually has five or six archegonia, but sometimes ten or more. When fertilization occurs in one of them, the others degenerate; but in rare cases when fertilization occurs in two or more archegonia, several embryos are developed up to certain stages. Occasionally megaspores and microspores were found in the same sporangium. In *I. asiatica* the $2x$ chromosome number is twenty-two, while in *I. japonica* it is "forty-three to forty-five." Hybrids of the two species were produced, an account of which is promised later.—J. M. C.

Complexmutation.—As the term mutation is now being used by geneticists, its application is restricted to "locus changes" on the chromosomes. At one place on one chromosome, mutation takes place, the effect of the change being so restricted as to involve only a single factor; other factors, although lying very close on the same chromosome, remain unchanged. Save for "deficiency," noted by BRIDGES[11] (which is evidently of a different category), all mutation seems to have been of this very localized type. It is perhaps surprising that no clear cases of mutations involving simultaneous changes in

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[10] TAKAMINE, N., Some observations in the life history of *Isoetes*. Bot. Mag. Tokyo **35**:184–190. *figs. 9.* 1921.

[11] BRIDGES, C. B., Deficiency. Genetics **2**:445–465. 1917.

several factors in one region of a chromosome have been discovered. NILSSON-EHLE[12] now claims to have such a case, and calls it "complexmutation." Normal wheat mutates to bearded speltoid, involving a simultaneous change in two closely linked factors. Among the F_2 progeny of normal×mutant appear a few bearded normal type and beardless speltoid, but only a very few, due to the very close linkage of the two mutated factors. In another case the investigator claims that three linked factors have mutated simultaneously.—M. C. COULTER.

Ozark forests.—The Ozark region, as covered by PALMER[13] in this reconnoissance, is defined as occupying the southern half of Missouri, a narrow spur crossing southern Illinois, the northwestern part of Arkansas, and a long triangular strip in eastern Oklahoma. The two topographic divisions of this uplifted region, lying midway between the higher mountains of the east and west, are the flat-topped dome of the northern plateau with an average altitude of 300–500 m., and the southern Boston Mountains with a few points above 600 m. It is a hill region surrounded by fertile plains, and possessing a rather abundant rainfall. Floristically there are no distinct floras corresponding to the topographic divisions, although the southern parts of the region, including the Boston Mountains, have a heavier forest growth richer in types than the northern, and include such southern forms as *Aesculus discolor*, *Tilia floridana*, *Rhamnus caroliniana*, *Ilex decidua*, and *Magnolia acuminata*.

The larger portion of the report is occupied by floristic notes on various sections of the flora and on certain genera and species. The author is convinced that in the region as a whole there is a demonstration of the gradual but actual encroachment of forest upon prairie lands.—GEO. D. FULLER.

Temperature and nodule development.—Using soil temperatures ranging from 12° to as high as 40° C., JONES and TISDALE[14] have studied the effect of these temperatures on the development of nodules by alfalfa, red clover, soy beans, and field peas. The results as to the number of nodules developed were not so very consistent, but when the dry weight of the nodules was determined, it was found that the greatest development, in the case of the soy bean, was at 24° C. This effect of temperature on nodule development is not correlated with a corresponding effect on root and shoot development. It is pointed out in the paper that the real question in a study of this kind is not the effect of temperature on the number of nodules developed by the plants, or on the volume of these nodules, but the effect on the amount of nitrogen fixed in the nodules.

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[12] NILSSON-EHLE, H., Multiple Allelomorphe und Komplexmutationen beim Weizen. Hereditas 1:277–311. 1920.

[13] PALMER, E. J., The forest flora of the Ozark region. Jour. Arnold Arboretum 2:216–232. 1921.

[14] JONES, F. R., and TISDALE, W. B., Effect of soil temperature upon the development of nodules on the roots of certain legumes. Jour. Agric. Res. 22:17–31. *pls. 1–3.* 1921.

Some data in regard to this point are presented. While this is a preliminary paper, the results obtained and the statements as to methods of attack on a problem of this kind are suggestive to workers in this field.—S. V. EATON.

Response of apple trees to nitrogen fertilizers.—Nitrogenous fertilizers have been found by many investigators to cause increased vegetative growth of fruit trees and increased yields of fruit, but the experiments establishing these facts have been largely empirical. A bulletin by HOOKER[15] records the results of experiments planned to get at the chemical changes in the tree resulting from nitrogenous fertilizers. He finds that the spring application of nitrogenous fertilizers causes increased setting of apples, accompanied by an increased nitrogen content of the spurs. In the case of non-bearing trees, increased vegetative growth is caused. Different kinds of quickly available fertilizers had much the same effect. At the time of fruit bud differentiation, the spurs of the spring fertilized trees showed less starch than the spurs of the check trees, so that the spring application of nitrogenous fertilizers would not be expected to favor this process. The accumulation of nitrogen in the spurs just before growth starts in the spring is the greater the later the nitrogenous fertilizers have been applied the preceding season. It is a pleasure to see the horticulturists thus attacking the fundamental problems of their subject.—S. V. EATON.

Vegetation of Illinois.—The recent publication of the 14th annual volume of the Transactions of the State Academy of Science[16] shows about one-third of the volume devoted to reports of botanical and plant ecological studies. The State Forester, R. B. MILLER, discusses some of the undeveloped forest resource, of the southern portion of the state, and in collaboration with GEO. D. FULLER examines in some detail the conditions of tree growth and the forest types existing in a portion of Alexander County. W. G. WATERMAN makes a preliminary report on the bogs of the northern portion of the state, while W. B. MCDOUGALL contributes an interesting key to some forest tree roots. A more extensive investigation is reported by H. DEFOREST, who has studied the woodlands along the Rock River in the northwestern portion of the state. MARY E. RENICH contributes a study of growth as related to size of seed, and A. B. REAGAN has some interesting notes on the plants of the Bois Fort Indian Reservation, Minnesota.—GEO. D. FULLER.

Mosaic disease of tobacco.—PALM[17] has investigated the mosaic disease of tobacco, and has reached the conclusion that it is due to a causal organism.

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[15] HOOKER, H. D., Certain responses of apple trees to nitrogen applications of different kinds and at different seasons. Mo. Agric. Exp. Sta. Res. Bull. 50. 1–18. 1922.

[16] Transactions of the Illinois State Academy of Science. Fourteenth annual meeting. 1921. 14: pp. 326. 1922.

[17] PALM, B. T., Is the mosaic disease of tobacco a chlamydozoonose? English translation by P. G. WILSON. Bull. Deliproefstation Medan-Sumatra. no. 15. pp. 10. 1922.

His preparations of the diseased tissue show fairly large irregularly shaped corpuscles and very small granules, both clearly foreign elements, being entirely absent in healthy tissue. The author agrees with IWANOWSKI as to the probable interpretation of the foreign elements, namely, "that the minute granules are very small bacteria, carriers of the virus, and further that the irregularly shaped corpuscles must be considered as a pathological product of reaction of the virus carrier on the cell plasm." This causal organism is thought to be a species of *Strongyloplasma*, and is named *S. Iwanowski* in honor of its original discoverer. —J. M. C.

Effect of age on plant structure.—Miss TELLEFSEN[18] has studied the effect of age upon certain tissues of *Salix nigra*. This species was chosen chiefly because of the comparative ease with which specialized tissues, as roots, will develop from the meristematic tissue of cuttings under laboratory conditions. Numerous tables of measurements are given, and some of the conclusions are as follows. Cuttings from younger trees rooted in less time than those from older trees, and also leaves appeared earlier. Epidermal and cortical cells of the root become smaller as the parent tree becomes older, and xylem and meristematic become larger. The average area of vein islets in leaves from older trees is smaller than average vein islet areas of leaves from younger trees, the amount of vascular tissue increasing with senility, thus reducing the average area of vein islets.—J. M. C.

Leaf-skin theory.—Miss SAUNDERS[19] has reached the conclusion that the surface tissue of the seed plant shoot is of foliar origin, meaning that the leaves are decurrent, not merely those that are usually called so, but all leaves. In the same way the superficial tissue of the hypocotyl are derived from the cotyledons. This so-called leaf-skin is formed by the "downward growth and extension of the leaf primordium, which keeps pace with the extension of the central axis with which it is fused. In the case of flowering stems the leaf-skin is formed by the bracts (when present) and the outermost sepals." Miss SAUNDERS has gone into many details as to the extension of a single leaf surface in relation to the different types of phyllotaxy, the various surface patterns developed, and other features.—J. M. C.

Intrafascicular cambium in monocotyledons.—Mrs. ARBER,[20] in continuing her investigations of the occurrence of intrafascicular cambium in monocotyledons, has added Alismaceae, Aponogetonaceae, and Hydrocharitaceae to the list of monocotyledonous families, in some member of which this tissue has been

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[18] TELLEFSEN, MARJORIE A., The relation of age to size in certain root cells and vein islets of the leaves of *Salix nigra* Marsh. Amer. Jour. Bot. **9:**121–139. 1922.

[19] SAUNDERS, EDITH R., The leaf-skin theory of the stem: a consideration of certain anatomico-physiological relations in the Spermatophyte shoot. Ann. Botany **36:**135–165. *figs. 34.* 1922.

[20] ARBER, AGNES, Studies on intrafascicular cambium in monocotyledons. V. Ann. Botany **36:**251–256. *figs. 8.* 1922.

observed, bringing the number of such families to twenty-two. Incidentally, she shows that LIGNIER was probably mistaken in attributing only phloem-forming activity to the cambium in the petiolar bundle of *Arum maculatum*, since she finds secondary xylem in the corresponding bundles of a closely related species (*A. italicum*), and LIGNIER's figures indicate that the same process takes place in *A. maculatum.*—J. M. C.

Heat of inversion.—A careful measurement of the heat of inversion of sucrose by invertase has been made by DIXON and BALL,[21] who used a thermocouple differential calorimeter in vacuum flasks for the determination. Their results confirm the value found by BROWN and PICKERING many years ago, the mean of all results being 3.83 calories for each gram molecule of sugar inverted. The method is more accurate than the heat of combustion determinations of this value.—C. A. SHULL.

Fungus in Pellia.—RIDLER[22] has described the life history of a fungus occurring in a definite zone in the thallus of *Pellia epiphylla.* It was found to occur in the cells of the sporophyte, from which it was isolated, and identified as a species of *Phoma.* The fungus kills the protoplasts of the infected cells of the gametophyte, which ultimately become brown. The effect on the sporophyte is twofold: the contents of the cells are killed, and the cell walls are also wholly or partially absorbed.—J. M. C.

Mycorhiza of conifers.—MCDOUGALL[23] has identified two mycorhizal fungi from the roots of *Picea rubra* as belonging to the genus *Cortinarius*, and described a tubercle-like mycorhiza of *Pinus Strobus.* He reiterates his opinion that these ectotrophic mycorhizal fungi are of no benefit to the trees concerned, and probably do them no great harm, although truly parasitic in their relationship.—G. D. FULLER.

Rocky Mountain flora.—RYDBERG[24] has continued his studies of the montane regions of the southern Rockies, already noted in this journal,[25] by investigating the aquatic and grassland associations, as well as the flora of the sand hills, dry ridges, and rock slides. The plants of these habitats are listed as eastern, western, and endemic.—GEO. D. FULLER.

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[21] DIXON, H. H., and BALL, NIGEL G., A determination by means of a differential calorimeter of the heat produced during the inversion of sucrose. Notes Bot. School, Trinity Coll., Dublin **3**:121–132. 1922.

[22] RIDLER, W. F. F., The fungus present in *Pellia epiphylla* (L.) Corda. Ann. Botany **36**:193–207. *figs. 8.* 1922.

[23] MCDOUGALL, W. B., Mycorhizas of coniferous trees. Jour. Forestry **20**:255–260. *figs. 3.* 1922.

[24] RYDBERG, P. A., Phytogeographical notes on the Rocky Mountain region. X. Grasslands and other open formations of the montane zone of the southern Rockies. Bull. Torr. Bot. Club **48**:315–327. 1921.

[25] BOT. GAZ. **71**:336. 1921.

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ECOLOGICAL FACTORS IN REGION OF STARVED ROCK, ILLINOIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 299

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(WITH FIVE FIGURES)

Introduction

The work of COWLES (5) in calling the attention of the then newly differentiated science of plant ecology to the concept of plant associations as stages in successions, not as entities complete and final in themselves, but rather as steps in the evolution of social life in the plant world, was of far reaching influence in determining the development of ecology in America as a dynamic science, as the study of a progress rather than as a mere set of methods for the description of states. COWLES was also the first to emphasize the importance of topography as a general control over other factors that directly influence the activities of plants.

The earlier work in this field contented itself with pointing out the general effects of topography as a modifier, within a given region, of the climatic factors. It indicated the direction of such modifications, but only estimated their extent; it was qualitative rather than quantitative. It was, however, only natural that as time passed students of ecology should desire to gain a more exact knowledge of the factors controlling plant communities and their development, and a good share of the work now being done in American and British ecology concerns itself with the measurement of such factors as soil moisture, soil chemistry, temperature of both soil and air, evaporating power of the air, and intensity of sunlight. With the cooperation of ecological plant physiologists, a number of more or less satisfactory methods for the measurement of these factors have been evolved. Among the many workers on the problems of soil moisture, BRIGGS and SHANTZ (3) may be cited for their development of the wilting coefficient concept, BRIGGS and MCLANE (2) for the moisture equivalent idea, ALWAY (1) for the hygroscopic coefficient, and LIVINGSTON and KOKETSU (11) for the invention of the so-called soil points. Thermometric data were among the earliest to be gathered, although they are still among the least satisfactorily interpreted; special citations appear to be superfluous. Exception might be claimed for the "life zone" idea best developed by MERRIAM (15), but this is regional-climatic rather than local and topographic.

Modern ecological work in the measurement of the evaporating power of the air dates chiefly from the re-invention and popularization by LIVINGSTON (8, 9) of the porous cup atmometer. From time to time numerous attempts have been made to develop photographic and other methods for a field study of sunlight intensity, but none of them has been very satisfactory to students of plant activities. PULLING (16) gives a concise review of the work in this field. About the only instrument at present used by anyone except its own inventor is LIVINGSTON'S radio-atmometer (10), which obtains an approximate measurement of the effect of direct solar radiation on evaporation from a free water surface. Other methods for the measurement of ecological factors have some local vogue, but those here outlined are the ones most frequently used.

So widespread has been the practice of factor measurement, and so many the workers, that a complete review of the literature would be impossible in this connection, and in view of the extensive literature cited in such standard works as those of LIVINGSTON and SHREVE (12), and of CLEMENTS (14), may well be omitted.

An important consideration in the development of a successional series, but one that is not always given the prominence it deserves, is the fact that the determining conditions that permit or bar the entrance of new species in a given area are operative first on the seedling. Liminal conditions act on the infant of the race, practically always. The operation of this principle may be seen in the cultivation of adult plants outside of their natural habitats. These often thrive, but do not produce offspring. By the simple law of chance, propagules of all sorts are constantly falling into every area within the range of flight or carriage from the parent, but only where conditions are such as to permit their germination and initial growth do they become established. Once established they may weather an unfavorable season, but they cannot gain a foothold at all in a place where the conditions are unfavorable all the time. This principle recognized, it becomes at once apparent that any measurements undertaken with a view to their bearing upon succession should be made with special reference to seedling seasons and places. The first worker to use this idea as a definite basis for his investigations was FULLER (7), who made a study of the water relations of several plant associations on and near the Indiana dunes. His results indicated that the water supplying power of the soil was fairly uniform, or at least adequate for growth, throughout the season at all of his stations. On the other hand, the evaporating power of the air varied markedly, showing a pronounced correlation with the type of vegetation. The rate of evaporation bore an inverse relation to the density of the vegetation, being greatest on the cottonwood dune and least in the beech-maple forest. FULLER concluded that the differences in evaporation rates were sufficient to account for the successional range between the relative xerophytism of the cottonwood dune and the mesophytism of the climax forest.

The present problem

TERRAIN

Conditions rivalling those of the dunes in diversity of vegetational associations to be found within narrowly restricted limits exist in numerous steep-sided river gorges and their associated canyons scattered across the whole Mississippi Valley, along the edges of the various glacial drift areas, and in the unglaciated areas adjoining them. These cliffs and canyons are invariably the habitats of disjunct groups of various kinds: glacial relicts left behind by the northern retreat of the first post-Pleistocene flora, outliers from the mesophytic southeastern forests, forerunners of western and southwestern plains, and desert types. This contact of outposts of such different plant hosts is in itself an argument for the existence of notably different environmental complexes in close juxtaposition, and hence for the advantageousness of such locations as critical points in the study of the physical factors of ecology.

The data for the present study were obtained at the Illinois State Park at Starved Rock, in La Salle County, Illinois. Here, during early post-glacial times, the Illinois River cut a steep-sided trench through the St. Peter sandstone (which at this point is thrust to the surface by the La Salle anticline), and through its overlying strata of Pottsville shales and clays and blanket of glacial till. The geography of this region has been treated in detail by SAUER, and the geology by CADY (6). The sides of the trench still remain as lines of steep cliff, about a mile apart, facing each other across the floodplain of the now much shrunken river. The cliff on the south side of the river, from a point opposite the village of Utica eastward for about seven miles, is unusually precipitous and high, reaching a maximum of 157 feet from crest to mean low water level at the Starved Rock itself. It is furthermore cut into by a series of remarkable box canyons made by small tributary streams. Their sides are as precipitous as those of the cliff itself, and for the most part their bottoms are either at or near base level. Since the recession of the river (which now washes the base of the cliff only in a few limited spots, notably Starved Rock, Lovers' Leap, and Pulpit Rock) erosional débris has collected in places as talus slopes at the foot of the cliff, both within and outside the canyons. In addition, there are at the top of the cliffs the steep slopes from the top of the sandstone caprock to the general level of the upland till, and at the base a number of fragmentary river terraces of varying age, including oxbows in various stages of filling, and finally the present juvenile (and largely treeless) floodplain. A cross-section through a typical location either over the cliff side or into a canyon, therefore, would reveal the following types of terrain: (1) level upland of glacial or Pottsville clay; (2) more or less steep slope toward the edge of the sandstone cliff, also clayey; (3) exposed edge of cliff, sandy or sand mixed with clay and shale; (4) precipitous cliff, usually with weathered crevices and shelves; (5) talus slope, generally very sandy, with much humus and some clay; (6) canyon bottom (alluvial), or river terrace (alluvial to sandy), or juvenile floodplain (alluvial).

VEGETATION

With so varied a terrain as that just outlined, presenting such widely diverse types of habitat, it is only natural to expect a very widely diversified vegetational development. This expectation is well realized, for within the limits of the scant thousand acres of the Starved Rock State Park there is a collection of plants that for floristic and ecological interest can hardly be matched. All the orthodox successional stages between *Quercus macrocarpa* of the prairie edges and *Q. velutina* of the upland woods, to *Q. bicolor* of the sloughs and the *Populus-Salix* thickets of the river edge are there as a matter of course; but the region offers all the groups of disjuncts mentioned in a preceding paragraph as well. There are places in this park where one can stand beside a white pine and throw a stone through the top of a pawpaw!

No attempt can be made in this place even to outline the vegetational types to be found in this region. This has been presented briefly by COWLES (**6**), and a more detailed description by the present writer is now in preparation. The present study concerns itself more with the physical factors of the environment, especially as they affect seedling growth and hence succession, in a number of typical locations in the park. Ideally these determinations should have been made in a considerable number of places, at least three for each clearly distinguishable type of association, but this was beyond the available resources in time and apparatus. In all, seven stations were maintained throughout the major portion of one growing season, two on the upland, three on talus slopes, and two at the bottom. In locating these, some diversity was possible, overcoming in at least a slight degree the unavoidable inadequacy of data. Stations 7 and 6 were located on the upland, no. 7 on the upland proper, in a second growth *Quercus velutina-Q. alba-Carya ovata* association, and no. 6 on a gentle slope from this toward the edge of the cliff (but away from the exposed sandy terrain), in a second growth *Quercus rubra-Q. alba* association, with considerable undergrowth of *Cornus*, *Viburnum*, and seedlings of *Prunus*, etc. Stations 5, 3, and 2 were located on talus slopes, no. 5 about one-third of the way down one, on the face of the cliff, and nos. 3 and 2 near the tops of slopes on opposite sides of Hennepin Canyon. The tree growth at station 5 was predominantly *Quercus rubra*, with some *Q. alba*, *Juglans cinerea*, and *Tilia americana*, and a scattering of other mesophytic hardwood species. The shrubby undergrowth consisted mostly of *Hamamelis virginiana*, and the herbaceous situation was dominated by a magnificent "fernery" of *Osmunda claytoniana*. The soil here was a sand rich in humus, but with little clay. Station 3, within the canyon, was on a rather newer slope, the soil containing a considerable proportion of clay. The tree growth consisted largely of *Prunus serotina*, and there was a dense undergrowth of *Psedera quinquefolia*, *Ribes cynosbati*, *Lonicera Sullivantii*, and *Hydrangea arborescens*. There was also a fair development of mesophytic herbs. Station 2 was on a slope that was newer still. The soil was sandier than on station 3, but raw and poor in humus. It was full of stones, with large, moss-covered rocks protruding. There were fewer trees, and these were mostly young and small. A very considerable growth of *Hamamelis virginiana* was present, but few other shrubs, and almost no herbs. These three stations represented fairly well the state of the more mesophytic talus slopes. Stations 4 and 1 were on representative bottomlands, no. 4 being on the river floodplain and no. 1 on the floor of the canyon. The soil in both places was a black alluvium. The association at station 4 was dominated by *Acer saccharinum* and *Ulmus americana*, with many seedlings and saplings. Other undergrowth was mostly herbaceous, with *Laportea canadensis*, *Boehmeria cylindrica*, and *Campanula americana* as the conspicuous species. The alluvial floor of the canyon, where station 1 was located, bore an association of *Ulmus*, with some admixture of *Juglans*, *Fraxinus*, *Populus*, *Salix*, etc. Seedlings and young trees were numerous, and there was a fair amount of undergrowth, consisting largely of *Sambucus canadensis*, and at one place a notable thicket of *Euonymus*. There was an exceedingly rich, moist-mesophytic herbaceous flora, typified by *Impatiens pallida*, *Pilea pumila*, *Campanula americana*, and *Lobelia syphilitica*. Lianas were abundant, more so than at station 4. In general, this was by far the most mesophytic of all the stations. These three groups of stations were thus fairly typical of upland, slope, and bottomland respectively, and represented the principal plant associations reasonably well.

Instruments and methods

An effort was made at each station to secure some measurement of each of the following physical factors: soil moisture, evaporating power of the air, intensity of solar radiation (in terms of its effect on evaporation from a free water surface), and temperature of soil and air.

SOIL MOISTURE.—Soil moisture data were obtained by (1) the usual determination of the oven-dry weight percentage in samples of about 200 gm. each, compared with the wilting coefficients, as derived by the indirect method of BRIGGS and SHANTZ; and (2) the "soil point" method of LIVINGSTON and KOKETSU. All data were obtained for a level of approximately 7.5 cm. below the soil surface. This was the shallower of the two depths used by FULLER in his dune studies, and represents the level at which most seedlings begin their adventures. The soil points were used in sets of four, and results recorded as the average, to eliminate as far as possible the errors due to variability in instruments and soil. Holes were dug to the proper depth with a trowel, care being taken that none of them was within one-half meter of any hole in the same set or any previous set. As a rule, soil from the holes into which the points were set was taken for use in the moisture percentage determinations. From April 30 until July 1 it was possible to visit the stations only three or four times a month. From July 1 until September 20 determinations were made every forty-eight hours, except when rain intervened.

EVAPORATING POWER OF AIR AND SUNLIGHT INTENSITY.—Data on the evaporating power of the air and on the effect of direct solar radiation on evaporation rate were obtained by means of a pair of standard Livingston spherical atmometer cups at each station.

These were equipped with the rainproofing valves described by LIVINGSTON and THONE (13). Their reservoir bottles were partly buried, so that the cups were about 20 cm. above the ground level. From April 30 until July 1 readings were made weekly or fortnightly, during the month of July they were made daily, and from August 6 until September 20 they were made every two days. All readings, after correction, were reduced to mean daily rates for ten-day periods.

TEMPERATURE.—Air temperatures were obtained from a Sixe-type minimax thermometer at each station. The thermometers were placed at the same level as the atmometers. No artificial shelter was used except at station 1, since there was sufficient natural cover to protect the instruments from direct insolation at all the other stations, and even at the latter place the growth of the *Impatiens* thicket soon made artificial shelter unnecessary. Readings were made on the same schedule as that used in the atmometer observations. The stations were visited each day just before the period of maximum temperature, so that the maxima and minima for the preceding twenty-four or forty-eight hour period were obtained.

Soil temperatures were obtained by means of test-tubes sunk into the soil to a depth of 10 cm. The lower end was kept filled with water, and the tube kept stoppered. When an observation was to be made, a thermometer was lowered into the tube until the bulb was immersed in the water, and read after sufficient time had been allowed for an equilibrium to be reached. Since the readings were always made shortly before the period of maximum air temperature, it may be assumed that the soil temperatures thus obtained were a little below the maximum for the day. Since, however, the total diurnal fluctuations in soil temperatures are known to be small, and especially since in the present study they seemed in the end to have no particular significance, this source of inaccuracy, as well as the rather crude method employed for obtaining the data, may be overlooked.

For both air and soil temperatures, it may be remarked that the figures up to July 1 can have but little significance, since they apply to periods of seven or fourteen days. During July, of course, true diurnal data were obtained. From August 6 until September 20 readings were made every forty-eight hours.

Discussion

SOIL MOISTURE (figs. 1, 2).—It is obvious that topography cannot affect the availability of soil water for plant growth until two other factors have operated. The first of these, the amount and distribution of precipitation, is climatic, and thus affects all locations about equally. The second factor (or more properly group of factors) is strictly edaphic, having to do with the effect of the mechanical makeup of the soil (size, arrangement, and packing of soil particles) upon the capacity of the soil to absorb and hold precipitation water, and to deliver it to the roots of the plants when they demand it. Finally, the topography is of importance in its effect upon such factors as run-off, subsurface drainage, and exposure to factors that influence evaporation, both from the surface of the soil itself and through plant transpiration.

The effects of the climatic factors are plainly evident in the general conformity of all the curves in the soil moisture graphs, both those representing the growth water (fig. 1) and those representing the results of the operation of the Livingston-Koketsu soil points (fig. 2). After the cessation of the spring rains in early June, and until their resumption in early September, the season was one of the hottest and driest summers on record in recent years. There was but one brief period of precipitation, heavy rains occurring during the first few days of August. The soil water percentages show a notable correlation between the rainfall distribution and the water content of the soil at all the stations. Beginning with moderate but varying amounts during the late spring rains, all stations showed a falling off throughout June, increasing in amount and rate of loss through the July drought, and ending in a sudden increase at the time of the early August rain. Following this was another sharp decrease throughout August, until a period of rains in early September brought a rise, somewhat resembling that of early August, but less in amount. Fair weather during the last observation period of the season brought the beginning of another decrease. The ready responses of the curve to both drought and rain are very notable features.

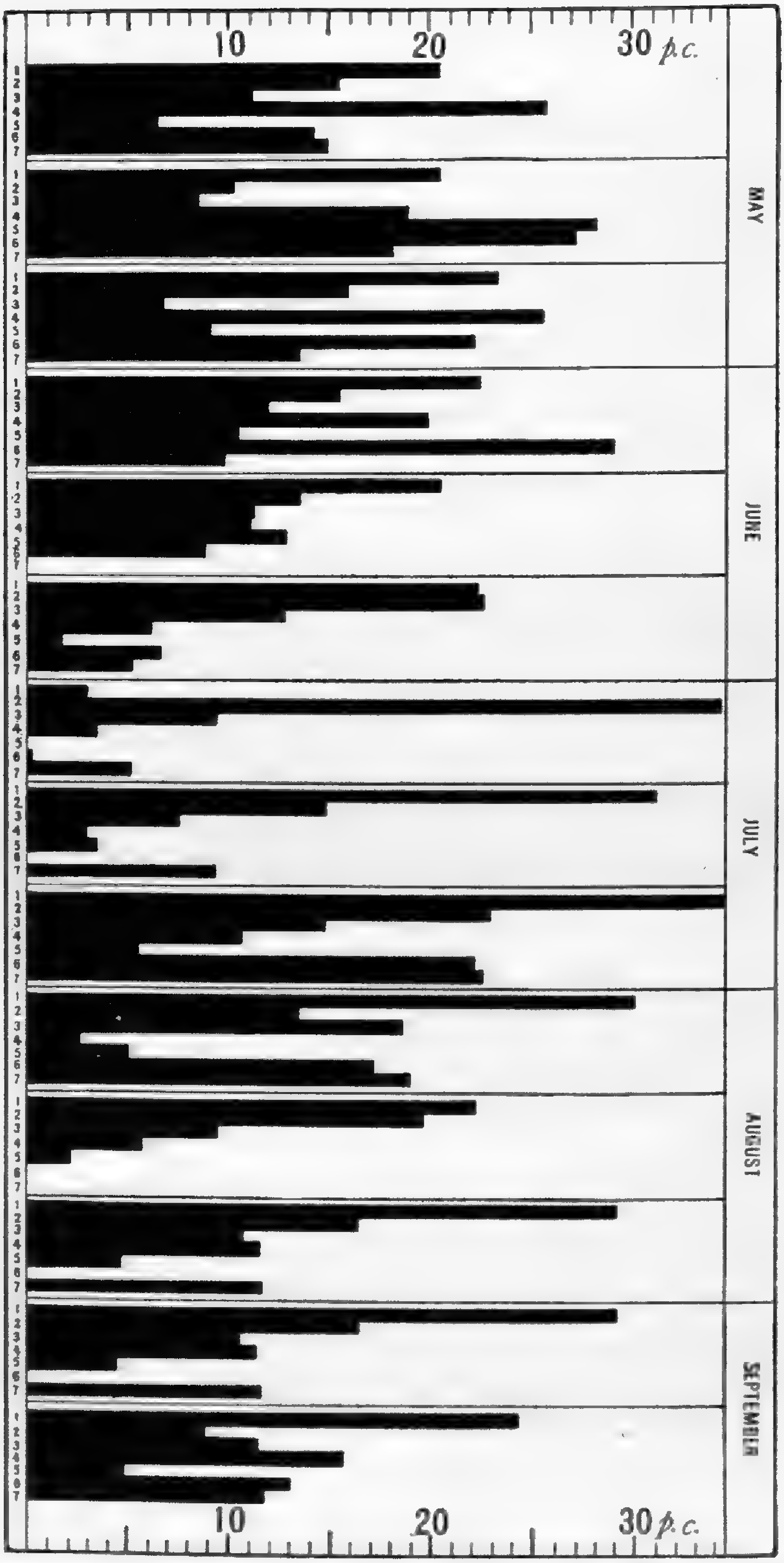

FIG. 1.—Percentages of growth water present in soil at seven representative stations, during ten-day periods from May 1 to September 20.

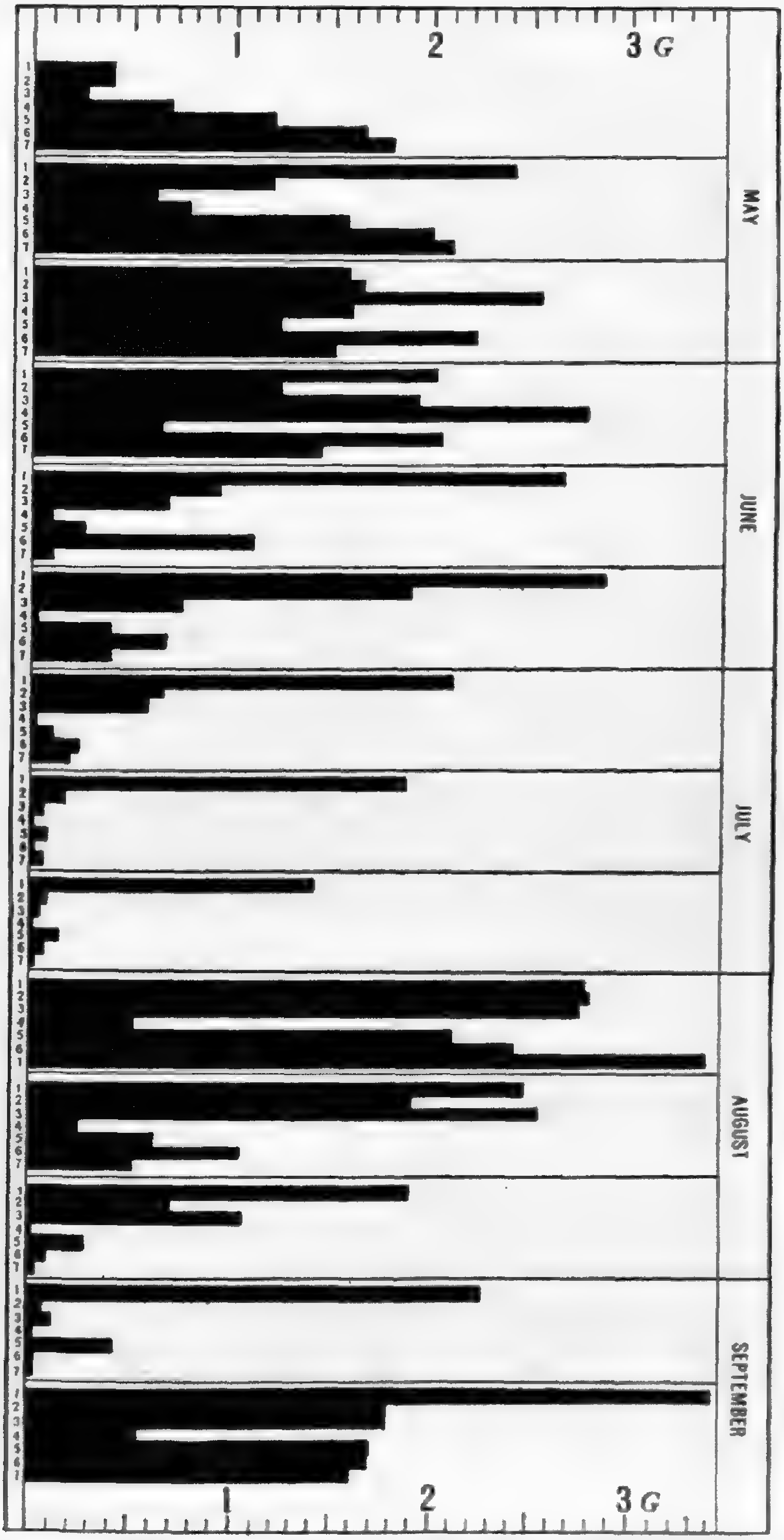

FIG. 2.—Amount of absorption in gm. per two-hour period by Livingston-Koketsu soil points at seven representative stations, during ten-day periods from May 1 to September 20.

The same fluctuation in the water-supplying power of the soil, as shown by the Livingston-Koketsu soil points (fig. 2), is even more marked. Both the individual determinations and their ten-day means show fluctuations closely parallel to those of the growth water percentage data. The close "bunching" of the ten-day means during July and the period ending September 8 is especially suggestive. Since the soil point method was devised in an effort to determine directly the water-supplying power of any kind of soil, independently of its wilting coefficient or any other physical constant, this shows clearly that during such periods of drought all the soils in the locations studied, save one, dropped to a very critical water-delivering power. In the Livingston-Koketsu experiments with wheat and *Coleus* under winter greenhouse conditions, permanent wilting ensued when the water-delivering power of a soil had fallen to a point between 0.04 and 0.11 gm. per two-hour period (as compared with about 15.0 gm. for the same period in a nearly saturated soil). The soil points used in the present determinations, however, have a somewhat greater absorbing power. According to data as yet unpublished, obtained by H. C. DIEHL, the new points have 1.25 times the absorptive power of those used by LIVINGSTON and KOKETSU. The water-delivering power of the soil at the permanent wilting point should therefore be between 0.05 and 0.14 gm. for a two-hour period. This is the case, at least, if we do not take into account the evaporating power of the air at permanent wilting. Since, however, the evaporating power of the air throughout the droughty periods was greater at the stations considered than it was in LIVINGSTON'S greenhouses, we are safe in doing so, and in taking as an approximate water-supplying power at wilting point 0.15 gm. for the two-hour period. It will be seen that during the drought periods all of the stations save one either approached or passed this critical point, and that several of them were well beneath it for a period of thirty days during July and one of twenty during August, with an interval of only ten days between these two prolonged droughts. Further, a comparison with figs. 3 and 5 will show that these were the periods of greatest stress from extremes of temperature and evaporation. It is fairly evident, therefore, that seedlings that are to survive throughout most of the park must be of species able to get an early start or make sufficiently rapid growth to have well established root systems before the advent of the usual summer droughts.

A more detailed examination of individual stations serves to emphasize the facts already noted in general, and also brings out several edaphic phenomena of considerable interest. It is here that topograph factors appear to function. Thus, station 1 is the least exposed, and also the least well drained, being on the flat floor of a canyon. It is also subject to flooding when there is heavy rain, and the run-off from the tributary gullies comes over the canyon falls. It is not surprising, therefore, to find that it is constantly well above both the wilting coefficient and the Livingston-Koketsu wilting point. It is not surprising, either, to find here the largest number of young seedlings, and a herbaceous vegetation dominated by annuals. On the other hand, the highest upland station, no. 7, is both well drained and quite exposed; in correlation therewith it rapidly loses what water it gains, and persistently holds a place near the bottom of the column during the droughty periods. Few seedlings develop, and the herbaceous vegetation consists largely of grasses and sedges and of perennating prairie plants. The stations on the more or less sloping terrain (no. 6 on the upland and nos. 5, 3, and 2 on talus slopes) present phenomena intermediate between these extremes.

Station 4, on an alluvial flat beside the river, in a maple-elm-ash association, presents an anomalous situation. Starting in the spring with a moderate amount of soil water, it loses rapidly and fails to recover, during the summer rains, to anything like the degree displayed by the other stations. The soil point data confirm its bankruptcy. After it has lost its water during June, it falls to the bottom of the heap during the droughty periods and stays there more persistently than any other station. During the rains, when the others rapidly increased in their water-supplying power, this station rose but little, and then quickly dropped to the bottom again. This behavior may be accounted for in several ways. The soil here is a heavy, silty alluvium, very tenacious of the water it gets. It does not receive the benefit of periodic flooding, as does station 1. The thicket of saplings is so dense that only the heaviest rains ever penetrate the foliage sufficiently to wet more than the surface of the ground. A large proportion of the soil surface is bare, puddling easily, and thus facilitating direct evaporation. These, and possibly other unnoted factors, can account for the condition here.

EVAPORATING POWER OF AIR (fig. 3).—The effect of the climatic factors that influenced soil moisture conditions is plainly evident in the atmometric data also. There was more rainy and cloudy weather during May than during June, and the rates of evaporation show a corresponding decline during the latter month. Then, with the beginning of the hot, dry weather of July the rates increased suddenly and enormously. There was a sudden drop after the oncoming of the early August rainy period. Throughout August the daily temperatures were markedly lower than those for July (fig. 4), with much cloudy weather, although actual precipitation was confined to brief local showers; with this was correlated a reduction in the transpiration curves to one-half or less of their July heights. A short heated period at the beginning of September brought an increase in the rate, but the ensuing cool, humid days that closed the period of observation brought the curve down, and the season came to an end with a period of very low transpiration rates.

A feature of some interest in the set of atmometric curves is presented by the deep "dip" during the month of June. It was natural that evaporation rates should be higher during July, as already pointed out, but the sharp decrease from the May rates seems at first somewhat anomalous. One possible reason suggests itself in the comparative amounts of general exposure due to the foliation of the trees. The spring of 1921 was late and cool, and during May the trees, especially the oaks, were still nearly naked. As the foliation increased, changes in three of the four main factors controlling evaporation might naturally be expected; direct insolation and air movement would be reduced, and relative humidity probably increased. All of these changes would be in favor of reduced evaporation. The fourth main factor, temperature, increased somewhat during this time. This would have worked in the opposite direction, but presumably the operation of the other three had the greater effect.

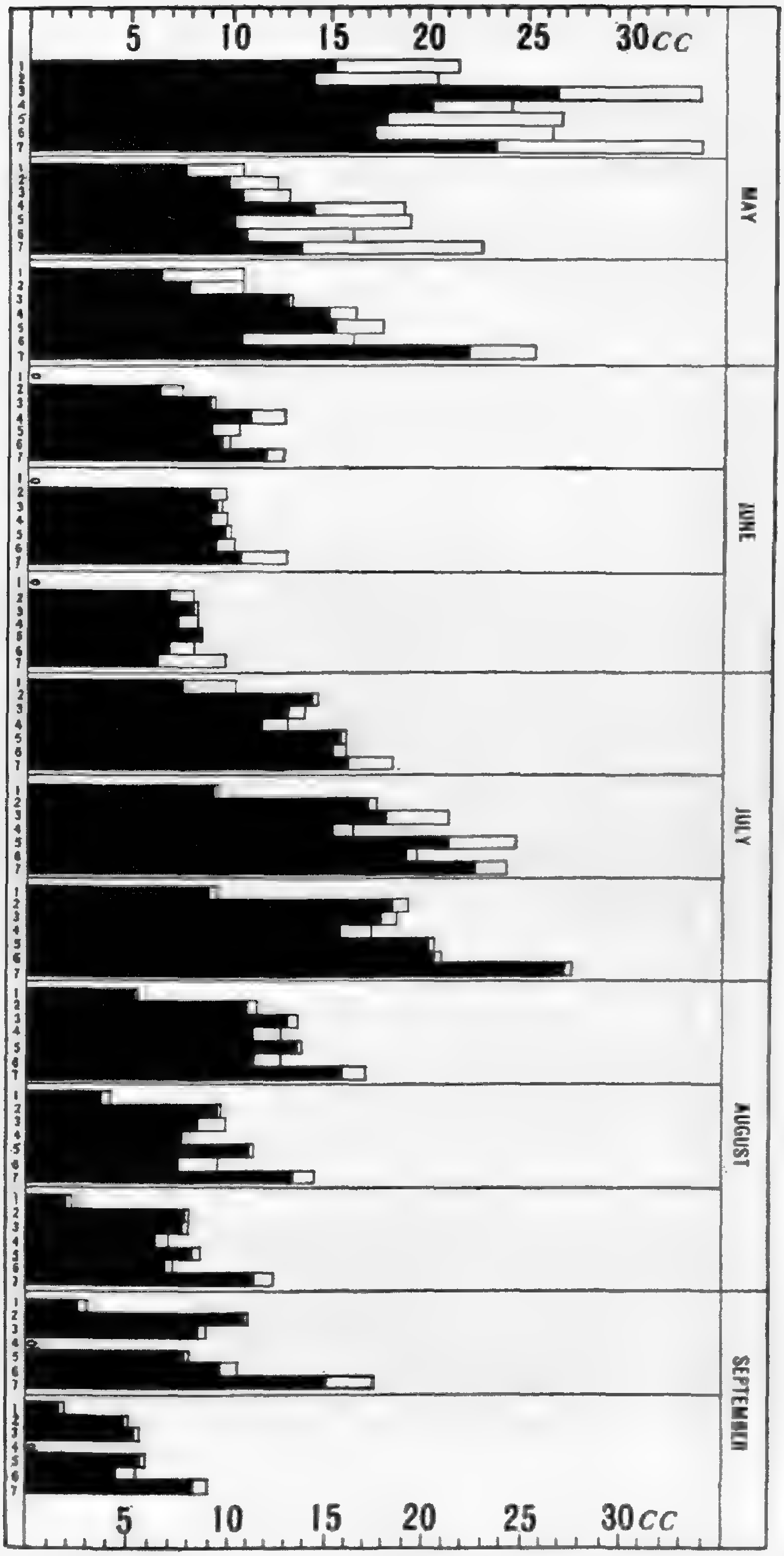

FIG. 3.—Mean daily amounts of evaporation in cubic centimeters from standard spherical black and white atmometers; black columns indicate simple atmometric effect; white extensions, increased evaporation due to direct insolation; a zero at base of column signifies data lacking for the period.

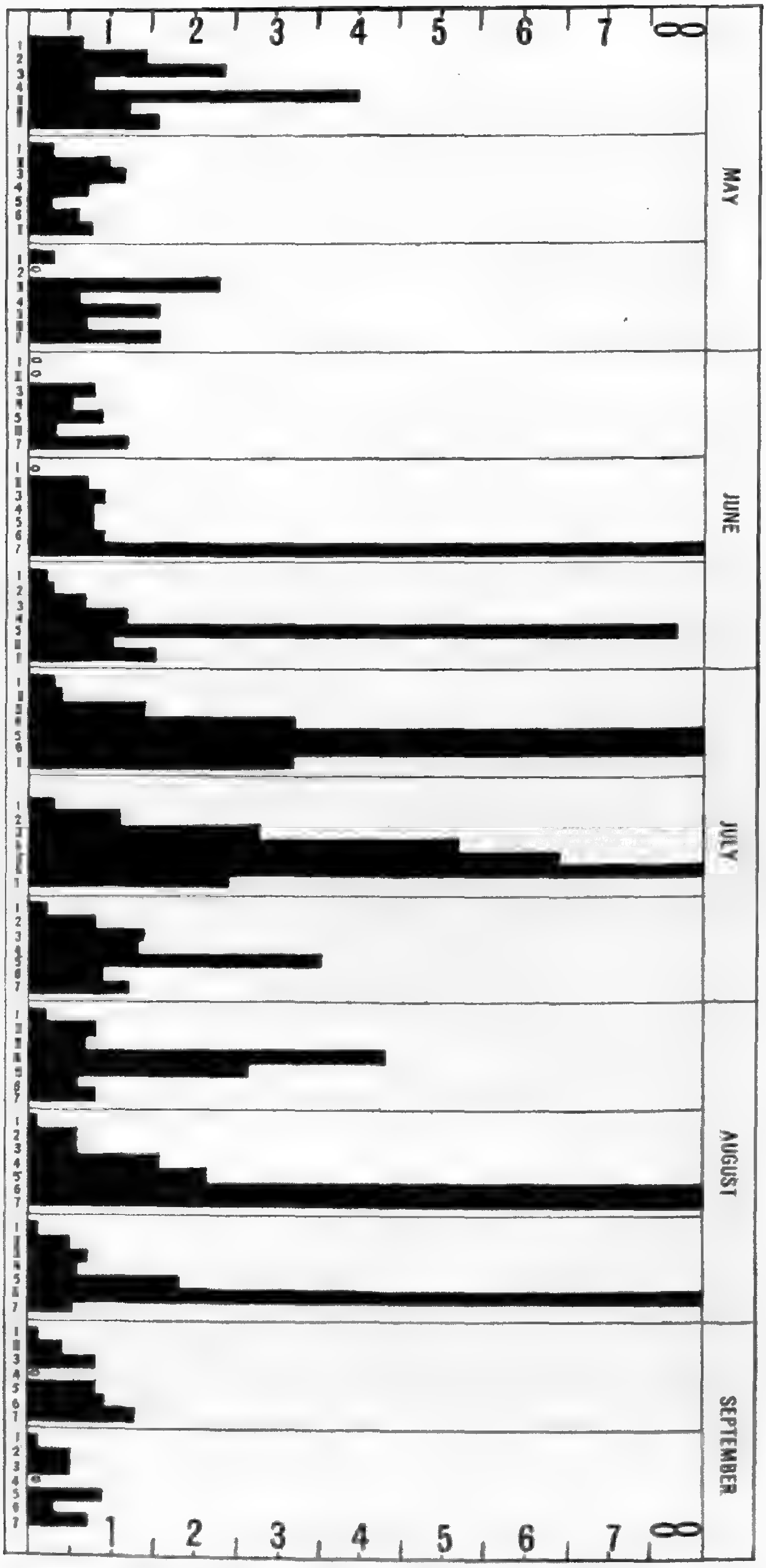

FIG. 4.—Relative xerophytism (ratio of evaporation rate to growth water) at the seven stations; a zero at base of column signifies data lacking for the period.

An examination of the differences between the curves of the separate stations brings to light certain topographical correlations. In the first place, there was a general correlation between the exposure of a station and its evaporation rate. Thus, station 7, the most exposed of the set, shows consistently the highest evaporation rates, while station 1, the least exposed, shows consistently the lowest. Even between these extremes the correlation holds, for of the three talus slope stations, no. 5, the most exposed, shows higher rates than nos. 3 and 2, which were partly sheltered within the canyon.

Again, the more exposed stations showed much more marked variation, both seasonal and diurnal, than did the more sheltered stations. The highest mean daily rate for a ten-day period (26.9 cc. per day, July 21–30) exceeded the lowest mean daily rate (8.1 cc. per day, September 9–20) by 18.8 cc. This may be compared with the excess of the maximum mean daily rate at station 1 (9.2 cc., July 21–30) over the minimum mean daily rate (1.3 cc., September 9–20), which is 7.9 cc. The excess at station 7 is 10.9 cc. greater than the excess at station 1, or a ratio of 2.4. Another notable thing is the relative stability of the daily rates at the more sheltered stations as compared with the greater fluctuation at the more exposed stations. Thus, at the two stations and for the two periods noted, the greatest mean daily rate at station 7 (26.9 cc.) exceeds the greatest mean daily rate at station 1 (9.2 cc.) by 17.7 cc., whereas the least mean daily rate at station 7 (8.1 cc.) exceeds the least mean daily rate at station 1 (1.3 cc.) by only 6.8 cc. The difference during the period of highest evaporation rate is 2.6 times as great as the difference during the period of lowest evaporation rate. The general effect of this factor is strikingly brought out by the "bunching" of the curves during June and September, the low evaporation periods. This greater variability displays itself even more markedly, of course, in the diurnal variations than in the variations of mean daily rates. During the period of high evaporation just cited, for example, the readings at station 7 on three successive days (July 14, 15, and 16) were 31.8 cc., 8.9 cc., and 19.1 cc. respectively, while on the same days readings at station 1 were 9.8 cc., 3.8 cc., and 10.4 cc. Similarly, even during a period of low evaporation, three successive readings at station 7 give 15.3 cc., 5.5 cc., and 11.6 cc., while at station 1 the corresponding readings are 4.0 cc., 1.4 cc., and 1.8 cc. Examination of the data from stations of intermediate exposure will show correlated results.

The ratios between growth water and evaporation rates, presented in fig. 4, serve to emphasize and render definite the ideas in the foregoing paragraphs. Of course, as FULLER (7) has pointed out, this ratio is a more or less artificial and arbitrary one, yet it is as good a method as we have at present for a quantitative statement of the relative xerophytism of different habitats, and inasmuch as it brings out strongly the cumulative effect of two factors operating in the same direction, it is of value.

The foregoing considerations of the atmometric and soil moisture data suggest strongly the essentially prairie-like summer conditions, even in this woodland island in the prairie, and the intensification of the summer xerophytism of the soil by the summer xerophytism of the air. The greatest evaporating power of the air prevails precisely during those periods when the plants are least able to obtain water from the soil to satisfy it. Summer rains relieve the tension, to be sure, but even after heavy rains the relief is short-lived, and slighter in degree than might be supposed. Only in places at once well endowed with a supply of water and at least fairly well sheltered from extreme transpirational conditions (represented in the present study by station 1) is there any chance for germination and growth of seedlings during the middle of the growing season. This theoretical conclusion is borne out by the existence in such places of annuals as the dominant herbaceous vegetation, and by the presence of large numbers of tree seedlings. Furthermore, it is here also that one finds "superclimax" trees, like *Acer saccharum* and *Asimina triloba*, belonging normally to more mesophytic regions. In the more exposed stations (the extreme being no. 7) the unfavorable summer conditions set in so early and become so severe as to discourage seedling growth. The canyon bottoms therefore receive strangers hospitably and permit of relatively rapid succession, while the exposed uplands conservatively cling to the climax vegetation they have, and permit, in places too xerophytic even for this, the survival of relict communities, like the conifers on the edges of the cliffs.

Insolation effects.—An examination of the radio-atmometric data serves to emphasize the atmometric phenomena already noted, in addition to its main purpose of getting some idea of the sunlight intensity as this affects evaporation. The evaporation from the black cups of course follows the same general seasonal curve as that from the white, the excess varying according to seasonal and local conditions. The radio-atmometric effect, as one might expect, was greatest during the season of greatest exposure, that is, during the month of May, before the leaves were on the trees. After the first of June the effect was much less marked. Even during the hot weather of July the evaporation rate from the black cups exceeded that from the white by but little.

The general differentiation in the radio-atmometric effect with the development of the foliar screen was accentuated by local variations. Thus, the tendency throughout the season was for a greater difference at station 7, the most exposed of the set, located in an open stand of second growth oak. Station 1, located on a treeless portion of the canyon bottom, in a stand of *Impatiens pallida*, showed a most notable radio-atmometric effect during May, before the surrounding herbage was well grown; after July 1 the effect fell almost to zero, and remained so throughout the rest of the season.

Of course it is not possible to take the radio-atmometric effect as a measure of solar radiation in all its effects on plants. It is intended only as an approximate determination of direct solar radiation on the evaporation of water from a free surface. Taking it as a rough index of the total illumination received, however, we find that during the season when other ecological factors are favorable for the growth of seedlings on the forest floor it is at its maximum, and that it falls off notably after the leafing out of the trees. Presumably then, sunlight conditions conspire with the ecological factors already discussed to make late spring the optimum season for seedling growth, and that at places like station 1, where other conditions are favorable even when they are most unfavorable elsewhere, sunlight intensity falls to an unfavorable level after about July 1, or possibly even earlier. It must be emphasized again, however, that so little is known about the sunlight relations of plants that attempts at close correlation are for the present unprofitable.

TEMPERATURE (fig. 5).—Temperatures of both air and soil show similar seasonal variations, with high points in July, and a falling off in August and September, interrupted by a brief period of high temperatures during the first few days of the latter month. The figures up to July 1 can be accepted only as approximations, since they are absolute maxima and minima for periods of more than a week, instead of mean daily maxima and minima. For this reason too much significance must not be attached to the greater spread between maxima and minima. Although a greater spread did undoubtedly exist, it probably was not so great as the thermometer readings at the ends of the periods would indicate.

So far as any significance may be attached to the figures before the period of daily readings began, the influence of the diminution of general exposure through the leafing of the trees seems to be at work here also, for during May the maxima both for air and soil show as marked a spread as that shown for the warmer months of July and August. This spread is especially noticeable in the data for soil maxima,[1] inasmuch as it amounts to eight or ten degrees throughout May, falls to a point at one of the June readings, and for the rest of the season never exceeds three degrees. These results agree fairly well with those of MCDOUGALL (14), who found a consistent seasonal variation of about 4° F. between the soil temperatures of typical upland and lowland stations in Illinois forests.

Topographical differences seem to have some influence also, although the correlation is not so clear here as it is in the case of the atmometric and radio-atmometric data. It may be remarked, however, that the high lying stations (typified by no. 7) show the highest maxima both for air and soil, and the low lying stations the lowest maxima. On the other hand, the lowest minima for the air are obtained at the high lying stations and the highest minima at the lower ones. This may be due, among other things, to the denser leaf covering and the greater amount of underbrush at the

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[1] Because of the close agreement between the readings for all the stations after July 1, it is not thought worth while to present the soil temperature data in detail.

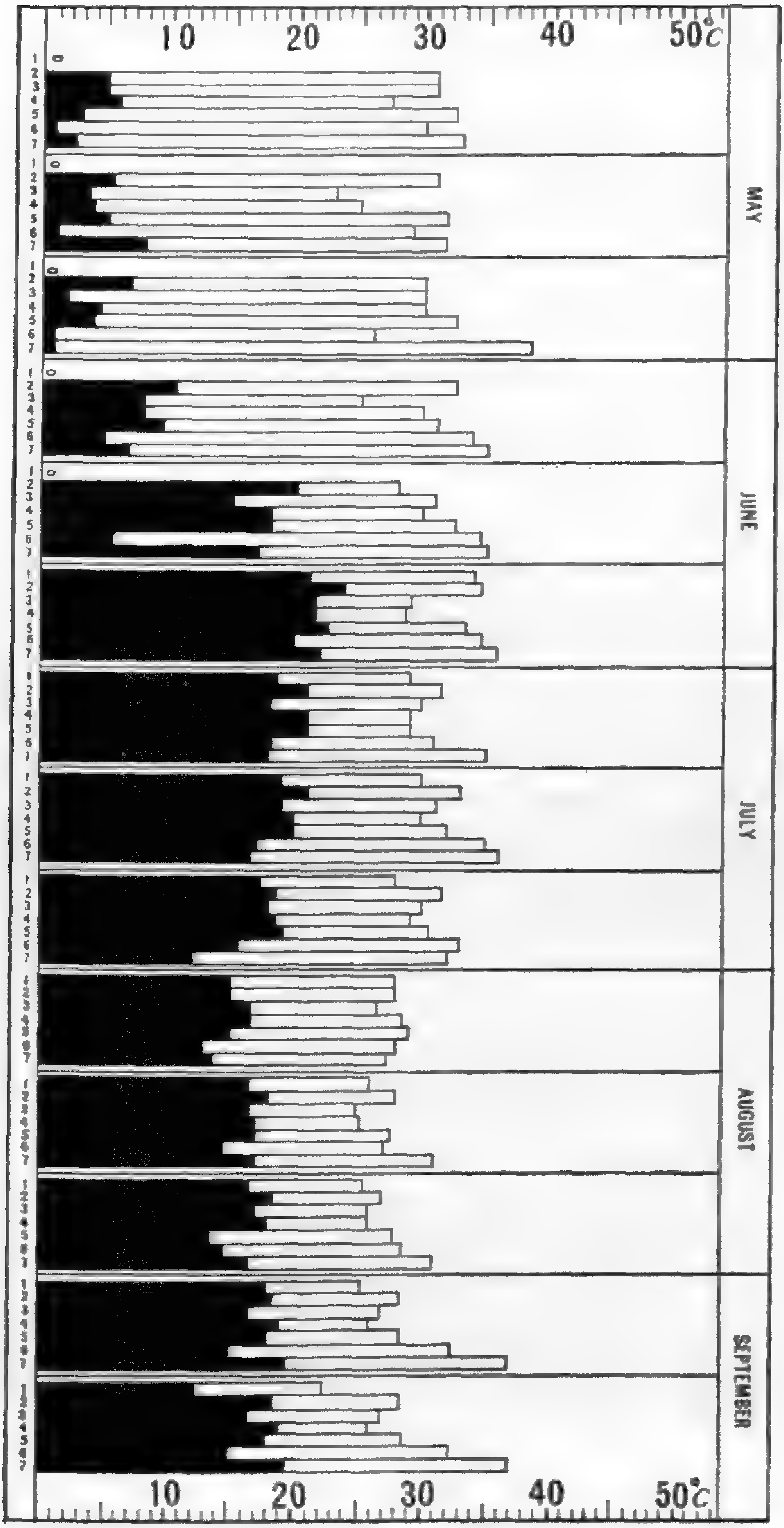

FIG. 5.—Mean daily maximum and minimum air temperatures at the seven stations; a zero at base of column signifies data lacking for the period.

lower stations, holding the lower stratum of air to a certain extent against displacement by air drainage.

One object in obtaining these temperature data was to ascertain whether temperature differences might be correlated with the invasion of lower latitude plants, like *Asimina triloba*, into the lower levels. The data obtained, however, are contradictory. The mean temperatures are undoubtedly higher at the higher lying stations, the last points of invasion, and also the last points of cession by relict northern species. On the other hand, the consistent higher minima at the lower levels might well permit a slightly longer growing period, and perhaps even milder winter conditions. Otherwise stated, the temperature optimum for southern species might not be so nearly approximated on the lower stations as at the higher, but a point above the minimum might be maintained throughout a longer period each year. The main reason for the confinement of southern invaders to the bottom lands, however, must probably be sought in the more favorable moisture conditions at these levels. The writer does not feel that attempts at close correlations between temperature and vegetation over such small differences in altitude would be very profitable at the present stage of development in ecological science. It is interesting to find, however, that fairly consistent small differences in temperature do coexist with small topographical differences.

Summary

1. This paper is a study of the ecological factors at seven representative topographical points in the Illinois State Park at Starved Rock, during the growing season of 1921. The factors studied were (*a*) soil moisture, (*b*) evaporating power of the air, (*c*) evaporating power of solar radiation, and (*d*) temperature of air and soil.

2. Observations were taken with special reference to their influence on seedling growth, because of the importance of the latter as a factor in succession.

3. Soil moisture was found to vary (*a*) seasonally, falling off after the close of the spring rains and reaching a point below the minimum necessary for plant growth during a considerable portion

of the summer, and rising again with the beginning of the fall rains; (*b*) according to the mechanical composition (and therefore retentivity) of the soil; (*c*) to a minor extent according to topography; and (*d*) according to the density of the foliage canopy.

4. The evaporating power of the air was found to vary (*a*) seasonally, increasing until midsummer and falling off afterward; (*b*) according to the state of tree foliation, declining after the forest had become completely clothed; and (*c*) topographically, being greatest for the same period in exposed stations and least in sheltered ones.

5. The evaporating power of solar radiation was found to vary in the same manner as the evaporating power of the air, complementing and emphasizing the data under the latter head.

6. Maximum temperatures were found to vary in much the same manner as the evaporating power of the air. Minimum temperatures of the air were found to be affected by topography in a mode inverse to that of the maxima, being highest at the low lying stations and lowest at the higher lying ones.

7. Certain vegetational phenomena showed a general correlation with the instrumental observations: (*a*) the density of ground cover, number of tree seedlings, and proportion of annuals in the total vegetation of any given association bore an inverse relation to the relative xerophytism; (*b*) in all but one of the stations, conditions were favorable for the development of seedlings only in spring and fall; (*c*) in the climax forest for the region (upland oak woods) the water-supplying power of the soil consistently fell nearly or quite to zero during the summer drought period; (*d*) the location of "subclimax" and "superclimax" associations showed closer correlation with water relations than with temperature.

The writer owes a debt of special gratitude to Professors H. C. Cowles and G. D. Fuller of the University of Chicago for their encouragement and assistance in the preparation of the data here presented. The courtesy of the Illinois State Park Commission should also be acknowledged.

University of Chicago

LITERATURE CITED

1. Alway, F. J., Studies on the non-available water of the soil to the hygroscopic coefficient. Res. Bull. 3, Agric. Exp. Sta. Nebr. 1913.
2. Briggs, L. J., and McLane, J. W., The moisture equivalents of soils. U.S. Dept. Agric., Bur. Soils, Bull. 45. 1907.
3. Briggs, L. J., and Shantz, H. L., The wilting coefficient for different plants and its indirect determination. U.S. Dept. Agric., Bur. Plant Ind., Bull. 230. 1912; Bot. Gaz. **51**:210–219. 1911; **53**:20–27; 229–235. 1912.
4. Clements, F. E., Plant succession, an analysis of the development of vegetation. Carnegie Inst. Wash., Publ. 242. 1916.
5. Cowles, H. C., The ecological relations of the vegetation of the sand dunes of Lake Michigan. Bot. Gaz. **17**:97–117; 169–202; 281–308; 361–391. 1899.
6. ———, Part III, Botany. In Sauer, Cady, and Cowles, Starved Rock State Park and its environs. Geog. Soc. Chicago, Bull. 6. 1918.
7. Fuller, G. D., Evaporation and soil moisture in relation to the succession of plant associations. Bot. Gaz. **53**:193–234. 1914.
8. Livingston, B. E., Atmometry and the porous cup atmometer. Plant World **18**:21–30; 51–74; 95–111; 143–159. 1915.
9. ———, Atmospheric influence on evaporation and its direct measurement. Monthly Weather Rev. **43**:126–131. 1915.
10. ———, A radio-atmometer for measuring light intensity. Plant World **14**:96–99. 1911.
11. Livingston, B. E., and Koketsu, R., The water-supplying power of the soil as related to the wilting of plants. Soil Science **9**:469–485. 1920.
12. Livingston, B. E., and Shreve, F., The distribution of vegetation in the United States as related to climatic conditions. Carnegie Inst. Wash., Publ. 284. 1921.
13. Livingston, B. E., and Thone, F., A simplified non-absorbing mounting for porous porcelain atmometers. Science **52**:85–87. 1920.
14. McDougall, W. B., A comparison of soil temperatures in upland and bottomland forests. Trans. Ill. Acad. Sci. **13**:249–254. 1920.
15. Merriam, C. H., Laws of temperature control of the geographic distribution of terrestrial animals and plants. National Geog. Mag. **6**:229–238. 1894.
16. Pulling, H. E., Sunlight and its measurement. Plant World **22**:151–171; 187–209. 1919.

NOTES ON NEOTROPICAL ANT-PLANTS

I. CECROPIA ANGULATA, SP. NOV.

I. W. BAILEY

(WITH PLATE XV AND EIGHT FIGURES)

Introduction

In a previous paper (1) the writer discussed the significance of the anatomical peculiarities of a number of Ethiopian ant-plants. So many features of unusual interest were encountered in studying these plants, that it seemed desirable to extend the scope of the investigation, and to include certain neotropical myrmecophytes for comparative purposes. With this end in view, the writer spent the summer of 1920 at William Beebe's Tropical Research Station in British Guiana, where the following ant-plants (*Tococa aristata* Benth., *Triplaris surinamensis* Cham., *Tachigalia paniculata* Aubl., *Cordia nodosa* Lam., and *Cecropia angulata*, sp. nov.) grow in close proximity to the laboratory.

Since the publication of SCHIMPER'S (8) much quoted investigations, *Cecropia adenopus* Mart. has been considered one of the most classical illustrations of myrmecophytism. SCHIMPER interpreted the "Müllerian food bodies" and the "prostomata" of this ant-plant as adaptations for enlisting the services of an aggressive army of Aztecas, which protect their host against the attacks of the destructive, leaf cutting, Attine ants; a conclusion that has been assailed by VON IHERING (4), ULE (9), RETTIG (7), FIEBRIG (3), and other critics of the BELT-DELPINO theory of myrmecophily. In view of the important rôle that has been assigned to *C. adenopus*, in discussions concerning the significance of ant-plant symbioses, the writer welcomed the opportunity of studying a somewhat similar species of this interesting genus. The results of the investigation are summarized in the following pages.

Taxonomy

One of the first difficulties encountered in studying a neotropical biocœnose, in which representatives of the higher plants, ants, coccids, and other insects are closely associated, is the identification of the organisms concerned in the complex. Of course, the principle of significant figures must be considered in biology, as in physics and mathematics. The most detailed field observations and carefully planned experiments may lose much of their significance if an investigator fails to secure adequate information concerning the identity of the plants and animals with which he is working. In most cases, therefore, it is advisable to prepare museum specimens which may be preserved as a record for verification by other investigators. Typical herbarium specimens of all of the plants to be discussed in this and in subsequent papers have been deposited in the Gray Herbarium of Harvard University. Specimens of the insects have been preserved in the collections of Professor W. M. WHEELER, who collaborated with the writer in the investigation of the myrmecophytes of the Kartabo region. The ants were identified by him, the coccids by Mr. HAROLD MORRISON of the United States Bureau of Entomology, and certain parasitic Hymenoptera by Professor C. T. BRUES.

In dealing with *Cecropia*, one is concerned with a group of plants which present many taxonomic difficulties. The leaves of mature plants frequently are much too large for herbarium sheets of standard dimensions, and the inflorescences are difficult to season for museum purposes. Thus many of the descriptions of species are based upon the study of more or less fragmentary material. Furthermore, there appears to be as yet no consensus of opinion as to which of the foliar and floral characters are of the greatest diagnostic value. In view of these facts, the writer devoted considerable attention to the investigation of the variability of the morphological characters of the Kartabo species of *Cecropia*.

There proved to be two distinct species, a myrmecophytic species, with a well developed trichilium and numerous food bodies, and a non-myrmecophytic species, which is entirely devoid of these structures. The latter is considered by Dr. E. H. SNETHLAGE to be a variety (*decurrens*) of *C. sciadophylla* Mart. The former does not agree with any previously described species, and owing to its association with ants, was studied more intensively.

During the earlier juvenile stages the leaves of this species are small, ±6 cm. long, ±2 cm. wide, simple, lanceolate, finely serrate, pilose above and densely albido-tomentose below. The subsequently formed leaves increase rapidly in size, forming first three, then five, and ultimately nine to eleven lobes (text figs. 1, 2). As the juvenile leaves develop lobes, they lose their marginal serrations and become sharply asperate upon the upper surface. The transitions from the juvenile to the typical adult foliage are gradual, and may be deferred until relatively late stages in the ontogeny of the plant. Thus the large deeply lobed leaves of tall saplings may retain many of the juvenile characters, that is, asperate upper surface, chartaceous texture, conspicuous white tomentum on the under surface, acuminate lobes, etc. The leaves of adult indi-

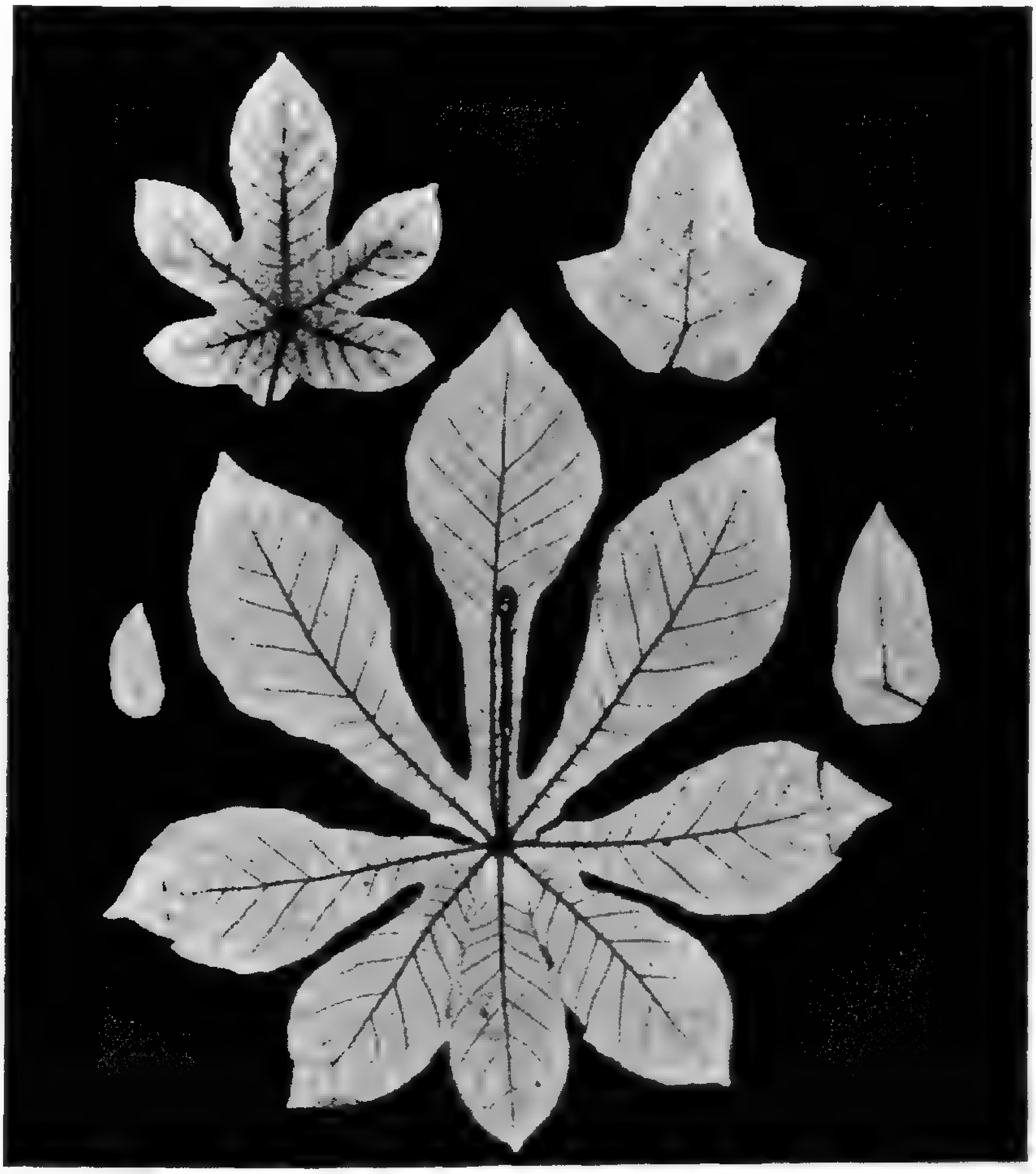

FIG. 1.—*Cecropia angulata:* leaves from juvenile plants of different ages, showing white tomentum and formation of lobed lamina; ×⅙.

viduals are huge, 56–98 cm. long, 56–84 cm. wide, coriaceous, glossy glabrous above, and are provided with only a microscopic layer of closely appressed white hairs in the areoles of the lower surface. They are divided to within 4–7 cm. of the base into 9–11 cuneate-obovate or spatulate lobes which have undulate margins (text fig. 2).

FIG. 2.—*Cecropia angulata:* typical leaves from tall tree, showing variability in size; $\times\frac{1}{14}$.—Photograph by JOHN TEE-VAN.

Although at first sight most of the vegetative characters appear to be extremely variable, many of them are relatively stable during specific stages in the ontogeny of the plant. For example, the leaves of adult individuals always are deeply lobed and undulate on the margins, never scabrous on the upper surface or conspicuously tomentose below; whereas the leaves of juvenile plants always are hairy or sharply asperate above, and are densely albido-tomentose on the under surface. It is evident, therefore, that in distinguish-

Fig. 3.—*Cecropia angulata:* terminal shoot of tall, vigorous young tree, showing ♀ inflorescences, and foliar and florial bracts; $\times\frac{11}{30}$.—Photograph by JOHN TEE-VAN.

ing species of *Cecropia* it is essential to compare leaves from plants of similar age classes, and, so far as possible, to avoid generalizations based upon highly variable, transitional types of foliage, such as occur on saplings.

FIG. 4.—*Cecropia angulata:* terminal shoot of old, slow growing tree, showing ♀ aments in different stages of development; × $\frac{3}{13}$.—Photograph by JOHN TEE-VAN.

Certain of the floral characters are variable, whereas others are relatively stable. The bracts which envelop the young inflorescences are coated during the later stages of their development with dense, rufous tomentum, and are frosted with long white hairs. The male and female aments (text figs. 3, 4) vary in size during different stages of their ontogeny, but the former (text fig. 5*A*) retain their juvenile angularity, whereas the latter (text fig. 6*D*) become nearly cylindrical at maturity. The peduncles vary considerably in length, but are asperate in both sexes. The female inflorescences are characterized by having one or more perigones in the sutures between the short, stout, conferruminate pedicels (text fig. 7*C*). The clavate, pentangular or hexangular, apically cleft male flowers are not jacketed by a dense mat of long, interlacing trichomes, but are provided with a collar of short, stiff hairs (text fig. 5). The perigonial chamber is confined to the upper half of the flower, and the caudate anthers are borne on short filaments which become extraordinarily broad and membranaceous at the time of dehiscence (text fig. 5). The filaments, connectives, and anthers vary considerably in size and shape (text fig. 5*D*–*G*);

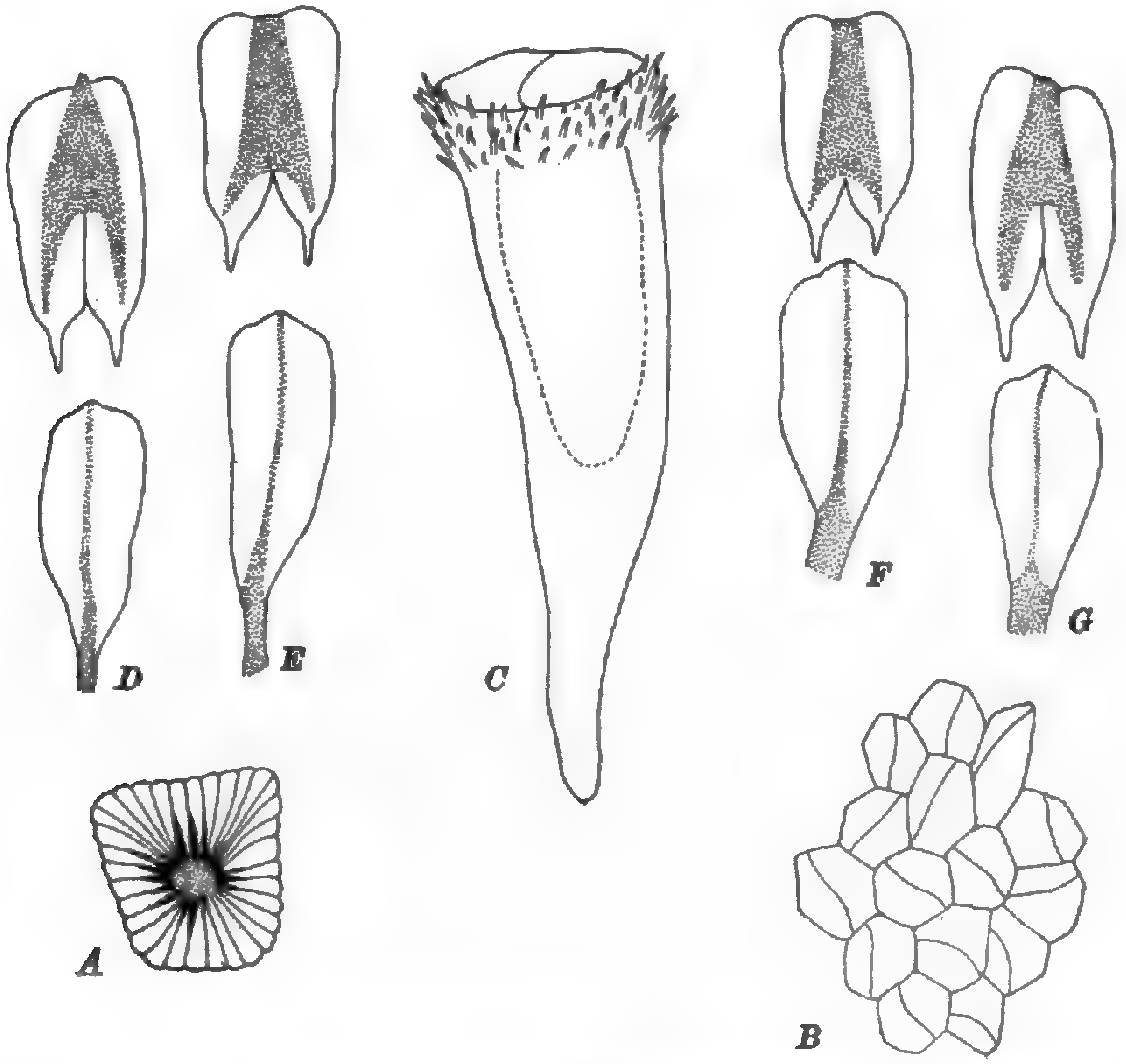

FIG. 5.—*Cecropia angulata:* *A*, cross-section of ♂ ament, ×2.5; *B*, surface view of portion of ♂ ament, showing pentangular and hexangular, apically cleft perigones, ×10; *C*, ♂ perigone, showing collar of short, stiff hairs, and position of perigonial chamber, ×22; *D*, lower stamen of pair; *E*, upper stamen of same pair; *F*, upper stamen of pair from another perigone; *G*, lower stamen of this pair; (*D*–*G*) ×22.

for example, the upper anther of each pair tends to be somewhat smaller, and to be attached nearer its base to a longer filament. The connective may or may not project beyond the apex of the anther. The pentangular or hexangular perigones of the female aments (text fig. 6) are characterized by having three distinct types of trichomes. They are jacketed both internally and externally by dense mats of long, interlacing hairs (text fig. 7). In addition, they have a crown of extremely short, stout bristles on the margins of their exposed apical surface, and a fringe of longer bristles which project into the constricted upper portion of the perigonial chamber (text fig. 6). The glabrous, almond-shaped ovary is mottled, dark gray, and bears a cylindrical style which terminates in a comose stigma (text fig. 7). The style is provided with short, stout bristles, such as occur on the apical portion of the perigone. During the development of the seed, the perigone

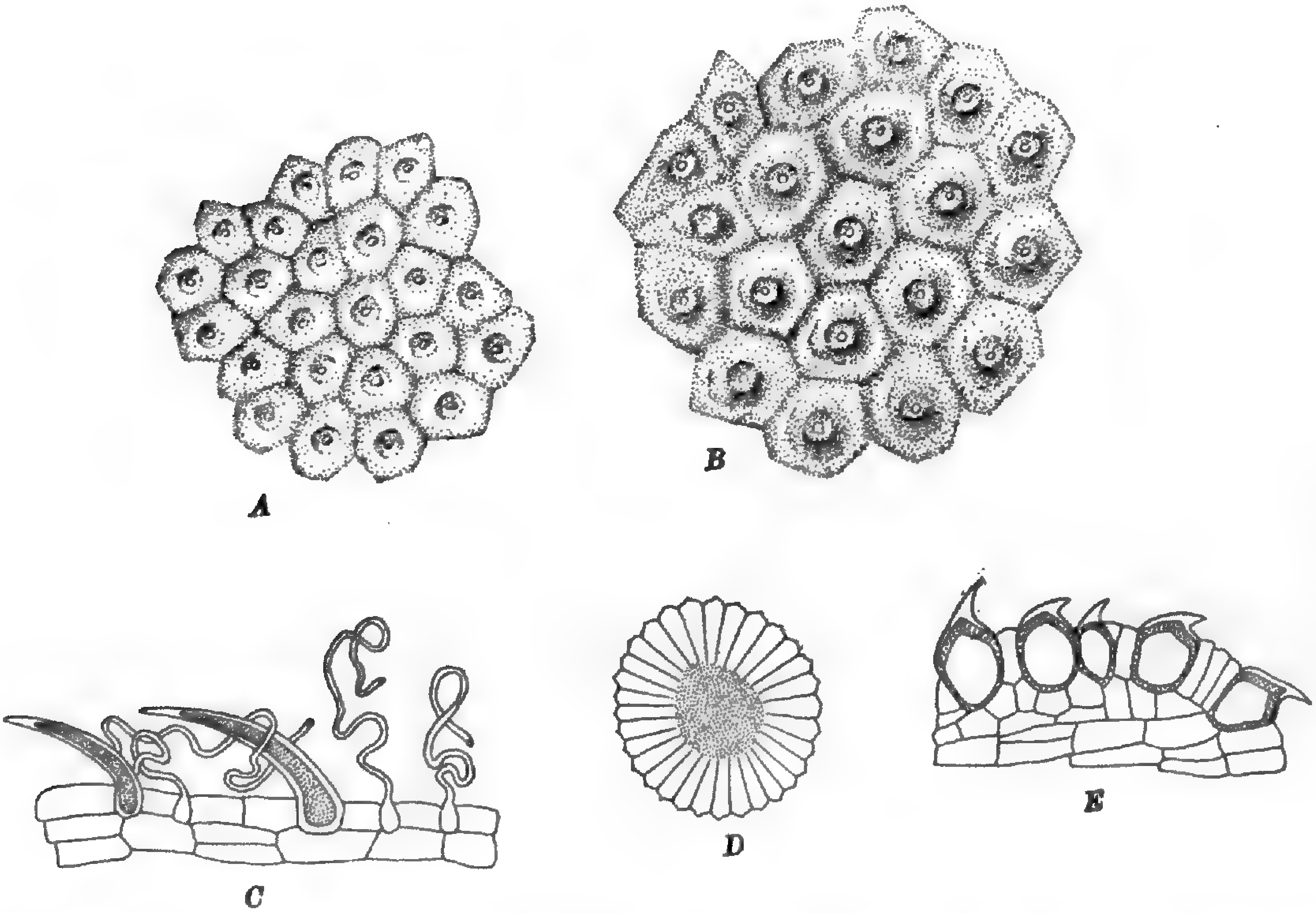

FIG. 6.—*Cecropia angulata:* *A*, surface view of portion of ♀ ament, showing pentangular and hexangular perigones at fertilization, ×10; *B*, surface view of portion of ♀ ament, showing enlargement of perigones during earlier stages in formation of seed, ×10; *C*, two types of trichomes from inner wall of perigone, ×315; *D*, cross-section of ♀ ament at fertilization, ×2.5; *E*, trichomes from outer apical portion of perigone (similar bristles occur on style); ×315.

enlarges and becomes somewhat modified in form and structure (text figs. 6, 7). The white tomentum is carried outward, so that the lower two-thirds of the flowers are not held together by interlacing hairs, and an annular depression is formed in the exposed outer surface of each perigone. At maturity the scurfy, dark reddish brown, oblong seeds (text fig. 7*E*) have a more or less symmetrical, lozenge-shaped, triangular or rectangular contour in cross-section.

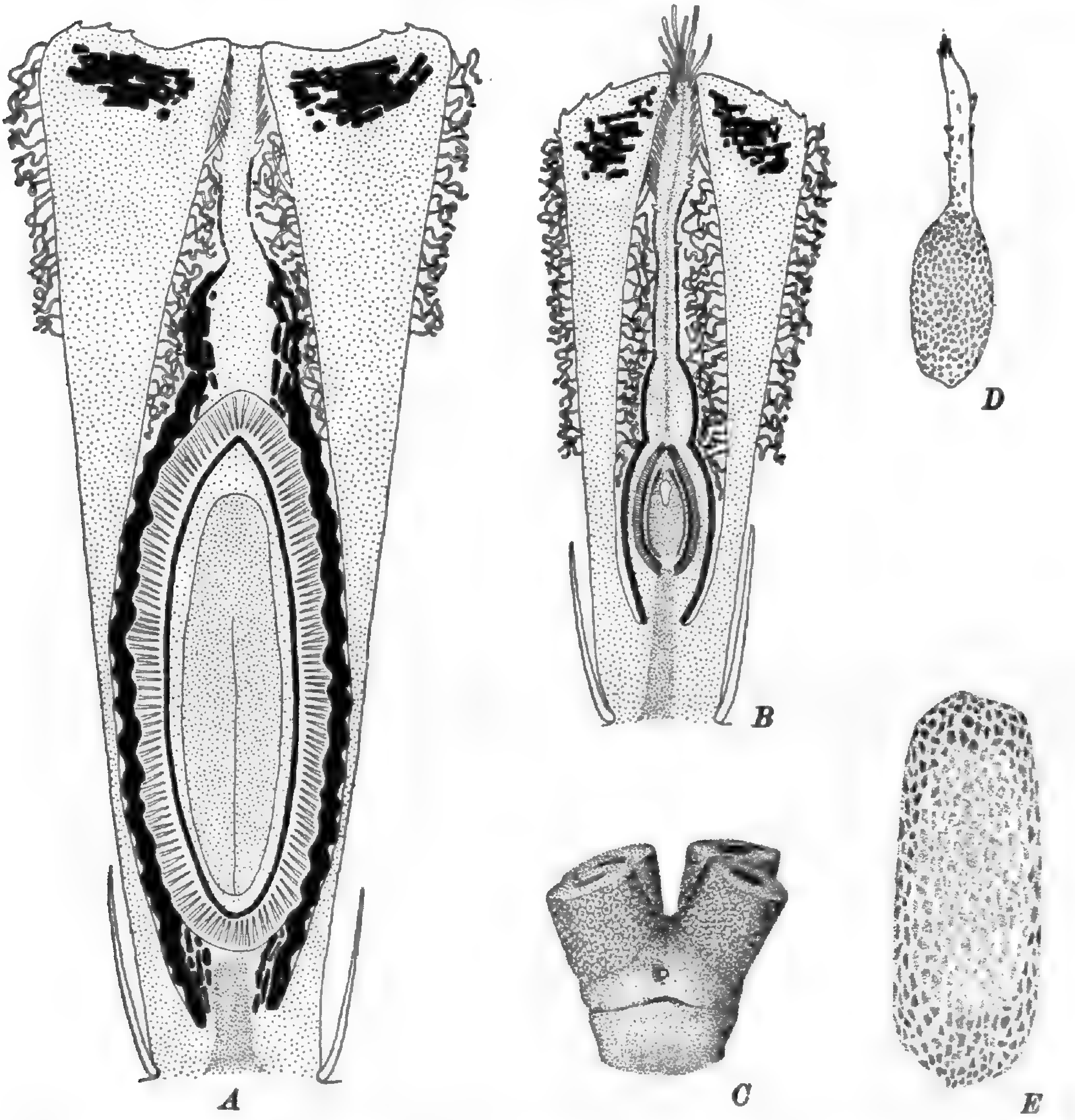

FIG. 7.—*Cecropia angulata:* *A*, longitudinal section of ♀ perigone and nearly mature seed, showing embryo and various seed coats, ×31; *B*, longitudinal section of ♀ perigone, style, ovary, and ovule, showing various types of trichomes, ×31; *C*, basal portion of ♀ inflorescence, showing perigone at point of contact of conferruminate pedicels, ×$\frac{4}{1}$; *D*, ovary and style, ×18; *E*, mature seed, ×20.

Owing to the variability of many of the floral characters during the enlargement of the aments, there is a considerable element of uncertainty in comparing inflorescences which are not in equivalent stages of differentiation. In the case of the male aments, the anthers, and especially the filaments, do not attain their most characteristic size and shape until just before dehiscence. Furthermore, there are two distinct stages in the differentiation of the female aments, one before and one after fertilization. The changes in the size and form of the perigones during the maturation of the ovules, and subsequently during the enlargement of the seeds, are reflected in the aments, whose external characters become correspondingly modified. The significance of these developmental stages does not appear to have been appreciated fully by a number of students of the species of *Cecropia*. The aments, perigones, stamens, seeds, etc. are described and are used for diagnostic purposes without reference to their variability or to their particular stage of development. Until more is known concerning the variability of the various species of *Cecropia*, and until their more stable diagnostic characters have been isolated and fully described, it is desirable to give rather comprehensive descriptions of new species. The following description of the myrmecophytic species of the Kartabo region is based upon the study of typical adult specimens. The juvenile characters have been referred to previously.

Cecropia angulata, sp. nov.—Arbor 10–25 m. altus, caulibus ramisque juvenibus argute asperatis formicosis (*Azteca*). Folia ampla rubella vel viridia 56–98 cm. longa 56–84 cm. lata profunde 9–11–lobata, lobis cuneato-obovatis vel spatulatis, apice obtusis vel rotundatis saepissime mucrontis, margine undulatis, lobo medio 36–60 cm. longo 13–24 cm. lato, nervis secundariis 15–20, lamina supra glabra nitente (post exsiccationem castanea obscurata) infra in nervis venis venulisque galbrata, in areolis solis tenuiter (microscopice) albido araneoso-tomentosa et in disco centrali breviter griseo-villosa, petiolo cylindrico (post exsiccationem costato) 50–120 cm. longo glabro vel albido-tomentoso ad basin incrassato et cum trichilio albido (tardius castaneo) instructo. Stipulae vaginantes maximae castaneae paullo griseo-tomentosae. Amentae ♂ 14–18 rectae rigidae *angulatae* 5–12 cm. longae 4–7 mm. crassae in pedicellis tenuibus 5–17 mm. longis gestae et in spatha castanea griseo-tomentella fusiformi ad basin constricta 14–16 cm. longa inclusae. Amentae ♀ 4 rectae rigidae vel apice paullo curvatae cylindricae ante anthesin 9–12 cm. longae 7–8 mm. crassae (tardius 13–17 cm. longae 11–13 mm. diametro) in pedicellis crassis conferruminatis 3–7 mm. longis gestae et in spatha castanea griseo-tomentella quadrangulata obtusa 10–12 cm. longa inclusae. Pedunculi argute asperati compresso-cylindrici, ♂ 9–12 cm. longi 8–11 mm. crassi, ♀ 8–10 cm. longi 7–10 mm. crassi. Perigonia ♂ clavata pentangularia vel hexangularia 2–3 mm. longa, filamentis in maturitate membranaceis 0.5–1.4 mm. longis 0.3–0.5 mm. latis, antheris caudatis 0.8–1.0 mm. longis 0.4–0.5 mm. latis. Perigonia ♀ pentangularia vel hexangularia cum albido tomento vestita ad anthesin 1.5–2.0 mm. longa deinde usque ad 3.0–3.5 mm. elongata. Stylus cylindricus ovarium aequans vel eo longior. Stigmata comosa. Fructus maturus furfurosus rubidus 2.0–2.5 mm. longus.

Kartabo region, British Guiana: *I. W. Bailey* (1920), nos. 3, 4, 5, 6, 8, 9, 14, 15, and 17; Kangaruma, British Guiana: *H. A. Gleason* (1921), no. 198.

The fistulose stems of *Cecropia angulata* are inhabited by four distinct species of *Azteca;* a majority of the plants are colonized by a black species, *A. constructor* Emery, a considerable number by a yellowish species, *A. alfaroi* Emery, and an occasional isolated individual by either *A. instabilis* F. Smith or *A. trigona* subsp. *mediops* Forel. The following observations upon the habits of the guest ants are based upon a study of the first two species.

Of the coccids which are associated with *Azteca*, *Pseudococcus rotundus* Morrison is the commonest species. *Akermes quinquepori* Newstead and *Pseudococcus bromeliae* Bouché are of more or less sporadic occurrence.

Are Cecropias protected by their guest ants?

In studying the remarkable leaf cutting and fungus growing habits of the neotropical Attine ants, Belt (2) became much impressed by the efficiency of these insects in defoliating large numbers of native and introduced plants, and he was led to wonder how tropical vegetation has survived their devastating attacks. He inferred that the leaves of many plants are distasteful to the ants or are unsuitable for their purposes, and that other plants are provided with special means of defense. He concluded that the so-called bull's-horn Acacias and other myrmecophytes are protected by their guest ants, which drive away their leaf cutting relatives. MÜLLER (6) and SCHIMPER (8) endeavored to prove that the myrmecophytic species of *Cecropia* are protected by the ants which inhabit their fistulose stems and branches. They found that plants which were colonized by Aztecas were not molested by Attine ants, whereas uninhabited individuals were more or less completely defoliated by them.

That BELT, MÜLLER, and SCHIMPER tend, on the one hand, to exaggerate the destructiveness of the leaf cutting ants, and on the other hand to overemphasize the protection afforded by the guest ants, has been suggested by VON IHERING (4), RETTIG (7), ULE (9), FIEBRIG (3), WHEELER (10), and other critics of the theory of myrmecophily. Several of these investigators call attention to the fact, previously noted by MÖLLER (5), that the leaf cutting ants feed upon a great variety of plants, and show no particular preference for the foliage of *Cecropia*. Thus, although the Attine ants frequently are troublesome pests in gardens and orchards, their feeding habits under normal conditions are such that they are not likely to exterminate indigenous species. According to VON IHERING'S computations, 183 nests of leaf cutting ants consume no more foliage during a year than does a single cow. In many cases the myrmecophytes grow in regions, such as swamps and periodically inundated areas, where the fungus growing Attas do not occur. Furthermore, during the earlier stages of its development *Cecropia* is not inhabited or protected by its putative guardians. In addition, it has been shown that plants which are not inhabited by Aztecas may remain unmolested by Attas for long periods, and that trees which are inhabited may be seriously injured by phytophagous insects and sloths. Of course it must be admitted in this connection that the discrepancies between the conclusions of MÜLLER and SCHIMPER and those of ULE, VON IHERING, and others, may be due to the fact that they were concerned with different species of Attine ants. Furthermore, it may be argued that the juvenile plants do not need the protection of the Aztecas, since they are immunized by various protective devices.

In the forests of the Kartabo region there are numerous colonies of the common leaf cutting and fungus growing ant, *Atta cephalotes* L. The writer found, as MÖLLER had previously done in the case of *A. discigera* Mayr., *A. hystrix* Latr., and *A. coronata* Fabr., that this ant utilizes the leaves of a great variety of plants in the construction of its fungus gardens. In virgin and second growth forests, it seldom works for any considerable length of time upon a particular type of plant, but continually shifts its activities from one species to another. Owing to this fact and to the rapid recovery from injury by plants in moist tropical environments, the effects of its attacks upon a given species appear to be more or less evanescent. Its normal leaf cutting habits, however, may be considerably modified under unusual or abnormal conditions. It frequently exhibits a strong preference for plants to which it has not previously been accustomed. Thus its attacks upon certain exotic plants in gardens and orchards at times may prove to be singularly persistent and destructive.

In many cases *Cecropia angulata* is not colonized by its guest ants until it has attained a considerable size. This is largely due to the fact that the young, fecundated queens, which initiate the new colonies, are killed by a Hymenopterous parasite, *Conoaxima aztecicida* Brues. Such plants are no more subject to defoliation than are the inhabited individuals. In order to determine whether the leaves of the juvenile *Cecropia* are distasteful to the leaf cutting ants, or are unsuitable for their purposes, a number of young plants were placed in close proximity to a large nest of *Atta cephalotes*. Leaves of adult Cecropias and of various other plants were used as controls. Although the ants worked upon this material in a more or less sporadic fashion, and showed a strong preference for certain types of leaves, they devoted no more attention to the mature than to the juvenile foliage of *Cecropia angulata*. In view of the fact that both BELT and SCHIMPER admit having seen young myrmecophytes defoliated by Attas, there appears to be little evidence in favor of the suggestion that juvenile ant-plants are less susceptible to the attacks of phytophagous insects than are the adult individuals.

In defending the theory of myrmecophily, SCHIMPER placed great emphasis upon the discovery, in the Corcovado near Rio de Janeiro, of a species of *Cecropia* which is devoid not only of ants, but also of prostomata and Müllerian food bodies. He inferred that this *Cecropia* possesses no adaptations for attracting a defending army of Aztecas, because it is protected by a waxy coating which prevents the leaf cutting ants from climbing its stems. *Cecropia sciadophylla* Mart. var. *decurrens* Snethlage is not colonized by Aztecas, but, as will be shown later, is provided with conspicuous and highly differentiated prostomata. The external surfaces of the plant are scabrous and afford a firm foothold for ants, as evidenced by the fact that several species of these insects were seen climbing its stems and branches. This species of *Cecropia* is no more subject to defoliation by Attas than is *Cecropia angulata*. That the leaves may be utilized in the construction of fungus gardens is shown by the fact that, when cuttings from plants of various ages are placed near nests of *Atta cephalotes*, the ants frequently transport a portion of the foliage into their subterranean dwellings.

SCHIMPER's critics place considerable emphasis upon the fact that the myrmecophytic Cecropias may be inhabited simultaneously by two or more distinct genera of ants. To infer from this, however, that the Aztecas are indifferent to the presence of other ants is somewhat misleading. Although species of *Cecropia* were found that were inhabited by *Cryptocerus*, *Crematogaster*, stingless bees, etc., these insects were always confined to the older and lower internodes, which had been abandoned by the Aztecas. In spite of this marked segregation of the colonies in different levels of the stem, fierce conflicts may be waged for possession of the intervening internodes. Thus, the internodal cavities, where the colonies come in contact, frequently are filled with the corpses of dead warriors. The Aztecas are dominant, and in general do not tolerate the presence of other ants, in those portions of the stems and branches which are provided with leaves and food bodies. SCHIMPER found that when leaf cutting ants were deposited upon the terminal portions of a *Cecropia*, they were quickly seized by the Aztecas, if their presence was discovered, and were hurled to the ground. How then may one account for the defoliation of trees which are inhabited by Aztecas? When a *Cecropia* is touched or shaken, the angry and aggressive Aztecas rush out of their nests and swarm over the whole plant, but under normal conditions only a limited number of workers are visible on the stems and bases of the petioles. Furthermore, there are periods during which the entire colony is quiescent, that is, has withdrawn into its nest. It is well known that these periods of quiescence are not the same in the case of different species of ants. Thus, certain ants are nocturnal, others are active at temperatures when other species are inactive, etc. In other words, as suggested by MÜLLER and MÖLLER, a *Cecropia* may be defoliated by leaf cutting ants during periods when the Aztecas are quiescent.

Prostomata

Above the insertion of every leaf in *Cecropia adenopus* there is a shallow groove, which terminates just below the next node in a roundish depression (text fig. 8). As the external depression corresponds to an internal one, the wall of the fistulose stem is very thin at this place, and is a mere diaphragm in a tube. SCHIMPER showed that this diaphragm is devoid of hard and tough elements (fibrovascular bundles, collenchyma, lignified parenchyma, etc.), such as occur in the adjoining portions of the stem. He inferred from this that the diaphragm originated as an adaptation for facilitating the entrance of ants. At the phylogenetic commencement of symbiosis, the ants bored an entrance through the groove, evidently because the wall of the stem was somewhat thinner there. In accordance with a custom that is almost invariably followed and is connected with the domestic arrangements in their nests, they tended to penetrate into the internodal cavity at its upper extremity (at the apex of the groove). All features which facilitated their entrance at this place were retained in the struggle for existence, and were accentuated through natural selection. This led eventually to the differentiation of a thin, weak diaphragm or prostoma (VON IHERING). In other words, although SCHIMPER admitted that the groove is due to the pressure of the axillary bud, he maintained that its terminal portion is a highly specialized adaptation, acquired through the action of natural selection. On the contrary, RETTIG and FIEBRIG assert that the prostoma is merely the youngest or less highly differentiated portion of the groove, and that it is produced by the pressure of the axillary bud and other growth phenomena in the elongating internodes. The former investigator is of the opinion that the ants are deterred from excavating in the lower portion of the groove, not by mechanical obstructions, but by the occurrence of "laticiferous vessels" which are absent in the prostoma. It is to be emphasized in this connection, however, that RETTIG'S and FIEBRIG'S statements do not necessarily invalidate SCHIMPER'S conclusion that the diaphragm is an adaptation which originated as a modification of a previously existing structure.

Under most growth conditions *Cecropia angulata* does not form a shallow groove which terminates in a conspicuous circular pit (text fig. 8). The more or less fusiform depression or rill is somewhat deeper in the upper than in the lower portions of the internode, but the differentiation of specialized or mechanical types of tissue is retarded throughout its extension (fig. 1). As the walls of the internode increase in thickness, after the initiation of secondary growth, this fusiform diaphragm of delicate parenchyma is slowly reënforced by tougher and denser tissues (fig. 3). The metamorphosis begins at the base of the groove, and gradually extends upward, but the ants excavate their exits before these changes have progressed very far. It is evident, therefore, that in the case of the myrmecophytic *C. angulata*, the whole groove is a potential prostoma. The exact location of the aperture is determined, not by the presence or absence of resistant tissues or of "latex" vessels, but by the relative thickness of different portions of the diaphragm. That the whole groove is a potential prostoma, and that the ants merely excavate their exits and entrances in its thinnest and most easily perforable portion, are indicated by the behavior of young queens in juvenile plants. In many instances, several queens attempt to occupy the same internode, and as many as five entrances were found cut at different levels of a single groove. Not only do the queens cut through the basal portions of the diaphragms under such conditions, but they may even excavate entrances in portions of the internodes which are not provided with preformed depressions.

It may be argued that in *C. angulata* the whole groove has been modified as an adaptation to ants. Such an assumption is not warranted, however, when important facts in the anatomy of the non-myrmecophytic *C. sciadophylla* var. *decurrens* are taken into consideration. Although this species is not inhabited by Aztecas, it is provided with more tenuous and highly specialized diaphragms than is *C. angulata* (text fig. 8; fig. 2). The internodal groove is very broad and deep, and the diaphragm, which is entirely devoid of tough tissues and secretory vessels, is composed of extremely thin layers of delicate parenchyma. Such facts as these suggest that the so-called prostoma of *C. adenopus*, and of other myrmecophytic species of *Cecropia*, is not an adaptation for attracting ants, but is merely a structural peculiarity, produced by the pressure of the axillary bud, which is utilized by the ants in their parasitism upon the plants.

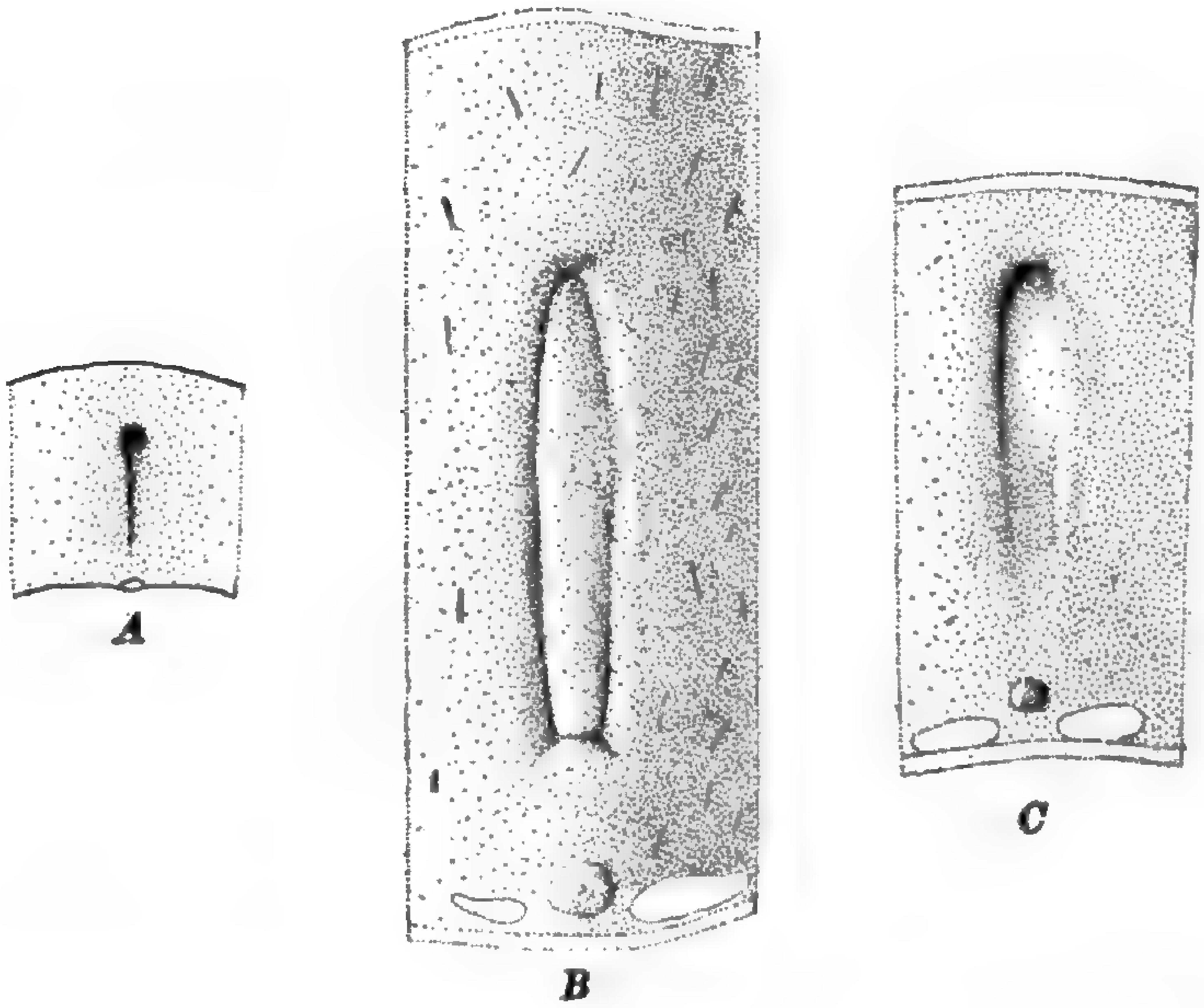

FIG. 8.—Prostomata or internodal diaphragms of various species of *Cecropia*: *A*, *C. adenopus*, after SCHIMPER; *B*, *C. sciadophylla* var. *decurrens*; *C*, *C. angulata*; ×½.

Feeding habits of guest ants

The *Azteca* colonies are initiated by young fecundated queens which cut entrances into the fistulose stems of juvenile plants. Although I examined hundreds of plantlets of *C. angulata*, none of any considerable size were found that had been entered by but a single queen. The queens are so numerous that many of the successive internodes become inhabited, and, as already stated, one not infrequently finds that several queens have penetrated into the same internodal cavity. So far as it was possible to determine, however, a large proportion of these queens perish before they have produced a brood. A very considerable number are killed by the parasitic *Conoaxima aztecicida*, others are killed by their rivals in conflicts for possession of a given internode, and, as soon as the workers become numerous, they cut through the nodal partitions and kill all but one of the surviving queens.

When a young *Azteca* queen enters an internodal chamber, she covers the entrance aperture with a layer of triturated pith. The entrance subsequently becomes occluded by callus, which continues to grow internally, and finally projects some distance into the medullary cavity (fig. 4). Thus the queen is sealed within the internode during the period when she is initiating the new colony. In view of the fact that the queen is unable to leave her nest in search of food during a period of two months or more, MÜLLER, VON IHERING, and FIEBRIG infer that she must feed upon medullary tissue and the inwardly projecting callus or "stomatome." It should be noted in this connection, however, that such an assumption is based upon two more or less fallacious premises: (1) that the queen must feed during her period of isolation, and (2) that tissues which are gnawed or excavated by ants actually are eaten by them. Most students of the Formicidae are familiar with the fact that female ants are able to do without food, except such as is stored in their own bodies, for the greater part of a year, while they are founding their colonies. Furthermore, it is well known that many ants tend to gnaw into and smooth the walls of their nests, regardless of whether they are composed of living tissues or of inert materials. I was unable to find any evidence that the *Azteca* queens feed upon the tissues in the young internodes of *C. angulata*. The so-called stomatomes, upon which MÜLLER and VON IHERING place so much emphasis, are not uncovered and cut back by the queens until just before the first workers are ready to emerge from the nest, nor do they excavate the medullary tissue to any considerable extent, except when in search of material with which to block up the newly formed entrance aperture. Although VON IHERING is of the opinion that the luxuriant growth of callus is due to the stimulus of some substance excreted by the queens, I found that homologous structures may be produced by purely mechanical injuries.

With the advent of the first workers, the entrance to the primordial chamber is reopened, and the young colony either migrates to a higher internode or cuts through the nodal partitions into adjoining cavities. VON IHERING states that the Aztecas always abandon the primordial chamber and never perforate its upper and lower walls. Such is not invariably the case in *C. angulata*, for primordial chambers were frequently found in direct communication with internodes which were not provided with prostomal openings. Regardless of its exact mode of origin, the permanent domatium soon becomes stocked with food bodies by the young workers. These small beadlike structures (fig. 6), which are packed with fat and protein, are formed in large numbers in a curious cushion or mat of hairs, situated at the base of each petiole. The ripe food bodies are so assiduously collected by the ants that it is almost impossible to find one *in situ*, except in young uninhabited plants. Indeed, the ants frequently trim away the surrounding hairs and dig out the immature food bodies. SCHIMPER interpreted these so-called Müllerian corpuscles, and similar structures which occur on the leaflets of certain myrmecophytic species of *Acacia*, as metamorphosed glands or highly specialized allurements for attracting ants. RETTIG and others, however, have called attention to the fact that such glands occur on plants that are not frequented by ants, and it is difficult for the adherents of myrmecophily to account for such occurrences without resorting to the purely gratuitous assumption that they are survivals from former symbioses. ULE is of the opinion, in addition, that the expenditure of carbohydrates and nitrogenous substances, contained in these corpuscles, is not compensated for by the protection which the ants afford to the plants.

Although most investigators agree that the food bodies are an important item of food in the diet of the Aztecas, it has been suggested that there are other potential sources of food in *Cecropia*. Thus, von Ihering and Fiebrig maintain that the imagines feed upon the succulent medullary tissues in immature internodes. It is true that the ants cut away the softer portions of the pith down to a hard, smooth peripheral layer of medullary tissue, but I found no evidence to indicate that this is not purely a process of house cleaning, such as occurs in many ant nests. In the moist, warm interiors of plants, ants have to contend with luxuriant growths of fungi which obstruct the cavities and interfere with the brood, unless they are held in check. The ants trim away the hyphae and cut back the substratum upon which these organisms tend to grow. Fiebrig records having seen Aztecas busily engaged in excavating the pith, and in casting fragments of medullary tissue from their entrances, but such observations cannot be interpreted as evidence that the ants actually feed upon the tissues that they are removing.

Most students of the myrmecophytic species of *Cecropia* have found coccids associated with the ants which inhabit the fistulose stems. Their presence has been variously interpreted. Belt, Müller, and Ule consider that they are tended by the ants which feed on their sugary exudations, but Fiebrig states that the insects "in keinen direkten Verhältnis zu diesen Ameisen stehen." Having found a very close and significant relation between ants and coccids in most Ethiopian ant-plants, I devoted particular attention to the investigation of their behavior in *C. angulata*. I did not succeed in finding a single large, ant-inhabited specimen which did not contain numerous coccids. When a tree is split open the ants are as solicitous for the welfare of the coccids as they are for that of their eggs, larvae, and pupae. They seize them in their mandibles and carry them about until some unopened portion of the plant is found where they may be deposited in safety. In artificial nests, the workers spend hours in tending and stroking the coccids, and in feeding upon their sugary exudates. In view of these facts, it cannot be doubted that the miniature milch cows are an important source of liquid carbohydrates for the ants.

As in many of the African myrmecophytes, the ants excavate pits in the walls of their domatia which enable the coccids to reach and feed upon the softer tissues of the *Cecropia*. Such excavations are essential, owing to the fact that the internodal, medullary cavity is entirely jacketed by a dense, horny layer of sclerenchyma. In the African ant-plants, the ants cut through to the cambium and induce the formation of a nutritive callus. In *C. angulata* the pits are not located in the sides of the internodal chamber, but in the nodal diaphragms. At the time when the ants begin their excavations, the nodal partition consists of five distinct layers (figs. 5, 7). The soft, internal layer, which is provided with strands of conducting tissue and which is fed upon by the coccids, is separated from the external layers of porous, medullary tissue by two layers of dense, thick walled tissue. The ants remove the two external layers and cut circular pits in the underlying sheets of horny sclerenchyma (figs. 8, 9). The coccids sit in these pits and thrust their setae into the succulent tissue which is thus exposed. That the pits are not made by the coccids, as suggested by von Ihering, is indicated, not only by the fact the delicate sucking mouth parts of these insects are not adapted for excavating in dense tissues, but also by the fact that I have actually observed the ants in the process of excavating them.

Summary and conclusions

The theory of myrmecophily, as modified by Schimper, interprets the structural peculiarities of myrmecophytic species of *Cecropia* as adaptations for enlisting the services of an aggressive army of Aztecas which protect their hosts against the attacks of the leaf cutting Attine ants. Cleverly conceived and suggestive as this Neo-Darwinian hypothesis undoubtedly is, it appears to be based upon a series of plausible deductions or teleological inferences, and is open to serious criticism. The distribution and feeding habits of the Attine ants in luxuriant, tropical forests are such that the ants are not likely to exterminate indigenous species. They show no strong preference for the foliage of *Cecropia*, and rarely attack either inhabited or uninhabited trees. Although Aztecas tend to prevent other ants from visiting the terminal portions of the adult Cecropias, they do not protect the juvenile individuals. That the curious prostomata and Müllerian corpuscles of *Cecropia adenopus* and of other myrmecophytic species of *Cecropia* are not allurements, acquired through natural selection, is indicated by the fact that such structures occur on certain species of *Cecropia* and other plants which are not frequented by ants. The *Azteca-Cecropia* associations, and ant-plants in general, are extremely interesting cases of parasitism, which illustrate the remarkable adaptiveness of ants in availing themselves of the potentialities of given environments. This is shown, not only in the utilization of the preformed food bodies, prostomata, and internodal cavities, but more strikingly in the structural modifications produced within the plants for the growth and tending of coccids. Thus, the ants are able to obtain food from the plant in two ways, fats and proteins directly from the Müllerian corpuscles, and carbohydrates vicariously through the coccids.

I wish to thank WILLIAM BEEBE for numerous courtesies during my visit to the Tropical Station of which he is in charge. To the directors of the Gray Herbarium of Harvard University, the New York Botanical Garden, and the Berlin Museum, and to Dr. EMIL SNETHLAGE I am indebted for many helpful taxonomic data. These investigations were conducted under a grant from the American Association for the Advancement of Science.

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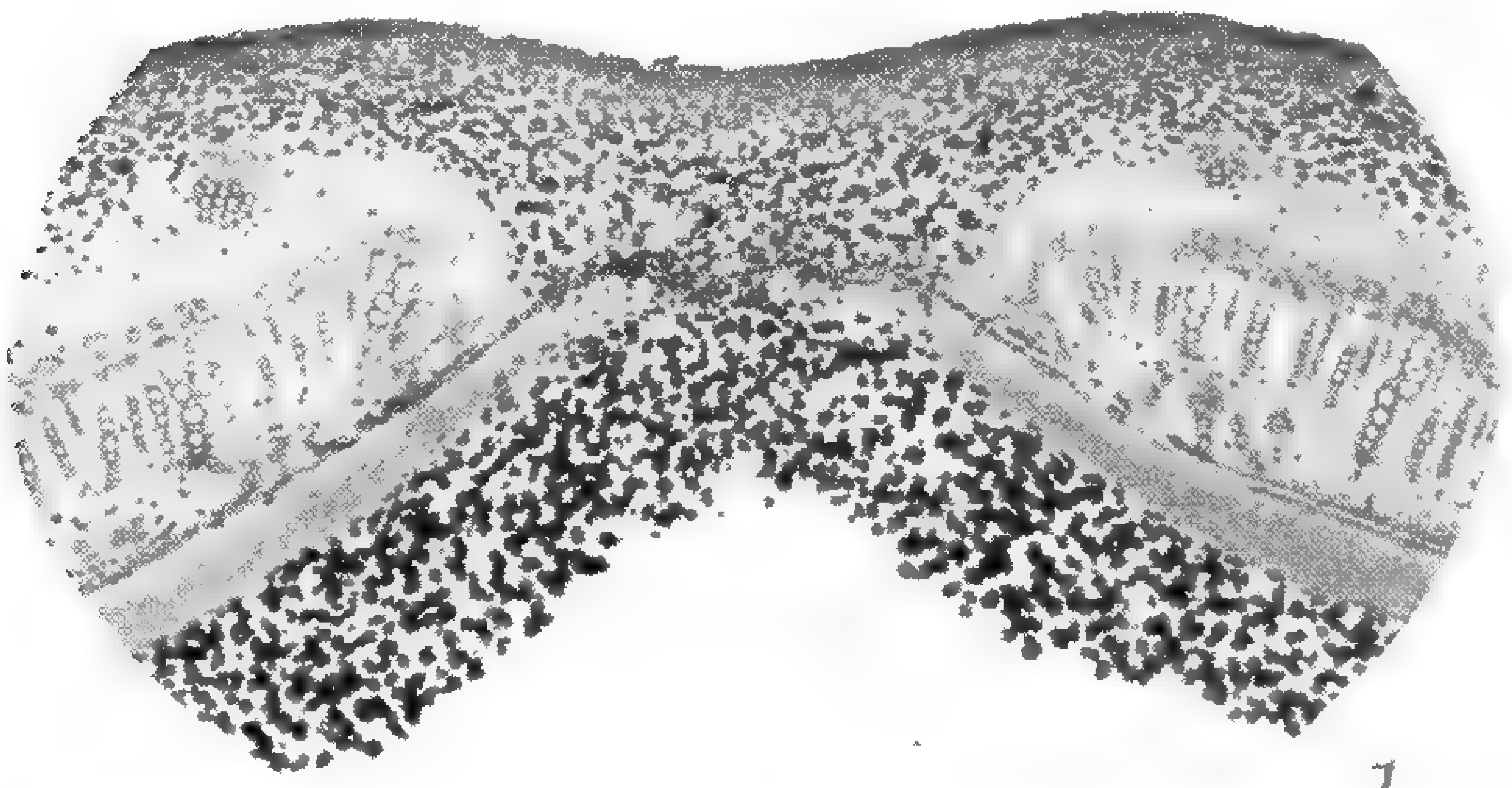
LITERATURE CITED

1. BAILEY, I. W., The anatomy of certain plants from the Belgian Congo, with special reference to myrmecophytism. Bull. Amer. Mus. Nat. Hist. **45**:585–621. 1921–1922.
2. BELT, T., The naturalist in Nicaragua. London. pp. xvi+403. 1874.
3. FIEBRIG, K., *Cecropia peltata* und ihr Verhältnis zu *Azteca alfari*, zu *Atta sexdens*, und anderen Insekten, mit einer Notiz über Ameisendornen bei *Acacia cavenia*. Biol. Centralbl. **29**:1–16; 33–55; 65–77. 1909.
4. IHERING, H. VON, Die Cecropien und ihre Schutzameisen. Engler's Bot. Jahrb. **39**:666–714. 1907.
5. MÖLLER, A., Die Pilzgärten einiger südamerikanischer Ameisen. Bot. Mitteil. aus den Tropen. Jena **6**:pp. vi+127. 1893.
6. MÜLLER, FRITZ, Die Imbauba und ihre Beschützer. Kosmos **8**:109–116. 1880–81.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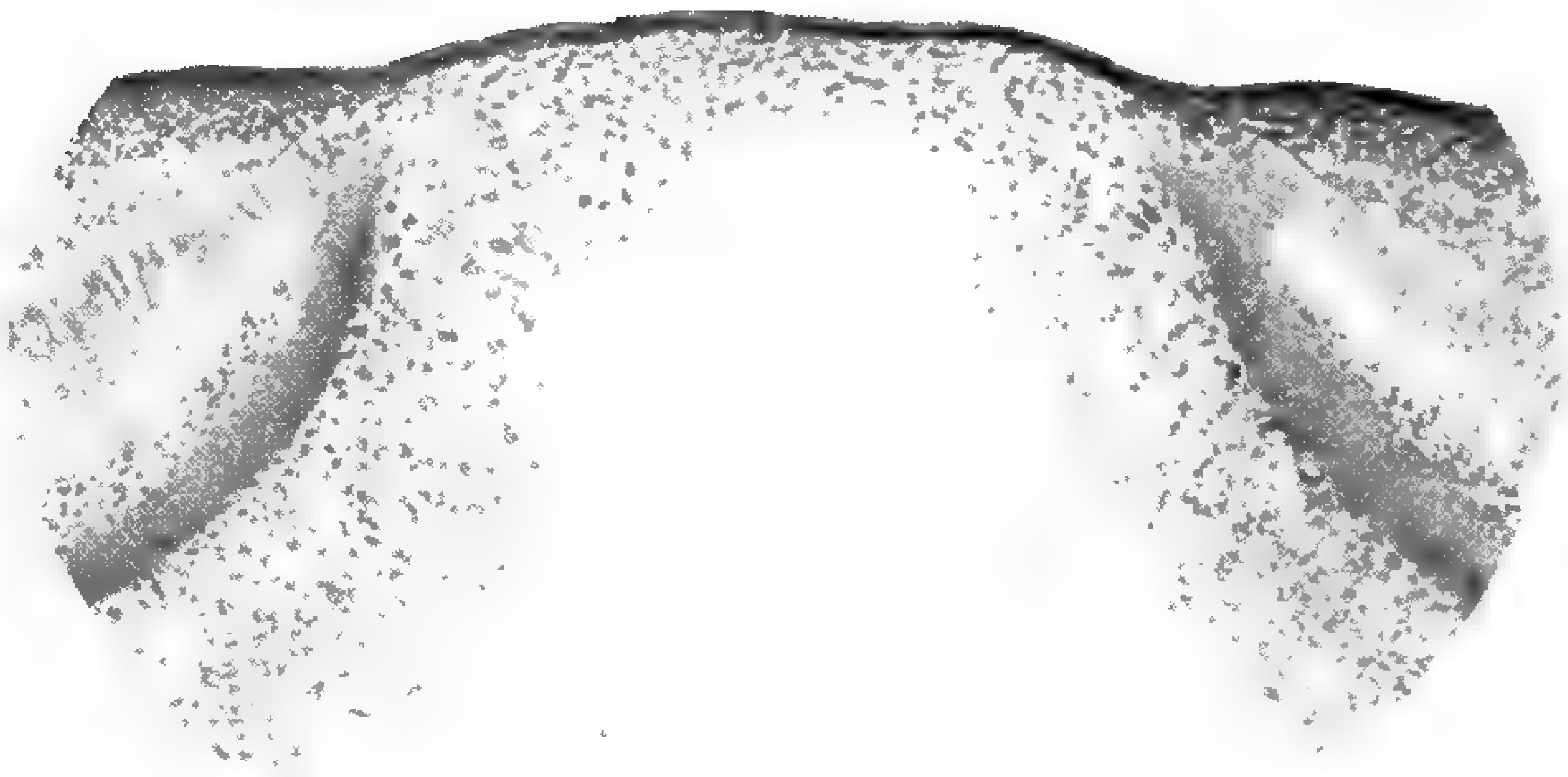
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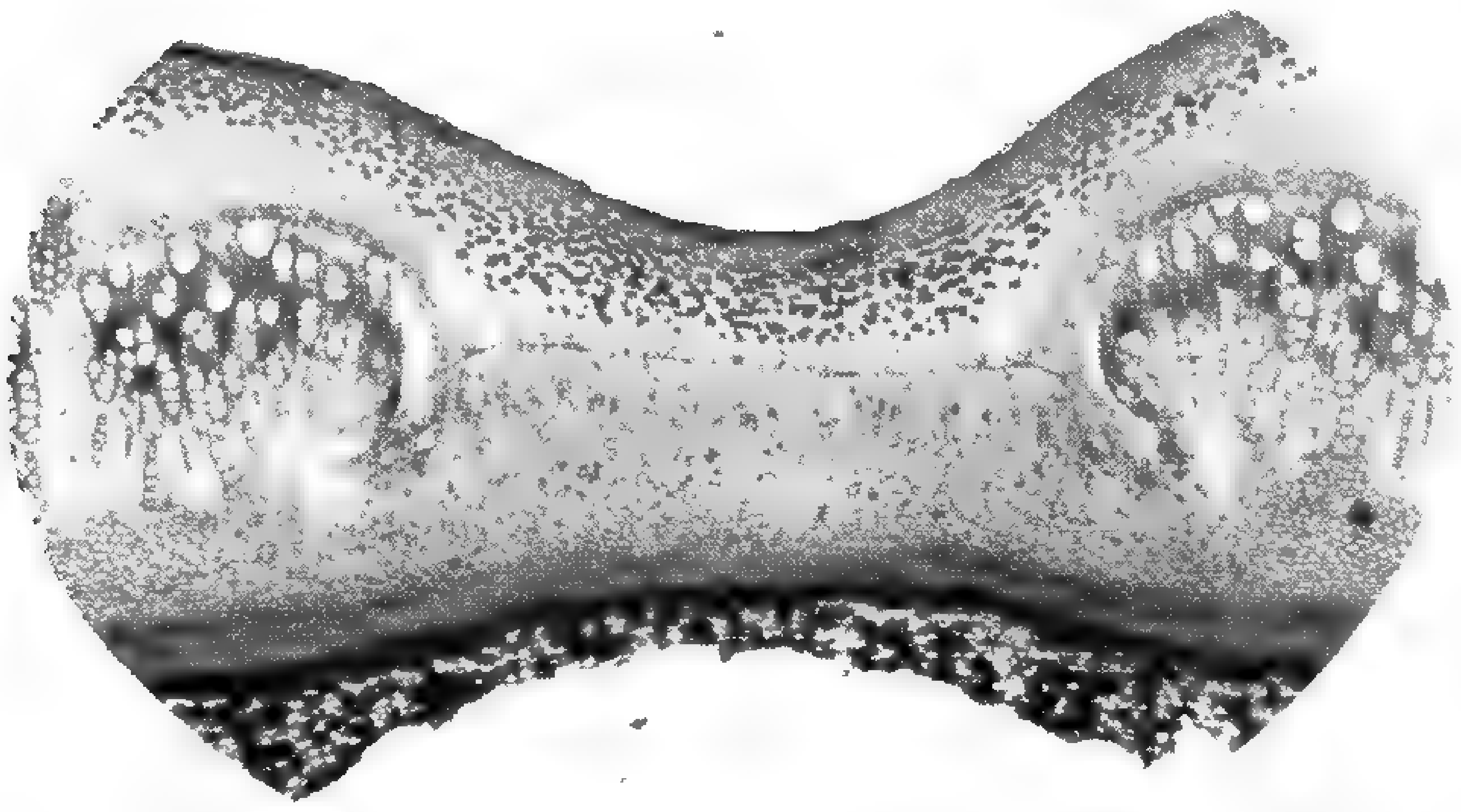
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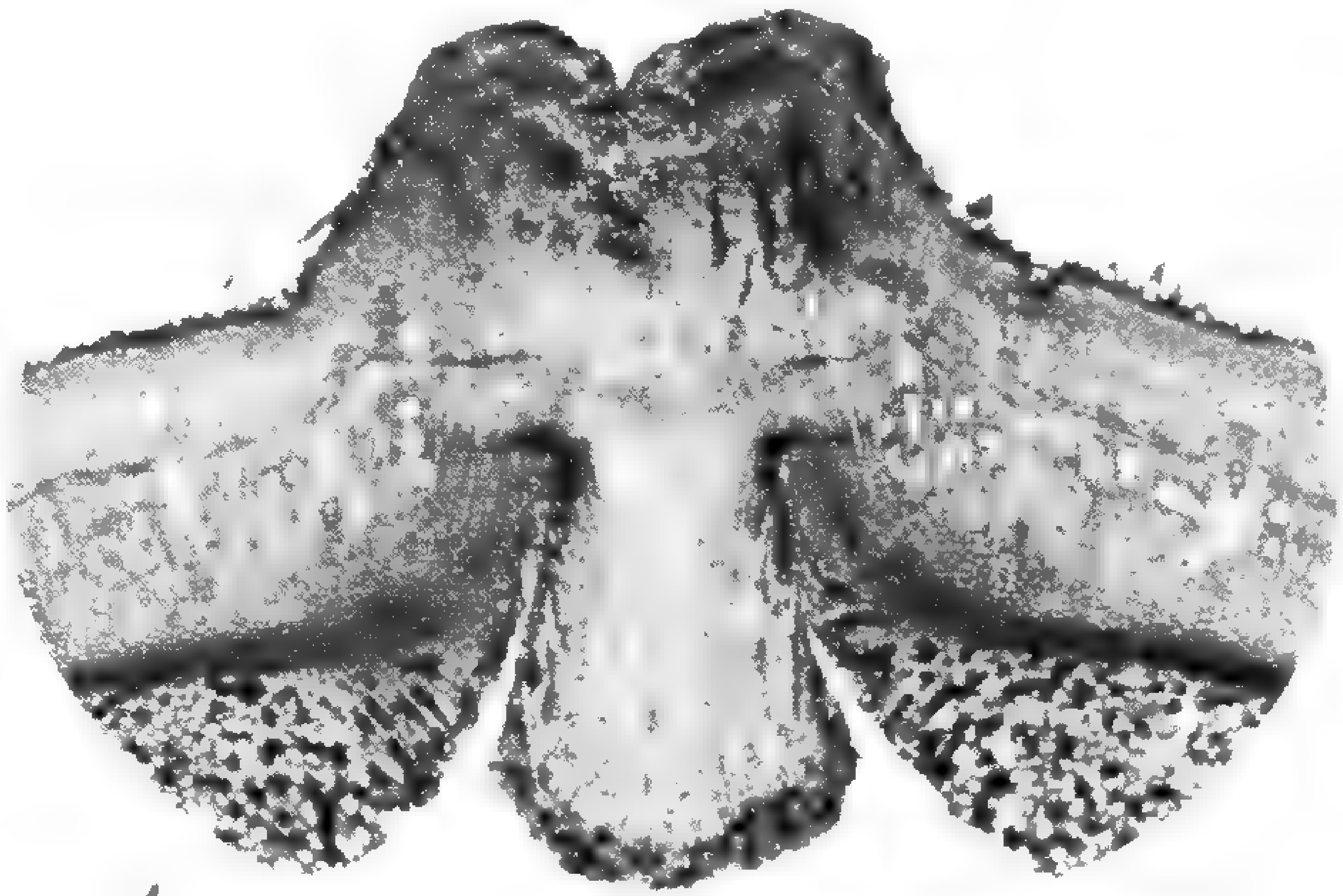
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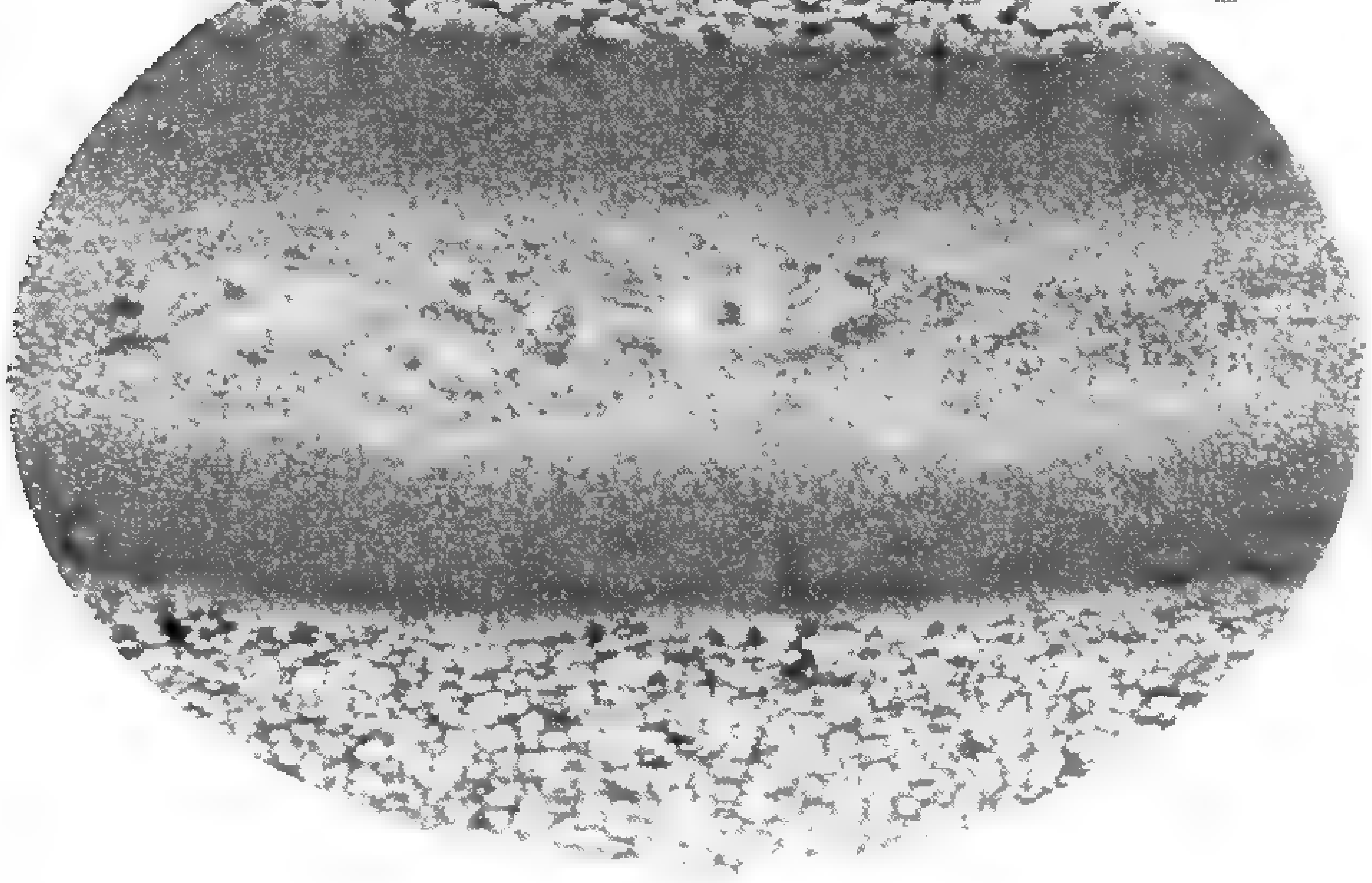
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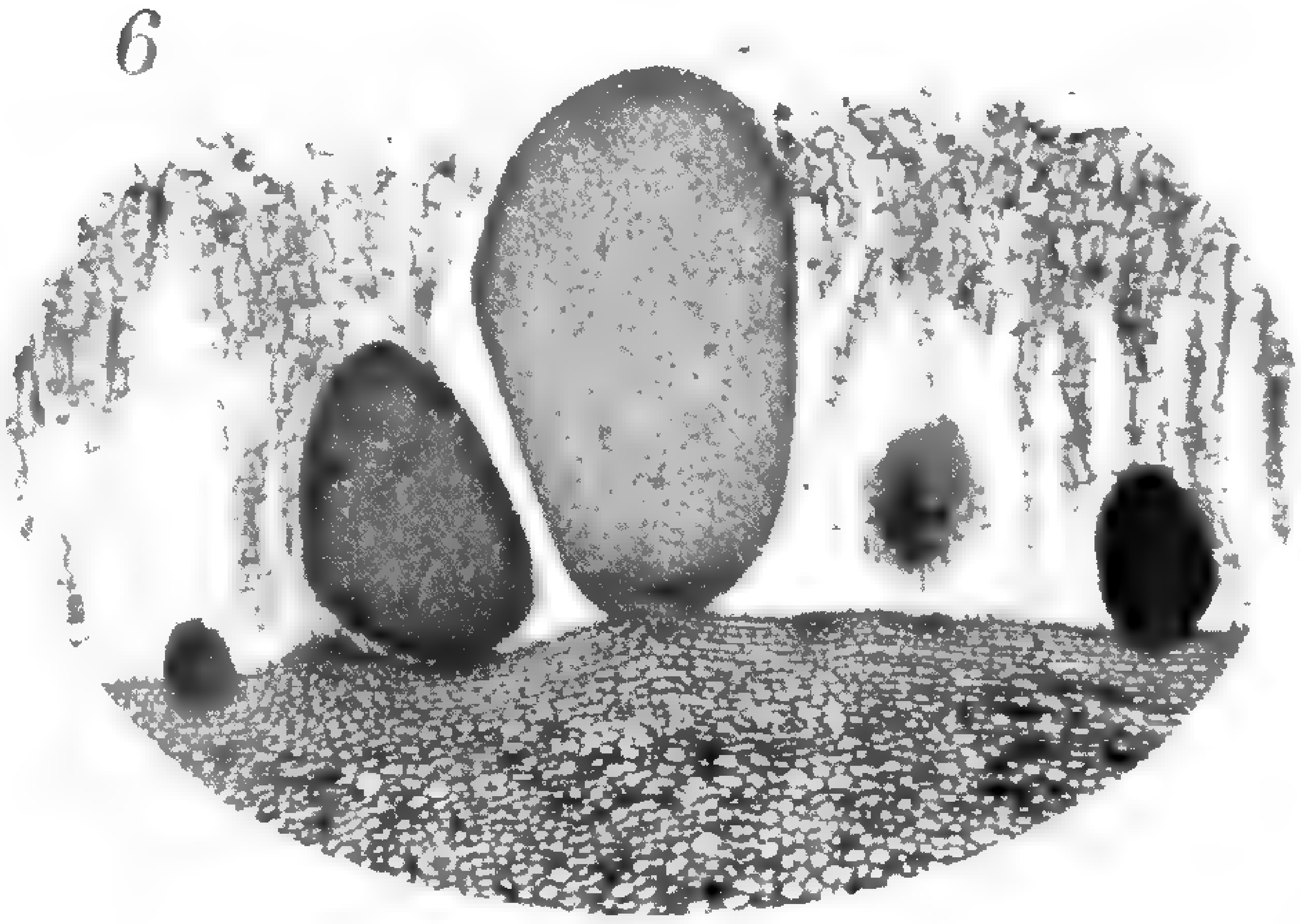
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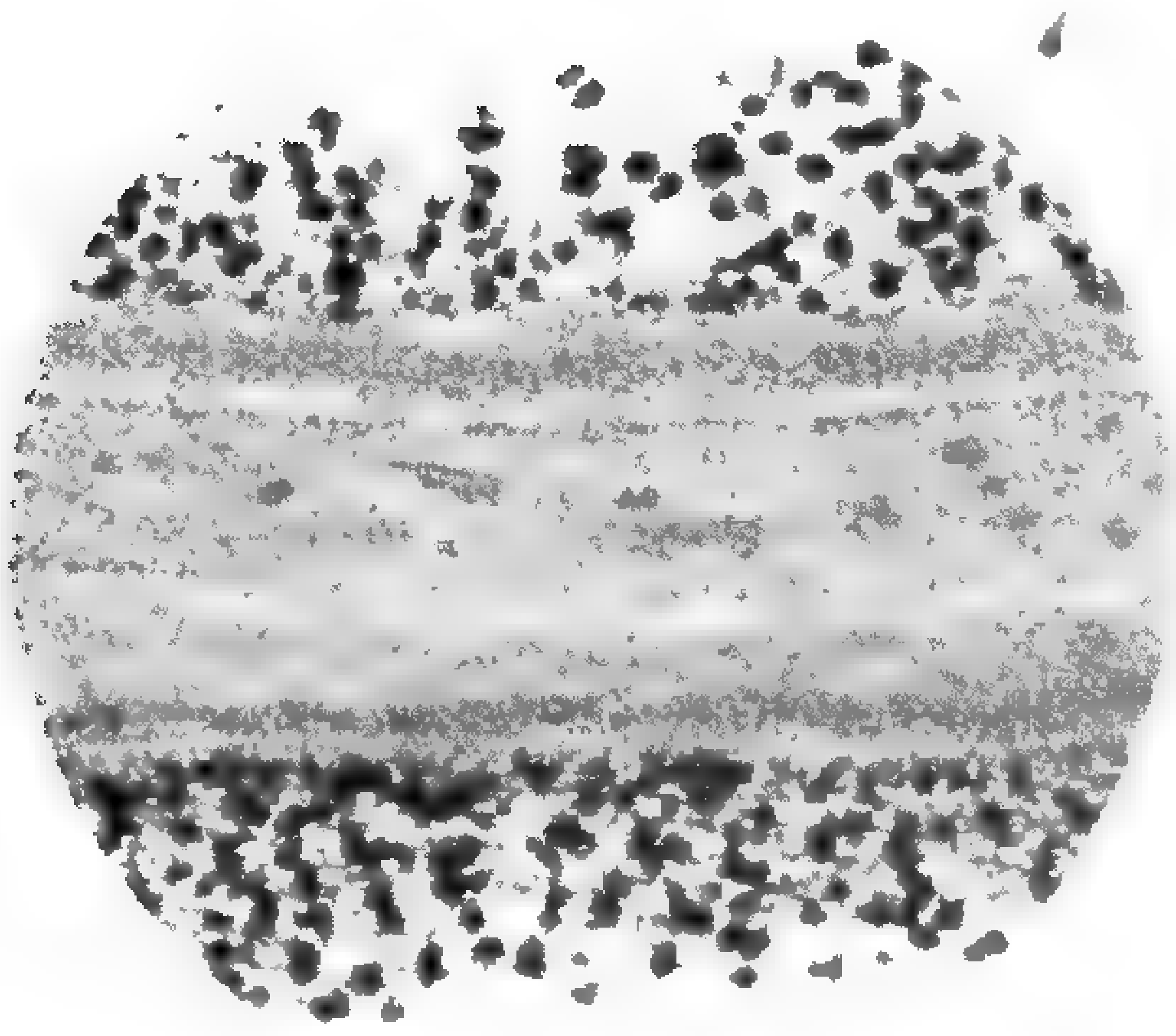
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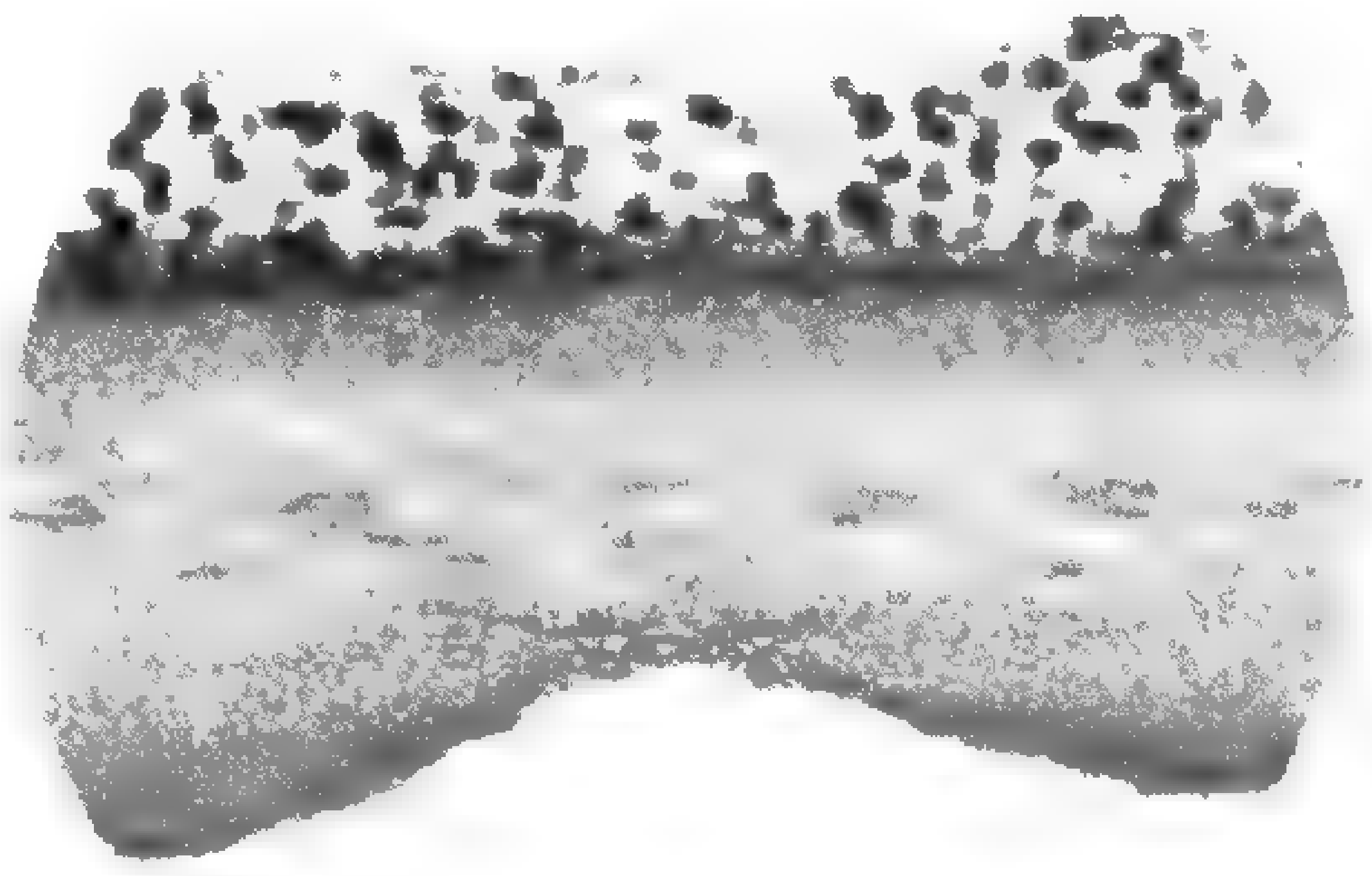
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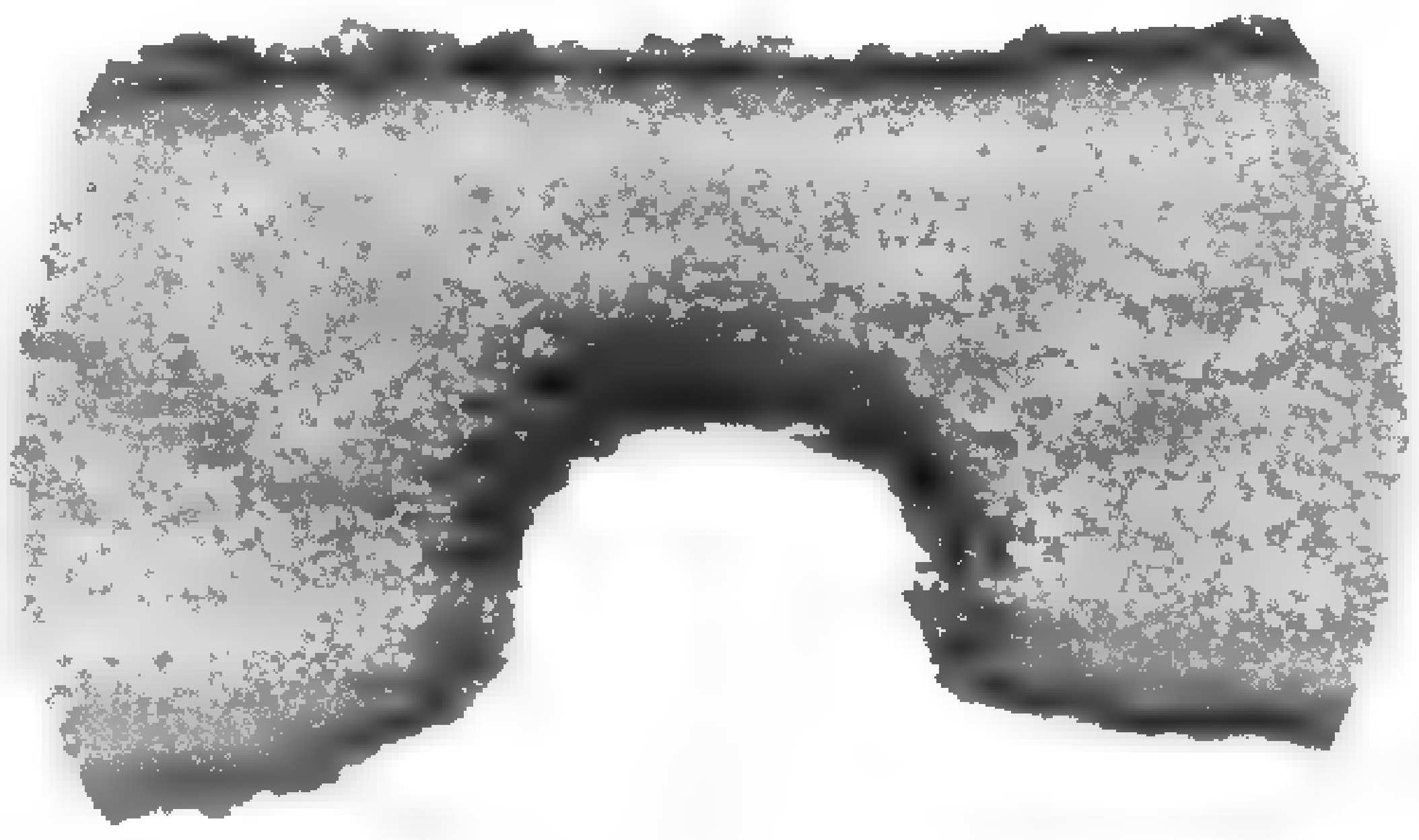
9

BAILEY on ANT-PLANTS

7. Rettig, E., Ameisenpflanzen-Pflanzenameisen. Beih. Bot. Centralbl. **17**: 89–122. 1904.

8. Schimper, A. F. W., Die Wechselbeziehungen zwischen Pflanzen und Ameisen im tropischen Amerika. Bot. Mitteil. aus den Tropen. Jena **1**: 1–95. 1888.

9. Ule, E., Ameisenpflanzen. Engler's Bot. Jahrb. **37**:335–352. 1906.

10. Wheeler, W. M., Observations of the Central American *Acacia* ants. Trans. 2d Congr. Entom. Oxford (1912) **2**:109–139. 1913.

EXPLANATION OF PLATE XV

Fig. 1.—*Cecropia angulata:* cross-section of upper portion of internode, showing "prostoma"; ×10.

Fig. 2.—*C. sciadophylla* var. *decurrens:* cross-section of internode, showing "prostoma."

Fig. 3.—*C. angulata:* cross-section of lower portion of internode, showing formation of secondary wood in "prostoma"; ×10.

Fig. 4.—*C. angulata:* cross-section of juvenile plant, showing occlusion of entrance aperture by callus or "stomatome"; ×11.

Fig. 5.—*C. sciadophylla* var. *decurrens:* cross-section of nodal diaphragm, showing five layers of three distinct types of tissues; ×18.

Fig. 6.—*C. angulata:* Portion of trichilium, showing food bodies and enveloping mat of hairs; ×43.

Fig. 7.—*C. angulata:* cross-section of nodal diaphragm, showing layers of thin walled parenchyma; ×11.

Fig. 8.—*C. angulata:* cross-section of nodal diaphragm, showing early stage in construction of a pitlike excavation; ×18.

Fig. 9.—*C. angulata:* cross-section of nodal diaphragm; soft external layers of medullary tissue removed on both sides, and circular perforation cut through horny layer on under side; ×15.

PROTHALLIA OF LYCOPODIUM IN AMERICA

II. L. LUCIDULUM AND L. OBSCURUM VAR. DENDROIDEUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 300

EARLE AUGUSTUS SPESSARD

(WITH PLATES XVI–XVIII)

In the first paper (4) there was included a short description of a single prothallium of *L. lucidulum;* also reference to what was thought at that time to be the prothallium of *L. obscurum* var. *dendroideum.* The question raised then was later settled in a short announcement published about a year later (5). It is the purpose of the present paper to describe fully the hitherto undescribed prothallia of these two species of *Lycopodium,* and to draw such comparisons as may seem justifiable.

Material and methods

The method of collecting described in the first paper has been followed almost entirely. It was found neither desirable nor practicable to sift or to wash the soil. The smallest desirable prothallia may be secured by the simple means of picking with curved forceps. A patch of soil in the form of a square or rectangle was removed to a depth of 4 or 5 cm., placed in a basket, and carried to the laboratory, where the prothallia were picked out. At frequent intervals small portions were placed under a dissecting microscope for the detection of the very youngest stages. Lying prone upon the ground is quite as good a method, save for the fact that one gets extremely tired after four or five hours of painstaking search.

For fixing, various mixtures of chromic-acetic-osmic acids were used, as well as formalin-alcohol. While the former proved to be somewhat better than the latter, it was not determined what is the best mixture.

The prothallia of *L. lucidulum* are ideal for a study of sex organs, since they are long dorsiventral plants with the organs mixed and growing acropetally from the apex. The main stages of both organs have been secured in a single section. This is rather rare, of course, but a comparatively few sections give one surprisingly good results. Since they can be secured very easily in the field because of their abundance, no other prothallia of *Lycopodium* offer so many advantages at once for class demonstration and laboratory material as these.

Lycopodium lucidulum

Since the prothallium of this species of *Lycopodium* was not known previous to my original brief description, it will not be out of place to give a rather detailed account of it.

LOCALITY

Out of a total of more than 500 prothallia of *L. lucidulum* which were found, only about half a dozen were gathered outside the small region just north by several hundred yards of the Ridge Street Cemetery at Marquette, Michigan. Evidence of the existence of them was found at Mid-Island Point, Sugar Loaf Peak, Negaunee, Munising, Ishpeming, and Michigamme. There is no doubt that any region of the upper peninsula of Michigan will yield prothallia, providing, of course, that it is in general a habitat for the species. It is only because the present investigations were confined to a careful examination of a very limited region that a larger range cannot be given here. This limitation was made primarily to determine the relative abundance of material.

OCCURRENCE

This species of *Lycopodium* produces prothallia in remarkable abundance. It is only necessary to become acquainted with the habitat and to exercise a due amount of patience and vigilance, to obtain a gratifying supply. All the prothallia were found within an area not more than 25 m. square. Within this area there were three especially rich patches, although these were not all discovered the same season. One was found in May, another in September, and the third in June of the following year. One patch measured

19 cm.×16 cm. and yielded 153 prothallia; another, measuring 18 cm.×14 cm., yielded 134 prothallia. The third patch was the most remarkable, being only 3 cm. square, and containing 74 prothallia. In the two larger patches there were 178 sporelings in all, 70 of which bore prothallia. Within the same general area in which the three rich patches of prothallia were found, were five other patches of the same general size, but which produced only sporelings. No effort was made to count them, but they were more numerous than the prothallia themselves, and were so closely crowded that many of them failed to produce more than the first set of leaves. In searching it was found to be much more profitable to seek patches of abundant sporelings than to wander about looking for isolated groups of them, even though these patches often failed to produce any prothallia in spite of the great number of sporelings. From this abundance of sporelings it is evident that not infrequently prothallia occur in even greater quantities than were actually secured from the richest patch found.

Relationship to spore-bearing plants

This investigation confirms the experiences of others who have found prothallia of *Lycopodium*, namely, that they occur most frequently where adult sporophytes are scarce. It seems probable that this situation must be explained by some difference in the soil. Since we know nothing of the conditions which govern the germination and development of *Lycopodium* spores, so far as the soil is concerned, a certain answer to the question is impossible. I have observed, however, that the soil in which prothallia grow is drier than that in which the adult sporophytes are found. This is especially true for the species under consideration. It is certain that an enormous number of spores must find their way to the soil beneath the cone-bearing plants, but the latter grow so thickly that sporelings would have little chance of survival, granting that prothallia had succeeded in growing. The sickly condition of many of the thickly growing sporelings found supports this idea.

As a matter of fact, prothallia do occasionally occur in even the densest growths of adult sporophytes, and it is not improbable that they might be found there in quantity. The fact that investigators consistently fail to uncover prothallia with adult sporophytes where the latter are growing thickly, in the opinion of the writer does not negative the probability, for it is most tedious to hunt the plants under these conditions. BRUCHMANN (1) has investigated old areas of sporophytes probably more carefully than any other investigator, but his method was first to remove the adult plants and then to look for sporelings. In doing this the majority of them would be destroyed. The writer has investigated the older plant areas, both by the method of BRUCHMANN and without first removing the adults. Four prothallia were found by the latter method and none by the former. Since the chief object was to secure prothallia, the waste of time necessary to secure only a few specimens among adult sporophytes caused the writer to abandon such areas.

The question naturally arises as to how the adult plants of *L. lucidulum* reach the habitats which are more moist than that of the prothallia. Let us assume that a prothallium has succeeded in growing on the side of a knoll or a hill, and that at the bottom of this knoll or hill is a moister habitat favorable for the growth of adult sporophytes. When the sporeling breaks through the surface of the ground it faces the hazards of drought and too much sunlight. If these are simultaneous or of sufficient duration, the plant must surely die. The observations of the writer are that this is a constant catastrophe in the struggle of these delicate plants. Occasionally, however, we may expect to find a plant growing under the conditions of a wet season. Then it progresses rapidly and becomes a hardy specimen. When it becomes an adult it is capable of producing spores and gemmae. It seems likely that it is the gemmae that play the chief rôle in further distribution, for when small clusters of these adult plants are found they almost invariably bear numerous gemmae, but rarely sporangia. When the gemmae fall in habitats favorable for the growth of prothallia, they may germinate well, and even form normal sporelings, but it is a significant fact that they appear unhealthy and generally show the incipient stages of extinction. On the other hand, those that reach a moister region, like that at the bottom of our assumed knoll or hill, grow vigorously.

The line of distributive succession is not easily shown in *L. lucidulum*, but a case of *L. obscurum* was found that well illustrates the idea. A sporeling of this species was found that was 10 cm. long, bearing six successive aerial branches and a distinct foot at the end of the underground trailing stem. The sporeling grew on the top of a small knoll, but instead of staying there, it took the shortest path toward the bottom, where adult sporophytes were growing in a moist hollow.

The rôle of prothallia in the life history of *L. lucidulum* is of slight value to the species, therefore, once a colony is firmly established. But for the opening up of new localities, and therefore for the geographical distribution of the species, the gametophyte plays the important part of getting a foothold in regions too unfavorable for vegetative parts, especially gemmae, to flourish. The sporeling itself rarely reaches maturity.

Two facts were observed in the collection of prothallia of various species which seemed to indicate that some agent other than wind takes part in the distribution of spores. The first of these was that the prothallia, in nearly all species, occur in bunches. The second was the frequent occurrence of prothallia in groups of four. The latter condition is explained by supposing that the original group of tetraspores was distributed bodily, and that the four germinated simultaneously. This phenomenon was observed very frequently in *L. clavatum*, *L. complanatum*, and *L. lucidulum*, most frequently in the first.

At the time of the shedding of spores the tetrasporic group is broken up, so that it does not seem probable that these groups are distributed by the wind. The only other explanation which suggests itself is that portions of the plant bearing sporangia are carried away by animals. The appearance of patches of prothallia and sporelings strongly suggests this. An isolated sporeling or gametophyte is rarely found.

HABITAT

Many searches for prothallia have been futile, not so much through a paucity of material as through a failure to learn, by a process of elimination, the exact type of terrain in which they grow most abundantly. It is difficult to describe what this is. In general one must follow the same principles involved while searching for the prothallia of any pteridophyte group.

For the prothallia of *L. lucidulum*, moisture is without doubt the most important factor. Countless millions of spores shed annually from an acre of these plants fail to produce prothallia, either because they are washed away into streams, driven too deeply into the soil, lodged in water logged depressions, or are subjected to dry conditions before and after germination. It is only when they fall upon a region whose sandy substratum is covered by a thin layer of leafmold, and is sufficiently protected from the seasonal drought and from inundation, that they germinate and produce prothallia. Such a place is a hillside or a hilltop with scattered trees, very little shrubbery, and a scattered representation of such herbs as the squawberry, wild sarsaparilla, *Clintonia borealis*, and *Polygala*. Very often grasses and *Polytrichum* are found in such a habitat. A sandy knoll with sparse vegetation and with a swamp at its base is an excellent locality.

Bruchmann found many of his prothallia of *L. Selago*, a related species, in mats of moss. The writer found very few in such places. The rich patches just described lay on a sandy hillside and were protected only by the leaves which fell the previous autumn. The soil was a rich humus for a depth of 1 cm., with almost pure sand beneath. Although hundreds of sporelings were found, many were in a desiccated condition, and only a very few would ever have become adults, for adult plants habitually occur in regions a little more moist than where the prothallia and sporelings were found.

Because the prothallia of this species of *Lycopodium* were found growing in such a variety of places, the following list of these may be valuable: (1) in a hole at the base of a living birch tree; (2) among the decayed needles of *Pinus resinosa;* (3) under a hard maple tree; (4) on the top of a rotten stump; (5) on partly shaded hillsides; (6) in the sand under a patch of *Polytrichum* and *Polygala;* (7) in sand scarcely covered at all with humus; (8) in moldy humus (most abundantly); (9) in muck, at the edge of a permanent pond; (10) in the wheel tracks of an abandoned forest road.

It might be well to add that the habitat of the prothallia of this species of *Lycopodium* is somewhat more shaded, more moist, and more protected from sunlight than that for *L. clavatum*, *L. complanatum*, and *L. annotinum*. The prothallia of five species have been found within a few centimeters of each other, however. They are most abundant at a depth of 1 cm., and seldom grow at a depth of more than 2 cm. Very frequently they occur upon the surface of the ground. The habitat of these prothallia agrees in all essential respects with that for *L. Selago*, with the exception already mentioned, which seems to indicate that they require a slightly drier place for growth than do those of the latter.

DESCRIPTION

The older regions of the prothallia are brownish, while the young growing tips as well as the very young prothallia are white. It is these very white tips that are first noticeable when digging in the soil. They are closely beset with multicellular hairs (fig. 49), which in some cases include as many as five cells. They are evidently of a glandular nature, for in fresh specimens of prothallia the region which bears them is covered with a thin mucilaginous secretion slightly denser than water. If the prothallia are placed in water, this substance quickly swells and is dissolved. Whatever its nature, no doubt it plays the important rôle of keeping the young growing region and the young sex organs from becoming dried. None of the fungus filaments which closely adhere to the exterior of other parts of the prothallium ever pierce it, nor do soil particles or leaf mold adhere to it. It is probable that the growing apical region is lubricated sufficiently by this substance to prevent injury during its upward passage through the soil and mold, which are often very compact and solid.

The adult prothallia vary very much in size and shape (cf. figs. 2–41). These figures are all drawn to a scale of four, so that they are just twice the size of the original specimens.

The form of the prothallium does not conform exactly to that of any type heretofore described. It approaches nearest that of *L. Selago* described by BRUCHMANN. Taking fig. 15 as the type form, it will be noticed that there is a lower cup-shaped primary region, from which has developed a more or less dorsiventral protuberance which becomes cylindrical near its apex only. In no case were there found the two forms of body as described for *L. Selago*, namely, the cylindrical or "ninepin" form and the flattened form. All the specimens of the vertically growing prothallia of *L. lucidulum* might roughly be considered as shaped like a "ninepin" or an Indian club, but upon close examination it has been found that sex organs grow only on one side, the rhizoids opposite and lateral to them, and that the symmetry is distinctly dorsiventral. It is only when active growth is taking place that the apical region ever becomes cylindrical at all. Fig. 51 shows a longitudinal section through the apex of a prothallium such as that shown in figs. 15 or 49. This bilateral symmetry is present from the very beginning of the differentiation from the meristem tissue.

If the prothallia grow upon the surface, however, they are distinctly of the *L. clavatum* or *L. annotinum* type (figs. 33, 35). Even when growth continues after a subterranean prothallium has reached the surface, this growth produces an expanded portion which rests upon the ground (figs. 34, 37, 39). In other words, when the length of the vertical portion is eliminated so that the primary tubercle lies immediately beneath the prothallium, we have a type in no essential respect different from that of *L. annotinum*. Figs. 32 and 37 show prothallia semisurface and semi-subterranean in development. In many instances (figs. 25, 28) horizontal growth is solely below the surface, and it is not until near maturity that the apical point turns abruptly vertical. In such instances the prothallia are evidently of the *L. annotinum* type originally, and later change.

In short, there are three methods of growth: one in which growth is entirely vertical; one in which growth is first vertical, then horizontal; and another in which growth is first horizontal and then vertical. In several instances growth was originally vertical, later assumed the horizontal, and still later resumed the vertical.

While a prothallium of *L. Selago* has never been examined, I cannot see from the figures of BRUCHMANN any evidence of a typically "conical" or "ninepin" shape for that species of prothallium. His fig. 32, plate VI, is the nearest approach to it, but the form is far from being typical, as the drawings to the left of it show. Even in fig. 32 the sex organs occur on one side, while the vegetative organs are mainly opposite and lateral to it. Apparently the only difference between the form of the prothallia of the two species in question is the alleged conical shape of *L. Selago* prothallia, and I am not able to see that BRUCHMANN'S figures admit even of this difference. If I were to use the same method of drawing as he has, it would be almost impossible to distinguish between the typical forms of prothallia of the two species. BRUCHMANN'S figs. 22, 28, and 29 are especially easy to match with prothallia of *L. lucidulum*. My figures merely accentuate the different regions of the prothallia, while his suggest them.

For the present, therefore, the prothallium of *L. lucidulum* is placed with BRUCHMANN'S type for *L. Selago*, but it is probable that the necessity for such a type is one of convenience rather than a morphological one. For the sake of reducing the growing number of types of prothallia for the genus *Lycopodium*, it might be well to include these representatives of the *L. Selago* type in the *L. clavatum* type.

Chlorophyll occurs regularly and abundantly in the subepidermal cells of all prothallia of *L. lucidulum* which were found growing upon the surface. Four prothallia were found which had bifurcated. The age of the prothallia varied greatly, as the figures show. There seems to be some evidence that the prothallia of *L. lucidulum* have a much shorter period of development than BRUCHMANN found for his European species. Solely from field observations I should estimate that adult prothallia for *L. lucidulum* probably mature within two or three seasons, but the period for *L. obscurum* must be much longer.

TISSUES

A longitudinal section of a prothallium like that shown in fig. 50 shows two main regions, an upper and a lower. The upper region is divided into a distinct epidermis, which bears the sex organs and paraphyses, and a subepidermal mass of cells of parenchymatous structure which contain chlorophyll (when this is present) in its upper half and reserve starch in the lower half. A cross-section is shown in fig. 64. This set of tissue, therefore, is reproductive and assimilative in function, in its upper portion, while its lower portion is employed for the storage of food material. The starch grains are compound. While they occur most abundantly in the lower half of the upper set of tissues, very frequently they are found also in the cells immediately below the epidermis, in the venter of the archegonium and the cells of the fungus regions (fig. 64). Approximately the lower half of the prothallium is inhabited by the endophytic fungus. This will be described later.

The meristematic tissue (figs. 51, 52) lies at the apical end of the prothallium in a depression between the two groups of tissues just named. A single apical cell is probably not present. Growth is apparently brought about by periclinal and anticlinal division of a small number of cells which form a meristematic plate between the youngest portions of the upper and lower sets of tissues. The mitotic figures of fig. 51 show that division continues in cells which lie at a considerable depth. The lobed-like mass of tissue which bends downward over the apical region and which bears the primordia and older cells of the sex organs is caused by this division of the interior cells, as well as by the rapid division of the more superficial ones. The cells of the meristematic plate, from which are cut off immediately the vegetative and reproductive primordia, are filled with dense protoplasm and numerous oil drops (fig. 53).

Rhizoids are very abundant, and are simple elongations of epidermal cells on the lower side. No case was observed in which a rhizoid was cut off by a cross wall.

Sex organs

The prothallia are monoecious. The sex organs appear in acropetal succession (fig. 51), the older ones being located (fig. 50) in the tissue lying above the primary tubercle. Both antheridia (figs. 54–58, 62) and archegonia (figs. 42–47) develop by the usual stages known for the genus. The largest number of canal cells found was four. A double row of these was not observed. The ventral canal cell is very much flattened at maturity. The egg is slightly oval at maturity, with the long axis perpendicular to the canal. It almost completely fills the venter. The neck of the archegonium is short, and the venter lies completely below the level of the epidermis. The neck is not provided with a cover cell. It opens by the dissolution of the inner walls of the four terminal cells which border each other at a common line. As soon as fertilization takes place further development of archegonia normally ceases. Consequently two embryos on the same prothallium are rarely found, and since fertilization occurs after the opening of the canal, the embryo therefore lies very near the meristematic region. The growth of the embryo causes activity there to stop, and as a result it is found as a rule at the end of the prothallium.

Antheridia appear earlier and more abundantly than archegonia, and are scattered throughout the entire upper surface of the prothallium. They are circular or oval in outline, and may be submerged entirely or may form a slight elevation on the upper part of the prothallium. They are isolated and never form antheridial masses similar to those very characteristic of *L. obscurum* to be described later. Figs. 58 and 59 show the latter stages in the development of the sperm.

The sex organ primordia lie so close together that it is impossible always to say what kind of organ will develop. They may be separated by a single layer of cells, or, as Miss LYON (3) observed in *L. annotinum*, they may touch one another. They are evidently of such a primitive nature that it is impossible to distinguish an antheridium from an archegonium in the very earliest stages of their development. There are some indications that the archegonial initial is slightly larger and longer than the antheridial initial, but this is so uncertain that it is useless as a criterion.

As a result of this primitive condition, mixed sex organs are very frequently found. These may be normally shaped antheridia in which a few cells have never divided into sperm mother cells, or they may be archegonial in form, the neck filled with normal spermatogenous tissue, and the ventral canal and egg cells of female appearance (fig. 60). The opposite condition may also occur (fig. 61). Figs. 60 and 61 are sketches of two archegonia constructed from camera drawings of nine sections in serial order. Six abnormal organs of a bisexual nature were found on one prothallium. They of course always occur near the apex of the prothallium. The fact that sexual organs of a bisexual nature occur on plants where primordia of sex organs are apparently alike in position, suggests that we may be dealing with one of the primitive stages of sex organ development. HOLFERTY (2) found the same indifferent situation in *Mnium cuspidatum*. Miss LYON brought together considerable information on several genera of pteridophytes regarding the condition in abnormal archegonia. A longitudinal section is best for the study of sex organs.

Only the first division in the development of the prothallia from the spore was observed. Fig. 1 illustrates the single specimen which was found in field material.

Lycopodium obscurum dendroideum

The prothallia of this species were most difficult to find. The sporelings have an appearance so much like those of *L. complanatum* that a certain abnormally growing prothallium of the latter species was described in my first paper (4) as that of *L. obscurum*. This error was corrected as soon as discovered (5). A total of thirty-seven prothallia were found. Twelve were dug up in a patch 2 feet square near the locality described for *L. lucidulum*, and very near to a juniper bush. The others were found near Mid-Island Point, Michigan. The exact spot lies just behind a row of cottages, about 300 yards from Lake Superior and in an alder clump between the lake and the marsh to the east of it. This spot was visited first in July and later in August of 1917. It was very dry at this time, but is probably water-logged for at least two months of the year. Twenty-five prothallia of *L. obscurum*, fifteen of *L. complanatum*, and six of an undetermined variety of *L. clavatum* were found in an area not more than 10 feet square.

The soil in the first locality contained very little humus in the sand. A species of grass and *Polytrichum* grew about the spot, which was fully exposed to the sun, and no leaf mold was present. The sand was yellowish a short distance below the surface, and rather compact. The spot rests on a ridge about 15 feet higher than the surrounding land. The lake bed once covered this point, and it is probably an old sand bar.

The soil of the second locality could hardly offer a more contrasting condition as to physical appearance. The subsoil of course was sand, but this was covered by a soil mixture of half leaf mold and half muck, covering lake sand. As a matter of fact, some of it was nearly pure mycelial threads. Other parts were as hard as dry muck itself. The prothallia found here grew for the most part in the intensely moldy soil. Some were found at the border between the humus layer and the sand lying beneath it. From this it will be seen that one cannot name a type of soil as a criterion.

If it is desirable to indicate the type of place to look for *Lycopodium* prothallia, I should say that one should look in neither the dry nor the wet places. For my own part I search in those spots a little less moist than the habitat of the adults. In the case of *L. obscurum* and *L. clavatum*, very often the reverse is true, but just as one cannot give a precise type of soil and moisture content for the sporophytes, so he cannot for the gametophytes.

Sporelings were found in a variety of other places near Marquette and Munising. The prothallia of *L. obscurum* probably grow abundantly wherever the sporophyte has been known to exist for a long time and in considerable quantity. Two prothallia and one sporeling were found in July 1922, near Rhinelander, Wisconsin. A sporeling and an old prothallium of *L. complanatum* were found in June 1922, near Pembine, Wisconsin.

DESCRIPTION OF PROTHALLIA

The prothallia are of the *L. annotinum* type and not of the *L. complanatum* type suggested in my first paper (4). They are about as smooth as the *L. annotinum* prothallia, and never as wrinkled or lobed as those of *L. clavatum*. While the first are yellowish brown and the second dark brown, those of *L. obscurum* are reddish brown. They are also somewhat larger than those of the other species. The largest one found measured 8×12 mm.; the smallest was about the size of a pin head. Figs. 65 to 71 show them just twice the natural size. Owing to the fact that the rhizoids are so few that the soil scarcely clings to them, they may be distinguished by this means also. The young of the three species just mentioned and *L. complanatum* cannot always be distinguished, but identity of species can generally be determined by the habit of growth of the endophytic fungus. Confusion of species is sometimes unavoidable, however, and this is especially true when prothallia of several kinds and of young age are found in the same lump of soil. The writer discovered such a mistake after a four months' study of a single slide. Certain features relative to the fungus were found on it that did not occur in any of the other specimens to which it purported to belong. In fact, it was this discrepancy that led to the discovery of the mistaken identity of the prothallia under discussion. The habit of growth of the endophytic fungus will be discussed later.

Fig. 70 shows a specimen of *L. obscurum* which looks like *L. complanatum.* The color and endophytic fungus both place it with the species assigned. Furthermore, the remarkable habit of growth of the antheridia was found present here, although the drawing does not show it. The enlarged antheridial mass shown in fig. 81 was taken from a specimen of a large *L. complanatum* prothallium. Both were rotted away at the lower end. Now no published figure of the last named species of prothallium shows that antheridia grow in such enormous masses. These facts can mean only one of two things, namely, that the fact of antheridial mass growth has not been observed, or that the form of *L. obscurum* prothallium is really intermediate between the *L. complanatum* and *L. annotinum* types. In view of the fact that other observers of *L. complanatum* prothallia were unlikely to overlook so conspicuous a feature, I am inclined to the view that the prothallia of *L. obscurum* occasionally take the intermediate position between the two well known types. After careful examination of the specimens found there is small likelihood of mistaken identity. If this be true, what I have called a prothallium of *L. obscurum* in my first paper (4) may need no modification. Moreover, the prothallia of *L. Selago*, *L. lucidulum*, and the peculiar specimen of *L. obscurum* all show a lateral groove more or less extensive, and are dorsiventral.

Sex organs

The antheridia grow in enormous masses of white beadlike knobs. These may half encircle the entire prothallium in the adult, or may cause the very young to resemble cornucopiae. Such structures have not been observed by the writer on any other prothallia with certainty. Certain prothallia previously figured (4) seem to suggest them, but at that time prothallia of *L. obscurum* were unknown, and identification was made upon comparison with figures published by other writers. It is entirely possible that those specimens may have been misnamed. There is also the possibility that antheridia may occasionally take the form mentioned in species other than the one under discussion. Figs. 79–81 show the habit of growth of these organs. The prothallia are all shown under a magnification of ten, so that a glance at the figures will give the relative size of the individuals figured. Fig. 81 shows a mass enlarged.

A cross-section of the prothallium through two antheridial ridges is shown in fig. 74. It is apparent that the masses arise from the tissue lying within the outer border ring. For sake of clearness, one region is represented as without the endophytic fungus. As a matter of fact the fungus does invade the antheridia through the opening shown by the opercular cells in figs. 82 and 89. It has not been located for certain in the cells of the antheridia walls, but it is possible that the fungus is present in the intercellular spaces. Because of the intimate relationship between the sperms, and possibly the wall cells, with the fungus, it seems very likely that the unusual growth of antheridial masses may be explained by symbiosis. There is no indication of a pathological condition of the sperms. The writer has never seen the fungus in the antheridia of any other species of prothallia of *Lycopodium*. It is well known that an endophytic fungus present in the prothallia of certain ferns will cause abnormal growth of tissue.

The individual antheridia arise acropetally from the groove. There is no primordium for the mass. This is a secondary growth, although it arises very early. Figs. 87 and 88 show the antheridium initial and the first division. The mature detail is shown in figs. 82 and 89. In this species four opercular cells may often be observed. I have never seen it for certain in any other species. The figures representing these cells were made by camera lucida.

In all of the prothallia sectioned, numerous retarded antheridia were observed. They were distributed throughout the older regions of the plant, and had the ordinary appearance of the male organs, but the mother cell walls were extremely weak and the nuclei small. They appeared starved. The endophytic fungus was not demonstrated to be present in them, and it is very probable that they originated in the usual way.

Mitosis does not occur simultaneously throughout the individual antheridia. The organ is divided into quarters, and all the cells of a single quarter will show the same phase, but one earlier or later than the neighboring quarter.

Archegonia

The stages in the development of individual archegonia are the same as those described for the genus. A few of the stages are shown in figs. 83–86. The neck is rather long, containing as many as fourteen canal cells. As a rule these are double, or at least part of them are. Even the ventral cell is involved in this division (fig. 86). In this organ the cells are beginning to disintegrate, as shown by the swollen walls. The position of such an organ relative to the prothallium is shown in fig. 90 on the cut edge. That the doubling of the neck canal cells occurs early is shown by fig. 85. One instance was observed of a very abnormal archegonial growth. Six organs arose from the prothallium (fig. 90), which were all apparently normal, near maturity, and about the same age. There were traces of the endophytic fungus in the canals. The fungus occurs normally in all the archegonia. It is probable that this peculiar massing of archegonia is to be explained on the same grounds as the antheridial masses. It would be instructive to know how frequently this occurs. Unfortunately the mass was not discovered until sections had been made, and the topographic view had to be made up from serial sections.

The exact morphological position of the sex organs, especially the antheridial masses, should be made plain. From a superficial view of fig. 80, it might appear that the upper mass is derived from the border, but the section shown in fig. 74 should make the matter clear. It was rather difficult to determine the origin of the mass. Antheridia of all ages are found in any one, but the primordia always are found at the outer junction of the mass and the prothallial tissue beneath. Nevertheless, certain sections showed that the primordia were slightly raised above the surface, as though dragged there by rapid growth of the surrounding tissue. While I am satisfied that there is no definite primordium for the mass, I am not at all certain that the mass itself may not begin development before the antheridial primordia appear; at least convincing evidence was not found. If the fungus is responsible for the growth, almost any order may be expected in the development of some individual organs. It was not possible to be certain of the relationship between the developing mass and the developing antheridia. The organs are so numerous that only one of two things can occur, either the whole group must spread itself, or most of the individuals must be absorbed. There is conclusive evidence in the figures that few are absorbed.

Endophytic fungus

The study of the endophytic fungus has delayed the publication of this paper considerably. The following account refers to that found in the two species of prothallia under discussion. Plate 00 has been added to show some of the structures observed. The identity of the fungus has not been established. It does not appear to be the same in both species. Both the reproductive structures and the habit of growth support this statement. Furthermore, there is evidence that they may both be Ascomycetes.

The illustrations are arranged so that the fungus of *L. obscurum* is represented in the upper half of the plate (figs. 91–110), and that of *L. lucidulum* in the lower half (figs. 111–126). Each group is subdivided into reproductive and vegetative structures. No attempt has been made to insure the morphological identity of any structure represented. In the *L. obscurum* group, figs. 94–105 are supposedly reproductive structures, and figs. 106–110 vegetative. In the *L. lucidulum* group, figs. 113–117 and figs. 122–126 are supposedly reproductive, while figs. 118–121 are vegetative. The structures shown in figs. 124 and 125 were found in both types of prothallia. They are all shown under the same magnification, and are collected from over 500 drawings.

All the reproductive structures, except in figs. 124–126, were intracellular. The vegetative structures were intercellular. The ascomycetous growth of fig. 124 was very common, but always was found just outside the epidermis. It was found connected with the intracellular mycelium, however. The mass shown in fig. 125 was often observed in decayed regions of the prothallia. Since the "spores" shown in fig. 126 were found in such a mass, it is probable that the fungus sometimes destroys the prothallium in the older parts, to distribute its own spores. At any rate, many prothallia of *L. complanatum* were found with the entire lower half rotted away, and all the prothallia of *L. obscurum* sectioned, and a few of *L. lucidulum* had rotten holes in them which were filled with the fungus.

HABIT OF GROWTH

The fungus enters the prothallium either through the rhizoids or between the epidermal cells. To say that it enters is not exactly accurate. It may be possible to show that many of the "entering" mycelia are in reality leaving the prothallium. It undoubtedly gets a foothold during the earliest life of the plant. To within two or three cells of the meristematic cells it is established (figs. 50, 51), which shows that it is eminently capable of taking care of itself, once it has obtained a foothold.

In *L. lucidulum* the fungus occupies the region shown in fig. 111, but there is one peculiar thing to be noted. Only about half of the cells contain the mycelial threads, the other half contain bodies which in some respects resemble spores (fig. 112). These show no nuclei under various stains. With safranin and aniline blue the walls reacted like cellulose. In a few cases minute red chromatin-like granules appeared differentiated, but these were never seen after iron alum haematoxylin; consequently, it is impossible to state just what these bodies actually are. In a few instances very minute mycelial threads were seen to radiate from pores in the walls of these bodies, as though they were germinating spores. These were always seen in section and cannot alone be considered conclusive evidence that they are indeed such structures. So far as this investigation goes, it cannot be said that they do or do not have any relation to the endophytic fungus. They are such a constant structure associated with the fungus, however, that some connection seems extremely probable. One other fact should be noted. The obviously reproductive structures, found abundantly in the cells which lie next to those containing the sporelike bodies, are found without these sporelike bodies in the region neighboring the meristematic tissue. In other words, the sporelike bodies are not found in the cells which abut the meristematic cells. Since the structures which looked like germinating spores and appeared to contain chromatin also appeared in those very cells where the typical sporelike bodies were wanting, the evidence is still stronger for the spore interpretation. A few microchemical tests were made to determine the exact nature of the cell wall of these bodies, but nothing definite was established.

In *L. obscurum* the habit of growth of the fungus is markedly different. The reproductive bodies are larger, and there are none of the small sporelike bodies so persistent in *L. lucidulum.* All the cells of the infected region contain the mycelium, but it is much less extensive than in *L. lucidulum* (figs. 91, 93). Here there are two lower cells with a very regular and tight coil of the mycelium, which is much denser than shown in the illustration, where clearness was desired. Above this layer is a region of finer coils, generally about one cell in thickness. The coils change their orientation slightly in the next layer above, until those of the uppermost layer are at right angles to those of the lowest layers. The reason for this is probably one of absorption, for the mycelial threads finely divide and closely abut the walls of the palisade tissue which lies above and which contains reserve starch. These fine threads were never observed to enter the palisade cells, nor did their tips show swelling. This very evident difference in habit constitutes my first reason for believing that the fungus in the two species of prothallia under discussion is not the same. The second reason involves the reproductive-like structures. A glance at these structures at the top and bottom of the plate will reveal organs drawn to the same scale, but vastly different in detail. The only possible comparison may be found in figs. 97 and 125. The latter may be only a later stage of the former. The lefthand cell of fig. 112 when compared with fig. 97 shows the contrast well. The organs of the last named figure occur only in the lower cells of fig. 93, but those of fig. 112 are equally distributed over the fungus region of *L. lucidulum.*

In spite of these observations, there is another which needs to be noted, and which may cast doubt upon them. The structures shown in fig. 124 were found on the epidermis of both kinds of prothallia. That shown in fig. 125 was never found except in the decayed portions of both kinds of prothallia. I suspect that the latter is only the maturer stage of the former. The "spores" of fig. 126 were drawn from a ruptured heavy walled structure shown in the lower lefthand corner of fig. 125. There were sixteen spores. All these facts point to the ascomycetous nature of the fungus, but since these last named structures were all found without the epidermis or within decayed regions where external fungi would have access, it is conceivable that they have no relation whatever to the endophytic fungus. The internal mycelium was so constantly segmented, however, that the Oomycete group has little chance of being recognized.

While the evidence presented is in no respect conclusive, it throws some doubt upon the *Pythium* theory as to the identity of the endophytic fungus of *Lycopodium* prothallia. The writer dislikes very much to leave so important a question unsettled, but it seemed best at least to record what had been observed.

Summary

1. The prothallia of two more species of *Lycopodium* have been discovered.

2. The sex organs of *L. lucidulum* are primitive, being frequently mixed in nature.

3. The prothallium of *L. obscurum* var. *dendroideum* shows a form transitional between the *L. annotinum* and *L. complanatum* types.

4. The sex organs of the last named species are invaded by the endophytic fungus and consequently show deformities. The antheridia and rarely the archegonia occur in enormous masses.

5. The sex organs develop individually as described for the genus, and acropetally in the two species.

6. The endophytic fungus is probably not *Pythium*. The reproductive structures point to this genus, but the vegetative and certain doubtful structures point to the Ascomycetes.

7. The habit of growth, and the appearance of the reproductive structures, indicate that the same species of fungus is not present in the two prothallia.

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LITERATURE CITED

1. BRUCHMANN, H., Über die Prothallien und die Keimpflanzen mehrerer Europraischer Lycopodien. pp. 119. *pls. 1–8.* 1898.
2. HOLFERTY, G. M., The development of the archegonium of *Mnium cuspidatum.* BOT. GAZ. 37:106–126. *pls. 5–6.* 1904.
3. LYON, FLORENCE, The evolution of the sex organs of plants. BOT. GAZ. 37:280–293. 1904.
4. SPESSARD, E. A., Prothallia of *Lycopodium* in America. BOT GAZ. 63: 66–76. *figs. 21.* 1917.
5. ———, Prothallia of *Lycopodium* in America. BOT. GAZ. 65:362. 1917.

EXPLANATION OF PLATES XVI–XVIII

PLATE XVI. PROTHALLIA OF L. lucidulum

FIG. 1.—Germinating spore, with first wall transverse; found in field.

FIGS. 2–41.—Habit sketches of various typical prothallia, some of which bear sporelings attached; ×2.

FIGS. 42–48.—Stages in development of archegonium; ×375.

FIG. 49.—Tip of prothallium, showing five antheridia and multicellular hairs.

FIG. 50.—Longitudinal section of prothallium.

FIG. 51.—Apex of same, showing acropetal succession of sex organs; meristem plate of cells lies just behind first initial.

FIG. 52.—Detail of meristem and initial plate; ×375.

FIG. 53.—Single cell from meristem region; ×775.

FIGS. 54–57, fig. 62.—Stages in development of antheridium and sperms; ×375.

FIGS. 58, 59.—Stages in sperm development; ×775.

FIGS. 60, 61.—Mixed sex organs, reconstructed from serial camera drawings; ×375.

FIG. 63.—Longitudinal section of embryo; ×85.

FIG. 64.—Cross-section of prothallium, showing distribution of starch grains.

SPESSARD on LYCOPODIUM

SPESSARD on LYCOPODIUM

SPESSARD on LYCOPODIUM

PLATE XVII. PROTHALLIA OF *L. obscurum*

FIGS. 65–70.—Habit sketches of typical prothallia; ×2.

FIG. 71.—Sporeling growing from prothallium which grew in inverted position; natural size.

FIG. 72.—Germinating spore containing oil drops; ×775.

FIGS. 73–80.—Moderately young and mature prothallia in topographic view; ×10.

FIG. 81.—Antheridial mass enlarged, as seen when pressed out under cover glass; ×85.

FIG. 82.—Opercular cells of antheridium; ×375.

FIGS. 83–86.—Stages in development of archegonium; ×375.

FIGS. 87–89.—Stages in antheridial development; ×775.

FIG. 90.—Diagrammatic reconstruction of abnormal archegonial group, constructed from serial camera drawings.

PLATE XVIII. ENDOPHYTIC FUNGUS

(All figs. ×775 diameters, except figs. 91 and 111, which are × about 80.)

Endophytic fungus of *L. obscurum*

FIG. 91.—Distribution of fungus in prothallium.

FIG. 92.—Two mycelial threads within rhizoid.

FIG. 93.—Three special layers of cells containing the fungus; mycelial threads of upper layer applied closely to walls of starch-bearing cells; reproductive cells, when present, found only in two lower layers.

FIGS. 94–105.—Intracellular reproductive structures.

FIG. 106.—Branch of intercellular mycelium leaving prothallium at base of rhizoid.

FIG. 107.—Cross-section of a rhizoid containing six mycelial threads.

FIGS. 108–110.—Intercellular vegetative structures.

Endophytic fungus of *L. lucidulum*

FIG. 111.—Distribution of fungus in prothallium.

FIG. 112.—Two neighboring cells; one at left contains mycelial threads bearing reproductive structures, while other has no threads, but contains numerous spherical structures whose nature has not been determined.

FIGS. 113–117.—Various reproductive structures, intracellular.

FIGS. 118–123.—Vegetative structures, intercellular.

FIG. 124.—Ascomycetous-like structure found on epidermis of both species of prothallia, but connected with internal mycelium.

FIG. 125.—Degenerated prothallia cells, with reproductive structures.

FIG. 126.—Sporelike bodies found several times in mass of fig. 125; suggestive of ascospores.

NEW SOUTH AMERICAN ASTERACEAE COLLECTED BY E. W. D. HOLWAY

S. F. BLAKE

(WITH PLATE XIX)

The new species of Asteraceae here described form part of a collection made by E. W. D. HOLWAY and Mrs. MARY M. HOLWAY in Ecuador and Bolivia in 1920. A few have previously been found by other collectors, notably by J. N. ROSE, who visited a part of the same region in 1918. Although Professor HOLWAY'S interest is primarily in rusts rather than flowering plants, his appreciation of the value of precise identification of host plants has resulted in the preparation of complete and well dried specimens which have considerably increased our knowledge of the phanerogamic flora of the regions in which he has collected. Not the least valuable result of his work in Central and South America is the discovery of many new species of flowering plants, particularly Asteraceae, represented by specimens far superior to those of many collectors who confine their attention to phanerogams.

Achyrocline glandulosa, sp. nov.—Perhaps suffrutescent below, 40 cm. high and more, branched; branches ascending, simple, like the stem densely glandular-pubescent, winged throughout by the decurrent leaf bases, the wings herbaceous, 1–1.5 mm. wide; leaves lanceolate or linear-lanceolate, 3.5–6 cm. long, 3.5–7 mm. wide, acuminate, not narrowed at the long-decurrent base, entire, green on both sides, densely glandular-puberulous, arachnoid-ciliate, tripli-nerved; panicles rounded or flattish, 3–7 cm. wide, dense, the heads densely glomerate on the branchlets, the glomerules at base loosely pilose-tomentose like the branches of inflorescence; involucre oblong-cylindric, 4–4.5 mm. high, whitish or pale straw color, the phyllaries somewhat graduated, scarious, oblong or elliptic-ovate, obtuse, glabrous; ♀ flowers 4 or 5, ☿ 2; receptacle alveolate; ♀ corollas tubular-filiform, whitish, glabrous, 4-dentate, 3 mm. long, the style included; ☿ corollas slender, glabrous, 3 mm. long, 5-toothed, the throat slightly ampliate above; achenes oval, glabrous, 0.7 mm. long; pappus 3 mm. long, the slender bristles denticulate above, deciduous singly or in pairs.

ECUADOR.—Cuenca, September 10, 1920, *E. W. D.* and *M. M. Holway* 982 (type in U.S. National Herbarium no. 1058640).

Allied to *A. alata* (H. B. K.) DC., which has fuscescent phyllaries and leaves with long scattered hairs above. No reference is made in the description of that species to the glands which are so abundant in *A. glandulosa*, and which give it the fragrant odor of *Gnaphalium obtusifolium*.

Achyrocline hyperchlora, sp. nov.—Slender herb, 40 cm. high and more, branching, the base not seen; stem and branches flexuous, wingless, stipitate-glandular and scantily arachnoid-tomentose, glabrescent; leaves elliptic-lanceolate, 3–5.5 cm. long, 6–15 mm. wide, acuminate or acute, cuneate into a petioliform margined base decurrent for about 1 mm., entire, membranaceous, triplinerved, glandular on both sides, above green, pubescent with several-celled hairs, loosely arachnoid, glabrescent, beneath persistently griseous-arachnoid-pilose; panicles loose, much branched, leafy bracted, the heads in small glomerules, involved in wool at base; involucre 3 mm. high, whitish faintly tinged with straw color, the outermost phyllaries brownish toward base, the phyllaries oval to oblong, obtuse, scarious, stipitate-glandular and lanate-pilose toward base; ♀ flowers 4, ☿ 1; ♀ corollas whitish, tubular-filiform, stipitate-glandular at apex, 1.8 mm. long; ☿ corollas similar but thicker, 5-dentate; achenes immature; pappus 2 mm. long.

BOLIVIA.—Cochabamba, March 14, 1920, *E. W. D.* and *M. M. Holway* 406 (type in U.S. National Herbarium no. 1058598).

This species has the same sweet odor as *A. glandulosa*. It is of the *A. vargasiana* group, and is distinguished especially by the character of its leaves and its loose panicle.

Polymnia eurylepis, sp. nov.—"Slender tree 4–5 m. high"; branches stout, sulcate, densely hirsute-pilose with several-celled sordid hairs and somewhat glandular-puberulous, glabrescent; leaves opposite; petioles rather narrowly margined, densely pubescent like the stem, 1–3.5 cm. long; blades broadly ovate or rhombic-ovate, 7–19 cm. long, 4.5–11.5 cm. wide, acuminate, at base broadly cuneate, then gradually cuneate to the base of the petiole, remotely denticulate (teeth minute, about 5 mm. apart), submembranaceous, above densely glandular-puberulous along the nerves, essentially glabrous on surface, beneath duller green, evenly but not densely hirsute-pilose, densely so along the veins and somewhat glandular there, triplinerved above the base and loosely prominulous-reticulate beneath, bullate in age above; heads 2 cm. wide, very numerous in panicles 9–19 cm. wide; bracts ovate, 6–15 mm. long; pedicels often decurved, glandular-puberulous and hirsute-pilose with several-celled hairs, 1–3.5 cm. long; disk subglobose, 6–8 mm. high, 8–13 mm. wide; outer phyllaries 5, broadly ovate, acutish, thin-herbaceous, ciliate, otherwise glabrous, 6–9 mm. long, 4.5–6 mm. wide; inner phyllaries (subtending the rays) oblong-oval, obtuse, membranous, hirsute-pilose, 6 mm. long; rays 12–14, yellow, exceeding the involucre, the lamina oval, 7 mm. long, 3.5 mm. wide, the tube densely hirsute-pilose; disk corollas yellow, sparsely hispid-pilose, 3.8 mm. long (tube 1.8 mm., throat broadly campanulate, 1.5 mm., teeth 0.5 mm.); pales acute, vittate, sparsely hirsute-pilose dorsally, 3.5 mm. long; ray achenes obliquely turbinate-subglobose, somewhat compressed, blackish, glabrous, 3 mm. long.

VENEZUELA.—Santo Domingo, altitude 2200 m., December 2, 1910, *Alfredo Jahn* 128.

ECUADOR.—Along fences, Cuenca, September 10, 1920, *E. W. D.* and *M. M. Holway* 974 (type in U.S. National Herbarium no. 1058639); Ficoa, near Ambato, February 1919, *A. Pachano* 135.

This species is related to *P. lehmannii* Hieron., which has broadly margined petioles, merely glandular-puberulous pedicels, smaller outer phyllaries (5.5 by 1.75 mm.), which are puberulous at base, and 7–10 rays. It is also near *P. arborea* Hieron., but in that species, represented in the National Herbarium by fragments from the type, the somewhat larger outer phyllaries are densely stipulate-glandular and sparsely hirsute-pilose on the back. HOLWAY'S label gives the height of the plant as 12–15 ft. PACHANO calls it a "slender tree," and gives the vernacular name as "polaco"; JAHN gives the name "aníme."

Monopholis, gen. nov.—Shrubs with large alternate serrulate or subentire leaves and many-headed terminal panicles of small pale yellow cylindric heads; heads homogamous or heterogamous, the disk flowers hermaphrodite, fertile, those of the ray when present pistillate; involucre about 3-seriate, graduate, passing into the pales, the phyllaries chiefly linear-elliptic or elliptic, indurated-subherbaceous, appressed; receptacle small, convex; pales firm, subindurate, persistent, all or the inner with thinner inflexed margins, more or less completely inclosing the achenes; ray corollas usually none, when present solitary, ligulate, fertile, the lamina oval, bidenticulate to trifid; disk corollas with slender tube, short broadly campanulate throat, and 5 equal or longer reflexed teeth; stamens with minutely sagittate base and ovate terminal appendages; style branches rather short, slightly recurved, with deltoid obtuse papillose-hirsutulous tips; achenes linear-fusiform, narrowed to the callous base, often shortly subrostrate, more or less compressed, lenticular or trigonous in cross-section, wingless, glabrous; pappus of a single usually broad deltoid to rhombic-lanceolate or oblong paleaceous persistent awn borne on the inner side of the achene, or rarely wanting.—Type species *M. hexantha* Blake.

A genus of four closely related species from the mountains of Ecuador and Peru, related on the one hand to *Verbesina*, on the other to the imperfectly known *Monactis* H. B. K. In habit and many other characters it makes a rather close approach to the Andean species of the section Lipactinia of *Verbesina*, but differs greatly in its linear-fusiform subrostrate wingless achene with a pappus of a single broad paleaceous awn. Its relationship to *Monactis* is closer, but the same characters of achene and pappus suffice to distinguish it. The short, broadly campanulate corolla throat equaled or exceeded by the spreading or reflexed teeth is also characteristic of *Monopholis*. The genus may be inserted in the system next to *Monactis*.

In addition to the two species here described as new, two species described by Hieronymus under *Chaenocephalus* but referable to this genus are represented by fragments in the National Herbarium. The following key will serve to separate the species.

- Heads chiefly pedicellate, the pedicels 1–7 mm. long.
  - Heads 6 or 7-flowered, 6–8 mm. high (excluding the corollas), 1.5–3 mm. thick in fruit; involucre 3.5–4.5 mm. high; broader phyllaries 1–1.5 mm. wide........ 1. *M. hexantha*
  - Heads about 12-flowered, 7–9 mm. high (excluding the corollas), 3–4 mm. thick in fruit; involucre 5–6 mm. high; broader phyllaries 1.8 to 2 mm. wide........ 2. *M. pallatangensis*
- Heads chiefly sessile, rarely short-pedicellate.
  - Branches loosely sordid-puberulous; leaves broadly ovate; ☿ flowers 8–11, ♀ 0; pappus awn 1.3–1.5 mm. long........ 3. *M. holwayae*
  - Branches "densely velvety-pubescent"; leaves lance-ovate or lanceolate; ☿ flowers 7 or 8, ♀ 1 or 0; pappus awn 0.4–0.8 mm. long 4. *M. jelskii*

1. **Monopholis hexantha,** sp. nov.—Shrub (or tree?); branches flexuous, striate-angulate, 4 mm. thick, obscurely griseous-puberulous with matted hairs; branchlets of the year fuscous, sordid-puberulous with loose several-celled subglandular hairs; internodes 1–2 cm. long; leaves alternate, often with fascicles of reduced leaves in their axils; petioles slender, naked, puberulous, 7–13 mm. long; blades lance-ovate or the smaller lanceolate, 6–12 cm. long or more, 1.8–5.5 cm. wide, acuminate, at base acutely cuneate into the petiole, serrulate (teeth depressed, less than 1 mm. high, 2–4 mm. apart) or the smaller entire or subentire, pergamentaceous, above green, harshly tuberculate-hispidulous with mostly deciduous hairs, beneath densely and softly griseous-tomentose-pilosulous with crisped hairs and gland-dotted, triplinerved above the base, bullate-rugulose above, prominulous-reticulate beneath; panicles terminating the branchlets, many-headed, flattish or rounded, 9–13 cm. wide, sordid-puberulous, the lower branches subtended by reduced leaves, the upper bracts linear, minute; pedicels 5 mm. long or usually less, often suppressed; heads discoid, 6 or 7-flowered, cylindric, 6–8 mm. high, 1.5–3 mm. thick; involucre graduate, about 3-seriate, 3.5–4.5 mm. high, passing gradually into the receptacular pales, the phyllaries linear-oblong to elliptic or sometimes elliptic-obovate, obtuse or rounded, subherbaceous-indurated, greenish-white, sordid-ciliate, somewhat glandular-puberulous, the outermost with narrow thinner sometimes purplish-tinged margins; corollas apparently pale yellow, the tube sparsely pilose with several-celled hairs, 1.8 mm. long, the throat broadly campanulate, glabrous, 0.8–1 mm. long, the 5 teeth lance-ovate, acute, spreading or reflexed, glabrous, 1 mm. long; pales linear-elliptic, obtuse, somewhat puberulous and ciliolate, about 5 mm. long, similar to the inner phyllaries but longer, their margins somewhat thinner, inflexed and inclosing the achenes, triangular or rhombic in cross-section; achenes linear-fusiform, 3.5–4.5 mm. long, 0.6 mm. wide, lenticular or trigonous in cross-section, shortly subrostrate, blackish-brown, with callous whitish carpopod, glabrous, finely papillose; pappus awn rhombic-lanceolate or oblong, flat, acute or obtuse, usually sparsely denticulate, 1.2–1.6 mm. long, about 0.3 mm. wide.

ECUADOR.—Cuenca, September 10, 1920, *E. W. D.* and *M. M. Holway* 973 (type in U.S. National Herbarium no. 1058646).

2. **Monopholis pallatangensis** (Hieron.) Blake.—*Chaenocephalus pallatangensis* Hieron. Bot. Jahrb. Engler **29**:47. 1900.

Known only from the type collection made by SODIRO (no. 38) in the Pallatanga Valley, Ecuador. Fragments are in the U.S. National Herbarium. HIERONYMUS describes the heads as 9 or 10-flowered, but I have found 12 flowers (all tubular) in each of two heads dissected.

3. **Monopholis holwayae,** sp. nov.—Branches stoutish, flexuous, striate-ridged, sordid-puberulous with crisped hairs; internodes 2–5.5 cm. long; leaves alternate; petioles pubescent like the stem, naked, 7–15 mm. long; blades ovate, 8–18 cm. long, 3–8 cm. wide, acuminate, acutely cuneate into the petiole, remotely crenulate-serrulate and somewhat repand or subentire, pergamentaceous, above deep green, densely and harshly tuberculate-hispidulous, along the veins sordid-puberulous, bullate in age, beneath rather densely and softly griseous-pilosulous-tomentose and gland-dotted, sordid-pilosulous along the chief veins, triplinerved above the base, prominulous-reticulate beneath; panicles terminating the branchlets, flattish or convex, many-headed, 8–10.5 cm. wide, sordid-puberulous with crisped subglandular hairs; pedicels usually obsolete, sometimes up to 2 mm. long; heads discoid, 8 to 11-flowered, cylindric, 9–10 mm. high, 2.5–3.5 mm. thick; involucre graduate, about 3-seriate, 4–5 mm. high, the phyllaries linear-elliptic or the inner oblong, obtuse, subindurate below and with thinner hyaline-subherbaceous greenish-yellow tip, sordid-ciliate, sparsely glandular, passing into the pales of disk; corollas apparently pale yellow, the tube sparsely long-pilose with several-celled hairs, 2 mm. long, the broadly campanulate glabrous throat 1 mm. long, the lance-ovate acute reflexed glabrous teeth 1.5 mm. long; pales elliptic, obtuse, sordid-ciliate, dorsally sordid-pilose and somewhat glandular, about 7 mm. long, the inner with inflexed margins more or less closely enveloping the achenes, the outer flattish; achenes similar to those of *M. hexantha* but with more acutely tapering base, 4.5 mm. long; pappus awn 1.3–1.5 mm. long.

ECUADOR.—Cuenca, September 15, 1920, *E. W. D.* and *M. M. Holway* 989 (type in U.S. National Herbarium no. 1058649).

The species is named in compliment to Mrs. MARY M. HOLWAY, who accompanied Professor HOLWAY on his South American trip.

4. **Monopholis jelskii** (Hieron.) Blake.—*Chaenocephalus jelskii* Hieron. Bot. Jahrb. Engler **36**:494. 1905.

Known only from the type collection by JELSKI (no. 698), made near Tambillo, Peru, August 10, 1878. Fragments are in the U.S. National Herbarium. The pappus awn is rarely wanting in some of the flowers of a head, as noted by HIERONYMUS, but I have seen no case in which there was a second shorter awn, such as he describes, present in the pappus. This appearance may have been due to a splitting of the awn into two, the possibility of which is indicated by the occasional occurrence of a deeply emarginate or bifid awn in this or other species.

Wedelia holwayi, sp. nov.—Shrub, trichotomously branched; branches slender, hispidulous and hispid hirsute with tuberculate-based spreading or reflexed hairs 1 mm. long or less; leaves opposite; petioles hirsute, 1–3 mm. long; blades lance-ovate, 3.5–6 cm. long, 1–2 cm. wide, acuminate, cuneate at base, remotely serrulate (teeth 4–10 pairs, 3–7 mm. apart), papery, above very harshly tuberculate-hispidulous and tuberculate-hirsute, beneath equally green, hirsutulous and hirsute, triplinerved, prominulous-reticulate beneath; heads in threes at apex of stem and branches, 2–3 cm. wide, on densely hispidulous and hirsute naked or 1-bracteate peduncles 2–6 cm. long; disk 1–1.3 cm. high, 1.3–1.6 cm. thick; involucre 3 or 4-seriate, graduate, 1–1.2 cm. high, the outermost phyllaries obovate-oblong, herbaceous throughout or indurated at base, acutish to obtuse, ciliate, tuberculate-glandular and sparsely tuberculate-hirsute, 5–8 mm. long, 3–4 mm. wide, the innermost elliptic-oblong or oblong with membranous-herbaceous ciliate rounded tip, on back essentially glabrous, the middle series intermediate in characters, all erect or with loose tips; rays about 12, golden yellow, fertile, the lamina elliptic, 8–14 mm. long, 3–4 mm. wide; disk corollas yellow, hispidulous toward base of throat, 6.5–7 mm. long (tube 2–2.5 mm.), the teeth papillose-hispidulous on margin within; pales subscarious, obtuse or acute, spinulose-denticulate above, 7–8 mm. long; ray achenes bluntly trigonous, not winged or auriculate, sparsely pilose, their pappus cyathiform, fimbriatulate, 0.8 mm. long, with a partly free awn 1 mm. long on the inner angle; disk achenes narrowly obovoid, thickened, densely pilose, wingless, without ears above, 3.8 mm. long, their pappus cyathiform, narrow, 1 mm. high, lacerate-fimbriate, with 1 awn 1.5 mm. long.

Bolivia.—Cochabamba, March 7, 1920, *E. W. D.* and *M. M. Holway* 376 (type in U.S. National Herbarium no. 1058597).

Nearest the Peruvian *W. forbesii* S. Moore, which has smaller lanceolate leaves, longer peduncles, and shorter involucre and rays.

Wedelia isolepis, sp. nov.—Apparently suffrutescent, dichotomously or trichotomously branched; stem and branches terete, evenly but not densely spreading-hirsute with scarcely tuberculate-based hairs 1 mm. long or less and somewhat puberulous, glabrate or glabrescent; internodes 8–16 cm. long; leaves opposite; petioles hirsute, 1.5–5 mm. long; blades ovate, 5–8 cm. long, 2.5–3.8 cm. wide, acute or acuminate, at base cuneate-rounded, remotely crenate-serrate or crenate-serrulate (teeth depressed, 3–8 mm. apart), papery, above deep green, harshly hispidulous and hirsute (the larger hairs with persistent lepidote-tuberculate bases), beneath paler green, evenly but not densely hirsutulous and along the veins spreading-hirsute, triplinerved above the base, prominulous-reticulate beneath; heads in threes at apex of stem and branches, 3.5–4 cm. wide, on naked densely hirsutulous and hirsute monocephalous peduncles 1.8–9 cm. long; disk 1–1.2 cm. high, 1.5–1.8 cm. wide; involucre 3-seriate, 10–11 mm. high, equal or the innermost series sometimes shorter, the two outer series of phyllaries equal, oval, herbaceous throughout or somewhat indurated at base, obtuse, rounded, or sometimes acutish, hirsute and minutely hirsutulous, the innermost equal or shorter, with ciliate, purplish, membranaceous tips, dorsally glabrous, all often reflexed at tip; rays 12, golden yellow, fertile, the lamina oval, tridentate, 15 mm. long, 7 mm. wide; disk corollas yellow, papillose-hispidulous on teeth, 6.5 mm. long (tube 2 mm.); pales acute, carinate, glabrous, yellowish and spinulose-denticulate above, about 5 mm. long; disk achenes obovoid, thickened, mottled, sparsely pilose, 3 mm. long; pappus cyathiform, 0.5 mm. high, fimbriolate.

Bolivia.—Soratá, April 14, 1920, *E. W. D.* and *M. M. Holway* 517 (type in U.S. National Herbarium no. 1058604).

Well distinguished by its involucral characters.

Helianthus hypargyreus, sp. nov.—Evidently tall, herbaceous at least above; stem stout, pithy, subterete, densely and softly cinereous-pilosulous with reflexed or spreading hairs, glabrescent or glabrate; internodes 2–13 cm. long; leaves alternate; petioles naked or margined above, those of the stem leaves 1–5 cm. long, densely cinereous-pilosulous; stem leaves ovate or broadly ovate, 6–15 cm. long, 3–8.5 cm. wide, acuminate, at base broadly rounded, then shortly cuneate into the petiole, crenate-serrate above the entire base (teeth 12–18 pairs, depressed-triangular, obtuse, 1–2 mm. high, 2–5 mm. apart), thin-papery, above dull greenish, densely and softly cinerescent-pilosulous with ascending hairs with small tuberculate-subglandular bases, beneath very densely and softly argenteous-pilose (sometimes yellowish-tinged) with subappressed lucid hairs, triplinerved just above the base, the primary veins evident beneath, the secondaries mostly concealed by the pubescence; branch leaves smaller, sometimes subentire; heads 2–3 cm. wide, in corymbose panicles of 3–9 at tips of branches and branchlets, the pedicels 1–5.5 cm. long, densely tomentulose-pilosulous; disk depressed-subglobose, 8 to (fruit) 14 mm. high, 1.2 to (fruit) 1.8 cm. wide; involucre 3-seriate, graduate, 9–15 mm. high, the phyllaries lanceolate or lance-elliptic to oblong-lanceolate, with indurate vittate base and shorter or equal to (outermost) longer reflexed or spreading acute herbaceous tips, densely sericeous- or cinereous-pilose-tomentose outside above the sparsely pubescent indurated base, the herbaceous apex densely pilosulous; receptacle slightly convex; rays 12, golden yellow, neutral, the lamina hirsutulous dorsally, elliptic-oblong or oval, 2 or 3-denticulate, 7–13 mm. long, 3–5.5 mm. wide; disk corollas yellow, hirsutulous chiefly at base of throat and on teeth, 5.5 mm. long (tube 1.6 mm., teeth 0.8 mm.); pales acute, carinate, hirsutulous at apex and very sparsely along keel, about 8 mm. long; achenes oblong-obovate, strongly compressed, blackish, glabrous, 2.5–3 mm. long, 1.2 mm. wide; awns 2, subequal, deciduous, narrowly lance-subulate, hispidulous throughout, 2.5 mm. long; squamellae none.

ECUADOR.—Huigra, Province of Chimborazo, August 3, 1920, *E. W. D.* and *M. M. Holway* 815 (type in U.S. National Herbarium no. 1058651); same locality, August 18 and September 6, 1918, *J. N.* and *G. Rose* 22171 and 22568.

This rather handsome species is related to *H. imbaburensis* and *H. lehmannii* Hieron., both of which are now represented in the U.S. National Herbarium by fragments from the types. The former differs in its narrower entire leaves and much smaller heads, the latter in its black-purple disk and longer, acuminate phyllaries.

Perymenium ecuadoricum, sp. nov.—Shrub(?); stem (above) and branches herbaceous, 4 mm. thick, harshly spreading- or ascending-hispidulous, or sometimes strigillose; leaves opposite; petioles naked, 7–20 mm. long, tuberculate-strigillose, hirsute-ciliate; blades ovate, 5–10.5 cm. long, 2.5–5 cm. wide, acuminate or acute, at base truncate-rounded or rounded to cordate, crenate-serrulate throughout (teeth about 32 pairs, obtuse, about 1 mm. high), thick-papery, above deep green, bullate-rugulose, harshly tuberculate-hispidulous, beneath densely and almost softly griseous-hirsutulous-pilosulous and along the veins hirsute, triplinerved, the veins and veinlets impressed above, densely prominulous-reticulate beneath; peduncles ternately arranged, terminal and from the upper axils, strigillose or erect-hirsutulous, 3.5–9.5 cm. long, bearing 2–4 heads on pedicels 1–5 cm. long, the bracts mostly very small, rarely leaflike; heads 2.5–3 cm. wide; disk subglobose, 7–8 mm. high, 8–10 mm. thick; involucre 4-seriate, graduate, 7–8 mm. high, the phyllaries broadly oval or the outermost oblong-ovate, with somewhat indurated base and shorter or subequal herbaceous or membranous-herbaceous rounded appressed tip, strigillose and ciliate, the inner glabrous above on back; rays 12, golden yellow, fertile, the lamina oblong-elliptic, 13 mm. long, 4.5 mm. wide; disk corollas yellow, papillose-hispidulous on teeth, 4.8 mm. long; pales obtuse to acute, minutely spinulose-denticulate on keel and toward apex, about 4 mm. long; disk achenes oblong, 2 mm. long, somewhat thickened but compressed, transversely rugulose, sparsely hispidulous above, often narrowly winged above on the inner side and there produced into an ear about 0.5 mm. high, sometimes also with a shorter tooth on the outer angle; pappus fragile, of about 10 unequal slender hispidulous awns 0.8 mm. long or less, and 1 awn twice as long.

ECUADOR.—Huigra, Province of Chimborazo, August 3, 1920, *E. W. D.* and *M. M. Holway* 828 (type in U.S. National Herbarium no. 1058652); same locality, August 18, 1918, *J. N.* and *G. Rose* 23820.

Closely related to the Peruvian *P. jelskii* (Hieron.) Blake (*Wedelia jelskii* Hieron. Bot. Jahrb. Engler **36**:488. 1905), of the type of which ample fragments are now in the National Herbarium. In that species, however, the leaves beneath are pale green rather than griseous, and much less densely pubescent, and their petioles are only 4–7 mm. long, while the stem is rather sparsely strigillose.

Steiractinia rosei, sp. nov.—Shrub; stem stout, densely sordid-pilose with matted erectish or loose hairs with small tuberculate bases, glabrescent; leaves opposite; petioles pubescent like the stem, naked, 7–17 mm. long; blades ovate or the smaller lance-ovate, 8–14 cm. long, 3.5–7 cm. wide, acuminate, at base cuneate-rounded, closely crenate-serrate or crenate-serrulate with depressed teeth, papery, above green, densely and harshly tuberculate-hispidulous, along the chief veins sometimes densely pilose, beneath rather softly and densely griseous-pilosulous or short-pilose, tripli-nerved above the base, impressed-veined and somewhat bullate above, prominulous-reticulate beneath; peduncles in threes at tip of stem and branches, pubescent like the stem, 3–8 cm. long, bearing 1 or sometimes 2 or 3 heads, usually naked; heads about 4 cm. wide; disk depressed-subglobose, in flower 8–12 mm. high, 8–15 mm. thick, in fruit 1.2–1.5 cm. high, 1.5–2 cm. thick; involucre about 4-seriate, graduate, 9 to (fruit) 15 mm. high, the outermost phyllaries suborbicular, herbaceous or with indurated base, acute or apiculate to obtuse, strigillose and somewhat strigose, 7–9 mm. long, 5–7 mm. wide, the middle ones very broadly obovate-oval, with rounded ampliate submembranous loose upper portion, ciliate, on back somewhat strigose or hirsute and strigillose, 8–11 mm. wide, the innermost similar but smaller and shorter, often nearly glabrous; rays 10, neutral, yellow, the lamina elliptic-oblong, when well developed 2.5 cm. long, 8 mm. wide; disk corollas yellow, 5.8 to (in age) 7.8 mm. long (tube 1.5–2.8 mm., throat 3.5–4.2 mm., teeth sparsely hispidulous near tip, papillose along margin within, 0.8 mm. long); pales narrow, acute, carinate, spinulose-denticulate above, about 8 mm. long; achenes obovate-oblong, compressed, blackish, narrowly 2-winged, 3.8 mm. long, sparsely hirsute-pilose, on the wings hirsute-ciliate, slightly contracted at apex into a collar about 0.3 mm. high, this bearing the caducous pappus of about 30 slender unequal hispidulous awns 3 mm. long or less.

ECUADOR.—Huigra, Province of Chimborazo, August 4, 1920, *E. W. D.* and *M. M. Holway* 832. Vicinity of Loja, September 29–October 3, 1918, *J. N. Rose, A. Pachano,* and *G. Rose* 23290 (type in U.S. National Herbarium no. 1023389).

Related to *S. sodiroi* (Hieron.) Blake, which has larger heads and outer phyllaries rather abruptly contracted into an indurated base.

Verbesina adenobasis, sp. nov.—Shrubby (?); branches stout, herbaceous, pithy, sordid-pilosulous with crisped hairs, glabrescent; leaves opposite or subopposite; petioles sordid-pilosulous, marginate above, stout, 1–2.5 cm. long; blades broadly ovate or rhombic-ovate, 12–17 cm. long, 7–10 cm. wide, sometimes with a short triangular lobe on one side near middle, acute, at base rounded-cuneate, then cuneately decurrent on the upper part of the petiole, serrulate above the base (teeth about 40 pairs, slender, acutish, 1 mm. high), papery-pergamentaceous, above deep green, densely scabrid-hispidulous with glandular-tuberculate-based hairs, very densely so along the veins, beneath rather densely hirsutulous-pilosulous with crisped somewhat deciduous sordid hairs (those along the veins dense and brownish), triplinerved above the base, loosely prominulous-reticulate beneath; panicle terminal, ternately divided, very many-headed, flattish, about 22 cm. wide, densely sordid-pilosulous with several-celled crisped sordid subglandular hairs; bracts minute; pedicels 3–14 mm. long, sometimes obsolete, naked or with minute bractlets; heads narrowly becoming broadly campanulate, the disk 8–11 mm. high, 3–4.5 mm. thick; involucre about 3-seriate, graduate, about 4 mm. high, passing into the pales, the outermost phyllaries small, linear-oblong or spatulate-oblong, herbaceous, obtuse or rounded, glandular-puberulous, ciliolate, 1.5–2 mm. long, 1 mm. wide or less, the inner oblong, obtuse, 1–1.3 mm. wide, ciliolate, otherwise glabrous, greenish-white, all appressed; rays 2 or 3, pale yellow, fertile, the lamina oval, 4.5 mm. long, 2.8 mm. wide; disk flowers 11 or 12, their corollas pale yellow, pilose on tube with several-celled hairs, 5.5–6 mm. long (tube 1.5–2 mm., throat cylindric-funnelform); pales obtuse, glabrous or somewhat ciliolate, 6 mm. long; ray achenes trigonous, 3-awned; disk achenes obovate, very flat, essentially glabrous, 5 mm. long, the body 1.5 mm. wide, 2-winged, the wings 0.5–0.9 mm. wide; awns 2, subequal, subterete, sparsely hispidulous, 3.5–4 mm. long.

Ecuador.—In shrubbery along roads, Cuenca, September 15, 1920, *E. W. D.* and *M. M. Holway* 991 (type in U.S. National Herbarium no. 1058641).

A member of the *V. arborea* group, related to *V. lloensis* Hieron., which has velvety-tomentose branches, oblong alternate leaves, 3–6 ray flowers or none, and 12–18 disk flowers.

Verbesina latisquama, sp. nov.—Shrub (?); branch stout, herbaceous, pithy, striate-subangulate, sordid-pilosulous with crisped hairs, glabrescent; leaves opposite; petioles stout, densely sordid-pilosulous, marginate above, the naked part 7–13 mm. long; blades ovate, 10–16 cm. long, 4.5–8.5 cm. wide, sometimes with a short obscure lobe on one or both sides near middle, acuminate, at base cuneate-rounded and decurrent into the petiole, serrulate (teeth about 40 pairs, depressed, apiculate, 0.5 mm. high), papery, above deep dull green, densely scabrid-hirsutulous (the hairs with lepidote-tuberculate not glandular bases) and along the veins very densely sordid-hirsutulous, beneath rather densely subtomentose-pilosulous with griseous matted hairs (probably finally deciduous) and along the veins densely sordid-pilosulous, triplinerved above the base; panicle terminal, ternately divided, pubescent like the stem, very many-headed, flattish, about 18 cm. wide; bracts small; pedicels 4–10 mm. long, or obsolete; heads narrowly campanulate, the disk 9–10 mm. high, 4–5 mm. thick; involucre 2 to 3-seriate, graduate, 4–5 mm. high, the outermost phyllaries oblong or obovate-oblong, 2–3 mm. long, 1.2–1.5 mm. wide, with subindurate base and subequal rounded herbaceous tip, ciliolate, somewhat glandular and puberulous, the inner similar but oval-oblong, thinner, 2 mm. wide; rays 2 or 3, pale yellow, fertile, the lamina oval, 6–7 mm. long, 3.5 mm. wide; disk flowers 8–12, yellow, pilose on tube with several-celled hairs, 6 mm. long; pales minutely apiculate from a rounded ciliate apex, sparsely pilosulous on back, about 6 mm. long; achenes (very immature) glabrous; awns subequal, hispidulous above, about 4.5 mm. long.

ECUADOR.—In shrubbery along roads, Cuenca, September 15, 1920, *E. W. D.* and *M. M. Holway* 994A (type in U.S. National Herbarium no. 1058643).

Allied to *V. adenobasis* described above, but with different pubescence and toothing of leaves, larger rays, and much broader phyllaries.

Calea huigrensis, sp. nov.—Shrub 1–3 m. high; stem terete, densely hispidulous-puberulous and hirsute-pilose with several-celled spreading hairs, the long ones mostly deciduous or sometimes entirely wanting; leaves opposite; petioles hispidulous-puberulous and gland-dotted, corky-thickened at base, 4–7 mm. long; blades ovate, those of the main stem 4–7.5 cm. long, 2–3.8 cm. wide, acute, at base rounded or cuneate-rounded, serrulate (teeth small, 4–7 pairs), slightly revolute-margined, papery, harshly tuberculate-hispidulous above, beneath equally green, densely gland-dotted, hispidulous along the veins, 3-nerved and prominent-reticulate, above impressed-veined and rugose; heads in umbelliform clusters of 5–9 at tips of stem and branchlets, on densely puberulous pedicels 3–12 mm. long, 21-flowered, campanulate, 9–10 mm. high, 4–6 mm. thick; involucre 6–7 mm. high, about 5-seriate, graduate, the outermost phyllaries ovate, 1.5 mm. long, with callous tip, puberulous and gland-dotted, the middle phyllaries oblong, rounded, brownish above, subscarious, vittate, sometimes ciliolate, obscurely puberulous above or glabrous, the inner obovate-oblong or oblong, rounded, vittate, yellow, scarious, glabrous, all appressed; rays 3, fertile, golden yellow, erectish, the lamina oblong, 3 mm. long; disk flowers 18, their corollas golden yellow, glabrous, 6 mm. long (tube 2.2 mm., throat cylindric, 3.2 mm., teeth 0.6 mm.); pales yellow, scarious, acute, obtuse, or bifid, glabrous, lacerate toward tip, 4.5 mm. long; achenes of ray and disk similar, hispidulous, 2.5 mm. long; pappus of 20 linear-lanceolate acuminate paleaceous awns 5 mm. long.

ECUADOR.—Banks of old railway grade, Huigra, Province of Chimborazo, August 7, 1920, *E. W. D.* and *M. M. Holway* 856 (type in U.S. National Herbarium no. 1058628); same locality, altitude 1525–1675 m., August 21, 1918, *J. N.* and *G. Rose* 22283.

A member of the section EUCALEA, related to *C. umbellulata* Hochr., which has glabrous achenes and only 6–9 disk flowers. The heads of this species impart a strong saffron color to the water in which they are boiled.

Gynoxys hypomalaca, sp. nov.—Shrub 3 m. high; stem stout, subterete, cinerous-tomentulose; young branches angled, densely cinereous- or ochraceous-tomentose; leaves opposite; petioles closely ochraceous-tomentose, 1.5–2.7 cm. long; blades ovate or oblong-ovate, 7–12 cm. long, 2.8–4.5 cm. wide, acuminate, apiculate, at base broadly rounded, obscurely and remotely serrulate with inflexed glandular teeth, appearing entire, coriaceous, above deep green, sparsely pilosulous, quickly glabrate except along the puberulous costa and sometimes the veins, beneath densely and softly griseous- or ochraceous-tomentose with rather loose crisped hairs, feather-veined, the lateral veins about 10 pairs, plane or impressed above, prominent beneath, the veinlets finely prominulous-reticulate above, loosely prominulous-reticulate beneath; panicles terminal, many-headed, 9–12 cm. wide, ochroleucous-tomentose, the linear-spatulate or linear tomentose bracts 1 cm. long or less; pedicels 5 mm. long or less; heads 11–14 mm. wide; disk 1 cm. high, 8 mm. thick; involucre 7 mm. high, bearing at base 3 or 4 linear bractlets about 4 mm. long, the phyllaries 8, densely ochraceous-tomentose on their exposed surface, oval-oblong, obtuse; rays 7, yellow, the lamina 6 mm. long; disk flowers 16–18, their corollas yellow, 7.5 mm. long, glabrous, the teeth papillose at tip; achenes (immature) glabrous; pappus bristles spinulose throughout, dilated at tip, 6 mm. long.

BOLIVIA.—Higher limit of trees, Soratá, April 22, 1920, *E. W. D.* and *M. M. Holway* 567 (type in U.S. National Herbarium no. 1058605).

This seems to be distinct from any of the very numerous species which have been described within recent years. Its closest ally is apparently *G. caracensis* Muschler, which, according to description, has leaves with appressed fulvescent-cinereous tomentum beneath, 8–10 ♀ and 11–14 ☿ flowers, and a somewhat different involucre. *G. hypomalaca* comes from the same locality where MANDON collected so many new species, but of the two new species properly belonging to the genus, briefly described by SCHULTZ BIPONTIUS from his collections, one, *G. mandonii*, is of a different group, while the other, *G. asterotricha*, agrees in most of the few characters given with *G. hypomalaca*, but is said to have cordate leaves.

Mutisia sagittifolia, sp. nov.—Stem slender, supported among shrubs, essentially terete, obsoletely winged in places, about 2 mm. thick, at first densely white-arachnoid-tomentose, soon glabrate and brown; internodes 6–30 mm. long; leaves alternate, terminated by a simple cirrhus 1–3 cm. long, the blades linear or linear-lanceolate, 6–8 cm. long, 5–8 mm. wide, usually falcate, acute and unequal at apex, sessile and sagittate-auriculate at base (the acute ears 3 mm. long), coriaceous, entire, revolute-margined, 1-nerved, above quickly glabrous, shining green, impressed-venulose, beneath densely and persistently white- or cinereous-arachnoid-tomentose; peduncles solitary at tips of branches, 2 cm. long, apparently decurved, bearing a single small bract, tomentose like the stem;

rays erect, the head 7.5 cm. high, about 5 cm. wide; involucre strongly graduate, about 7-seriate, 4.8 cm. high, funnelform-campanulate, the three outer series of phyllaries with deltoid or broadly ovate body and subequal or longer, abrupt, linear or linear-lanceolate, erect, herbaceous tip (the latter 8–12 mm. long, 1 mm. wide), glabrous, the body blackish-green, with thin slightly wine-colored margins; those of the 4 inner series oval or oblong-oval, at the broadly rounded apex apiculate and arachnoid-tufted, otherwise glabrous, the inner thin and wine-colored throughout, the outer similar to the three outer series in texture and color; pistillate flowers 13, glabrous, the corollas erect, the tube 2.2 cm. long, the outer lip elliptic, 4 cm. long, 1 cm. wide, acutely 3-toothed, apparently yellow inside and dark (reddish?) outside, about 14-nerved, the inner lip of two narrowly subulate segments 7 mm. long; disk corollas numerous, apparently yellow tinged with reddish, bilabiate, glabrous, 3.5 cm. long, the throat fenestrate below on both sides, the lips subequal, about 13 mm. long, one 3-denticulate at apex, the other 2-parted to base; achenes glabrous, those of the ray obcompressed, 4 mm. long, glabrous, their pappus of easily deciduous plumose setae 2.5 cm. long; achenes of the disk similar, the pappus of about 26 plumose setae united at extreme base; ray flowers with imperfect anthers and glabrous style branches about 0.6 mm. long; auricles of the stamens of the disk 5 mm. long, acuminate, somewhat denticulate toward apex.

ECUADOR.—On slopes of Mt. Pichincha, August 23, 1920, *E. W. D.* and *M. M. Holway* 941 (type in U.S. National Herbarium no. 1058634).

Allied to *M. mathewsii* Hook and Arn. in its sagittate-based leaves, but in that species the leaves are only 1.5 mm. wide, and the involucre only 3–3.5 cm. long. Only a single plant of the new species was found.

Hieracium pazense, sp. nov.—Perennial, with 2 or more stems, about 45 cm. high, the root not seen; stems rather stout, branched from the middle, rather densely hispid-pilose to middle with spreading or reflexed whitish hairs about 4 mm. long, with blackish tuberculate bases, above the middle hispid-pilose with wide-spreading blackish hairs 2–7 mm. long, with subulate bases, the shorter ones often tipped with blackish glands, and also not densely glandular-puberulous; stem leaves (below the inflorescence) about 5, the lower obovate, 7.5–13 cm. long (including the 2–3.5 cm. long petiole), obtuse, apiculate, acuminate into the petiole, sparsely and minutely glandular-denticulate or subentire, evenly but not densely long-pilose above (the hairs whitish, with brownish tuberculate bases, about 4 mm. long), beneath green, similarly pubescent; other stem leaves oblong-elliptic or oblong-ovate to elliptic, 5–7 cm. long, 1.3–2.2 cm. wide, sessile by a broad base, otherwise similar to the lower leaves; chief branches of inflorescence about 5, the lowest subtended by leaves similar to the upper stem leaves, the others by reduced lanceolate or lance-linear bracts 1–4 cm. long, 1–10 mm. wide; panicle loose, 20–48-headed, about 12 cm. wide; pedicels pubescent like the upper part of the stem, 7–17 mm. long; involucre campanulate, 7–8 mm. high in fruit, 2-seriate, equal or subequal, without evident calyculus, the phyllaries lance-subulate, acuminate, blackish-green (the inner with thin pale margins), evenly but not densely pilose with spreading blackish eglandular hairs about 2 mm. long and especially at base pedicellate-glandular, the hairs blackish below, yellowish above like the glands; heads about 43-flowered, the corollas not seen; achenes blackish-brown, 2.2 to 2.5 mm. long, columnar-ovoid, abruptly contracted downward near base, gradually narrowed to apex, not at all beaked, about 12-ribbed, minutely hispidulous especially toward base; pappus brownish-straw-color, fragile, 5.5 mm. long, equaling the involucre.

BOLIVIA.—La Paz, March 19, 1920, *E. W. D.* and *M. M. Holway* 425 (type in U.S. National Herbarium no. 1058601).

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EXPLANATION OF PLATE XIX

Monopholis hexantha Blake.—*a*, branch, ×$\frac{4}{5}$; *b*, head, ×8; *c*, disk corolla, ×8; *d*, stamens, ×8; *e*, style branches, much enlarged; *f*, *g*, two achenes in lateral and dorsal view, ×8.

BLAKE on ASTERACEAE

RECENT STUDIES OF PHAEOPHYCEAE AND THEIR BEARING ON CLASSIFICATION

WM. RANDOLPH TAYLOR

Introduction

During the past decade there has been so fundamental an advance in our knowledge of the reproductive processes and life history of the Phaeophyceae, that it seems to exceed in importance the change in viewpoint regarding any other group of plants during the same period. The work which must so largely overturn our ideas has mainly been done by European algologists, and has not been followed up in this country by any confirmatory studies; indeed, it seems to be little known. As each succeeding paper, and several from independent sources have now appeared, confirms the critical points of the others, it seems desirable at this time to review the situation and to indicate the necessary changes in the classification of the group. To date the only review in English of the studies in question is a short one by LEWIS (**11**), written at an early stage. Three have appeared in France, one by CONSTANTIN (**1**), and two by PECHOUTRE (**16, 17**), these latter of special value.

Historical

The pioneer in this field, to whom falls the honor of making the first clear advance, is SAUVAGEAU. Previous to the appearance of the standard texts, he, with other workers, had cleared up the normal life history of *Cutleria*, showing the relation of *Aglaozonia* as the sporophyte stage, and showing the variations in the life history (parthenogenesis, etc.) which appeared under various conditions (**18, 19, 22, 23, 24**). Further, his studies on members of the Sphacelariaceae and Ectocarpaceae have done much to help in the understanding of those families (**20, 21, 33**). Most important in the present connection, however, are his results from cultures of Laminariaceae (**25–30, 32**). In 1910 DREW (**2**) described the products of the unilocular sporangia on the surface of the *Laminaria* frond as functioning as gametes, and as producing a somewhat filamentous stage from which the mature *Laminaria* was vegetatively developed. A paper by KILLIAN (**5**) the following year, in discussing the development and structure of *Laminaria*, agreed, so far as it went, with the statements of DREW. This decidedly unexpected phenomenon of the sexual fusion of what from their origin should be zoospores met with considerable doubt, however, and was attacked by WILLIAMS (**37**), who claimed that it was not the motile zoospores of *Laminaria*, but other organisms, which had been seen to fuse by DREW. The first of SAUVAGEAU'S papers on the life history of the Laminariaceae appeared as a series on *Saccorhiza bulbosa* (**25, 26, 27**). Here he showed that in the case of the female the germinating zoospores from the unilocular sporangia produce a one to few-celled filament. The cells of this filament enlarge and emit a non-motile egg, which seemed to be fertilized *in situ* at the aperture, where it developed into a young sporophyte. The male plant is more complex, of five or six cells and slightly branched, with several more or less clustered antheridia. Germination of the zoospores within the sorus in which they were formed was seen, and it was found that the sporelings were both male and female, demonstrating that the sporangia on one plant produced both sorts.

Following this study appeared one on two species of *Laminaria*, *L. flexicaulis* (*L. digitata*) and *L. saccharina* (**28, 30**). SAUVAGEAU found that in germination the chromatophore of the spore divides (the zoospore on attaching itself rounds up and forms a firm wall), and one half passes into the germ tube as it elongates. The nucleus also divides, and one daughter nucleus with a chromatophore passes toward the inflated distal end of the tube, where it is cut off by a transverse wall. The nucleus which remains behind disorganizes more or less rapidly, while the cell with the other nucleus develops the gametophyte. Some of the male filaments are short, but others are elongate and markedly branched, forming the antheridia laterally on the branches toward the end. One sperm is formed in each antheridium, and the sperms are shed before the female gametophytes in the same culture reach maturity. The female gametophytes are from one to several cells in extent, all cells being able to form eggs. Each cell swells, elongates, and the egg emerges through a terminal rupture at the end of the protuberance. The opening of the oogonium forms a sheath around the base of the egg, which develops into the young sporophyte while attached to the gametophyte. Actual fusion of the gametes was not observed.

Promptly following the papers on *Laminaria*, SAUVAGEAU published a similar study of *Alaria esculenta*, belonging to a different section of the same family. The gametophytes differed from the previous cases in several particulars. The "embryo spore," or zoospore, which has passed into the resting stage, persists and may give rise to a second filament opposite the first. The gametophytes are also larger, and the female has elongate cells, part of which only seem to produce eggs. The fertile cells form irregular lobes instead of remaining of the usual simple ovoid form, but only one egg is extruded from each cell. Sometimes the female thallus is reduced to a single cell, as is not infrequent in *Laminaria*, but is more often of from two to four cells, with the terminal one becoming fertile first, and then occasionally some of the others.

In the same year that the *Laminaria* and *Alaria* studies of SAUVAGEAU were announced (1916), KYLIN published a paper on an independent study of the life cycle of *Laminaria digitata* which confirmed the statements of SAUVAGEAU in all essential respects (**8**). KUCKUCK (**7**) and PASCHER (**15**) also later published confirmatory accounts of studies on *Laminaria saccharina*. The latter describes a most interesting departure, where he found that occasionally cells of the very young sporophyte, or even the undivided egg, might function as unilocular sporangia producing 2, 4, 8, or 16 zoospores. Finally, IKARI (**4**) described the gametophytes of *Laminaria religiosa*, which are like the two species already studied in most points, but at times have the antheridia in rows at the ends of the branches of the thallus, and shed the sperm by a terminal pore.

In the year following his papers on *Laminaria*, SAUVAGEAU gave an account of the development of *Dictyosiphon foeniculaceus*, which demonstrated another unsuspected type of alternation. The evident plant of *Dictyosiphon* only produces unilocular sporangia.

SAUVAGEAU succeeded in getting good cultures in which the zoospores germinated quickly to form a branched filamentous thallus of considerable extent, reaching a millimeter in diameter and forming hairs. When mature, cylindrical gametangia are formed with two to twelve loculi. Each cell forms a single gamete. The septae disappear before dehiscence, and the isogamous gametes escape by a terminal pore. Conjugation was not observed, but was undoubtedly present, for a part of the rounded up, quiescent cells produced from the gametes had two nuclei and two chromatophores. Germination soon took place, and produced a short filament which in a few weeks gave rise to an erect thallus with the essential structure of *Dictyosiphon*.

It is a matter of peculiar satisfaction that the work of KYLIN (10) definitely shows *Chorda* to have the same sort of life history as *Laminaria*. The vegetative similarities which this genus shows to the kelps are not sufficient alone to place it in the same family, but the demonstration of a precisely similar life cycle removes all question of the relationship. The gametophytes are considerably larger than those of *Laminaria*. KYLIN was able to confirm the cultural studies by some incomplete cytological details. In the vegetative cells of the sporophyte tissue there are present forty chromosomes. Reduction divisions take place in spore formation, and are followed by two vegetative haploid divisions. Good fixation was prevented by the paraphyses, and countable metaphases were not found, but synapsis, diakinesis, and other conditions typical of the first reduction division were recognized. The number of chromosomes was about twenty, although it could not be determined precisely. In addition to the study of the preceding species, KYLIN has given details of at least part of the life cycle of several other genera in other families, which will be discussed in connection with the changes in the classification of those families.

Recently SAUVAGEAU has discovered what he believes to be the gametophyte of *Phyllaria reniformis* in the tissues of *Lithophyllum lichenoides* (32). Finally, there has appeared a preliminary note by WILLIAMS (38) relating to a detailed study of cultures of *Laminaria* and *Chorda*, with descriptions of the gametophytes of these genera essentially as previously outlined. In addition, he mentions having observed the fusion of the gametes and even of the gamete nuclei of *Laminaria*, completing the morphological evidence of alternation of generations in that genus.

Classification

It now remains to be seen what effect these recent discoveries will have on our ideas of the grouping of the genera of the brown algae. The standard text on the brown algae to date is that of OLTMANNS (**14**), published in 1904–1905. The classification used there differs from that of KJELLMAN of 1891 (**6**) in several features, notably in the reinclusion of the Dictyotales in the Phaeophyceae with the Fucaceae, and in the reduction of several groups of the Phaeosporeae from the rank of families, including them under the Ectocarpaceae. The classification accepted by LOTSY (**12**) is nearly that of OLTMANNS. A recent table by SCHAFFNER (**34**) disregards all the more recent discoveries, giving four orders in the Phaeosporeae: Ectocarpales (isogamous), Laminariales (zoospores only), Cutleriales (anisogamous), and Tilopteridales (oogamous). In the Cyclosporeae he includes Fucales and Dictyotales. The obvious fact that only a small proportion of the genera known have been fully studied, and even that some families are only understood in the most fragmentary fashion, need not deter us from taking full advantage of the knowledge which is at hand. It must also be borne in mind in all cases that parthenogenesis and other kinds of short cuts in the life cycle may be present, and may be so characteristic of the ordinary propagation of the plant that the fundamental type of alternation upon which the classification is based may be obscured.

The orthodox division into two major groups, Phaeosporales and Cyclosporales, is still acceptable, provided the former is understood to include anisogamous as well as isogamous forms, and a widespread morphological alternation. The Cyclosporales include all oogamous groups, and may show a reduction from a morphological to a mere cytological alternation of generations. The first division of the Class PHAEOPHYCEAE is then the

Order PHAEOSPORALES.—This may be defined as having gametes isogamous to anisogamous. It includes three suborders, as follows:

Suborder ECTOCARPINEAE.—Morphological alternation of similar generations shown or inferred to be present.

Family 1. *Ectocarpaceae.*—Reproductive organs formed by the metamorphosis of all or part of a branch; growth of the free filaments intercalary. This is the primitive family of the class. In *Ectocarpus* the fusion of the elements from the plurilocular sporangia as gametes has been known since the work of BERTHOLD. Recently KYLIN (**10**) has reviewed the work on *Ectocarpus*, and contributed a study of two species, *E. siliculosus* and *E. tomentosus.* It is to be considered that the plants with sporangia (unilocular) and those with gametangia (plurilocular) normally alternate in the life cycle. Cases of abbreviation of this are well known, and peculiar conditions, as in *E. Padinae* Sauv., have been reported (**33**). The thallus is always primitively branching-filamentous, and intercalary growth is typical, but this becomes localized in some species into definite regions, while the hapteron branches and other attached or endophytic parts grow apically. These features are of importance as indicating the source of similar characters in the following families.

Family 2. *Tilopteridaceae.*—Reproductive elements of two kinds, small motile cells which may function as isogametes, and larger non-motile cells often with more than one nucleus. These latter cells are of two kinds according to some accounts, representing eggs without a membrane and but one nucleus, and monospores with a membrane and usually four nuclei. The oogamous character of this family has long been tentatively accepted, but has never been proved absolutely. On the basis of vegetative characters and an assumed isogamy, this family would stand in close relationship to the Ectocarpaceae, but if oogamy is actually present it would need to be placed in the Cyclosporales as a suborder Tilopteridineae preceding the Dictyotinieae, differentiated by the thallus characters and the incomplete division of the spores. For a discussion of literature see KYLIN (**9**).

Family 3. *Sphacelariaceae.*—Reproductive elements formed by the metamorphosis of all or part of a branch, growth from an apical cell. The originally monosiphonous filament usually divides up by internal walls, and may develop a peripheral meristematic zone producing a very considerable increase in thickness. Special vegetative methods of reproduction are present in most forms. SAUVAGEAU (**20**) reports finding egg and sperm production on one individual of *Halopetris scoparia*, but could only find one type of gametangium in *Cladostephus*. If this observation is confirmed, it will either force *Halopetris* into the Cyclosporales, a removal of the whole family from its present position, or necessitate a wider interpretation of the Phaeosporales. The family as understood here includes the Choristocarpaceae of KJELLMAN.

Family 4. *Asperococcaceae.*—Reproductive organs formed by the metamorphosis of, or as an outgrowth from, a superficial cell; growth intercalary. This family corresponds to the Encoeliaceae, Striariaceae, and Myriotrichaceae of KJELLMAN, the latter two representing simpler forms with the same essential construction. Plants are derived from a simple filament, becoming parenchymatous, either cylindrical or flattened. All stages can be traced. Gametangia with isogametes and sporangia are present, and considerable portions of the life history are known, especially of *Asperococcus* and *Scytosiphon* (**10**). The statement of YENDO (**43**) that the products of the plurilocular sporangia of *Phyllitis* are not gametes, but give rise to a microscopic gametophyte, requires confirmation.

Family 5. *Chordariaceae.*—Thallus differentiated into axial and assimilative filaments, branched, meristem localized; sporangia replacing assimilative filaments or modified segments therefrom. Essentially filamentous, the final short branches of the axial filaments turn outward and form a close cortex rich in chromatophores. Since both sporangia and gametangia are known in these genera, the life history is probably the same as that of *Ectocarpus*. The Elachisteaceae of KJELLMAN represent a reduced epiphytic group, and may best be included in the Chordariaceae.

Family 6. *Desmarestiaceae.*—Reproductive organs formed by the metamorphosis of, or as an outgrowth from, a free (or corticating) branch cell; growth trichothallic. The main axis in these forms is ramified, and produces short corticating branches, as well as (in some) delicate free ones. Little is known regarding the development of these genera; unilocular sporangia only are present, and there may be the same life history as in *Dictyosiphon*.

Family 7. *Stilophoraceae.*—Reproductive organs lateral on special branched supports, thallus erect on the substratum. As understood here, this family includes KJELLMAN'S Stilophoraceae, Spermatochnaceae, and Sporochnaceae. The sporangia are borne on branched filaments developed from superficial cells of the thallus, and both sporangia and gametangia are known. Parts of the life cycle have been traced by KYLIN (10).

Family 8. *Ralfsiaceae.*—Reproductive organs at least in part lateral on special branched supports, thallus incrustating. Here we may include with the Ralfsiaceae also KJELLMAN'S Lithodermataceae. In both the gametangia are borne laterally on special branched filaments arising from the surface, but the sporangia are only so borne in the Ralfsiaceae, in *Lithoderma* being but modified surface cells. The life history is unknown from the experimental viewpoint.

Suborder DICTYOSIPHONINEAE.—Morphological alternation of dissimilar generations present or inferred, the sporophyte exceeding the gametophyte in size.

Family *Dictyosiphonaceae.*—Characters of the suborder. The forms are branching, have an apical cell, and differentiate axial and cortical areas. The life history, worked out by SAUVAGEAU (31), has previously been described. This is a very important group, as it indicates an intermediate step in the development of a microscopic oogamous thallus, such as is shown in *Laminaria.*

Suborder CUTLERINEAE.—Morphological alternation of similar or of dissimilar generations present, gametophyte when different larger than the sporophyte; growth trichothallic.

Family *Cutleriaceae.*—Characters of the suborder. The life history of this group is well known, thanks to the cultural studies of SAUVAGEAU, CHURCH, and several others, and to the cytological studies of YAMANOUCHI on *Cutleria* (40, 41) and *Zanardinia* (42). The alternation shown by the cultural studies has been shown to be associated with a haploid and diploid nuclear constitution, reduction taking place in the sporangia. In *Zanardinia* the two generations are essentially alike, but in *Cutleria* the reduced and flattened sporophyte was long known as *Aglaozonia,* and thought to be an entirely different genus.

Order CYCLOSPORALES.—Plants of this order are strictly oogamous.

Suborder DICTYOTINEAE.—Morphological alternation of similar generations present.

Family *Dictyotaceae.*—Characters of the suborder. The gametangia are aggregated into definite areas or sori, and the asexual reproduction is by tetrasporangia, motile zoospores being replaced by four non-motile elements. The life cycle of *Dictyota* is fully known. Cultural studies showing the course of the development have been made by HOYT (3), and cytological studies by MOTTIER (13) and WILLIAMS (35, 36), showing reduction in tetraspore formation.

Suborder LAMINARINEAE.—Morphological alternation of dissimilar generations present, gametophyte smaller than the sporophyte.

Family *Laminariaceae.*—Characters of the suborder. Members of this family are almost all large plants. Reproduction was thought to be strictly by zoospores until SAUVAGEAU showed that the zoospores produced a microscopic gametophyte. The results of the various studies have previously been described.

Suborder FUCINEAE.—Only cytological alternation of generations present.

Family *Fucaceae.*—Characters of the suborder. This family forms the spores within pits or conceptacles. Still inclosed, the spores undergo a few cell divisions, or even only a few nuclear divisions, forming the gametes which are then shed as egg and sperm. Cytological studies have been made by several workers, including YAMANOUCHI (39), confirming the morphological and cultural observations. This is the climax family of the brown algae, and represents the greatest reduction of the gametophyte possible while still retaining an alternation.

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LITERATURE CITED

1. CONSTANTIN, J., Travaux recentes sur les Thallophytes. Ann. Sci. Nat. Bot. X. xxx–xxxvi. 1919.
2. DREW, G. H., The reproduction and early development of *Laminaria digitata* and *L. saccharina*. Ann. Botany **24**:178–190. 1910.
3. HOYT, W. D., Alternation of generations and sexuality in *Dictyota dichotoma*. BOT. GAZ. **49**:55–57. 1910.
4. IKARI, J., Development of *Laminaria religiosa* Miyake. Bot. Mag. Tokyo **25**:207–224. 1921.
5. KILLIAN, K., Beitrage zur Kenntnis der Laminarien. Zeitschr. Bot. **3**:433–494. 1911.
6. KJELLMAN, F. R., Phaeophyceae and Dictyotales. In ENGLER und PRANTL, Die Naturl. Pflanzenfam. 1^2: 176–297. 1891, 1893.
7. KUCKUCK, P., Über Zwerggenerationen bei *Pogotrichum* und über die Fortpflanzung von *Laminaria*. Ber. Deutsch. Bot. Gesells. **35**:557–578. 1917.
8. KYLIN, H., Über den Generationswechsel bei *Laminaria digitata*. Svensk. Bot. Tidskrift **10**:551–561. 1916.
9. ———, Über die Entwicklungsgeschichte und die Systematische Stellung der Tilopterideen. Ber. Deutsch. Bot. Gesells. **35**:298–310. 1917.
10. ———, Studien über die Entwicklungsgeschichte der Phaeophyceen. Svensk. Bot. Tidskrift **12**:1–64. 1918.
11. LEWIS, I. F., Recent work on the life history of the kelps. Plant World **20**:190–192. 1917.
12. LOTSY, J. P., Vorträge über Botanische Stammesgeschichte **1**:257–306. 1907.
13. MOTTIER, D. M., Nuclear and cell division in *Dictyota dichotoma*. Ann. Botany **14**:163–192. 1900.
14. OLTMANNS, F., Morphologie und Biologie der Algen. Jena. 1904–1905.
15. PASCHER, A., Über diploide Zwerggenerationen bei Phaeophyceen (*Laminaria saccharina*). Ber. Deutsch. Bot. Gesells. **36**:246–252. 1918.
16. PECHOUTRE, F., La sexualité héterogamique des Laminaires et la reproduction des algues phéosporées. Rev. Gén. Sci. **27**:643–653; 688–692. 1916.
17. ———, Revue de Botanique. Rev. Gén. Sci. **30**:242–250. 1919.
18. SAUVAGEAU, C., Sur l'alternance des generations des *Cutleria*. Compt. Rend. Acad. Sci. **129**:555–558. 1899.
19. ———, Sur une nouvelle complication dans l'alternance des generations des Cutlerias. Compt. Rend. Soc. Biol. **63**:139–141. 1907.
20. ———, Sur la sexualité de l'*Halopteris*. Compt. Rend. Soc. Biol. **62**:506–507. 1907.
21. ———, Nouvelles observations sur la germination du *Cladostephus verticillatus*. Compt. Rend. Soc. Biol. **64**:695–697. 1908.

22. Sauvageau, C., Sur la germination des zoospores de l'*Aglaozonia melanoidea*. Compt. Rend. Soc. Biol. **64**:697–698. 1908.

23. ———, Sur la germination parthénogénétique du *Cutleria adspersa*. Compt. Rend. Soc. Biol. **64**:699–700. 1908.

24. ———, Nouvelles observations sur la germination parthénogénétique du *Cutleria adspersa*. Compt. Rend. Soc. Biol. **65**:165–167. 1908.

25. ———, Sur le développement et la biologie d'une Laminaire (*Saccorhiza bulbosa*). Compt. Rend. Acad. Sci. **160**:445–448. 1915.

26. ———, Sur les débuts du développement d'une Laminaire (*Saccorhiza bulbosa*). Compt. Rend. Acad. Sci. **161**:740–742. 1915.

27. ———, Sur la sexualité hétérogamique d'une Laminaire (*Saccorhiza bulbosa*). Compt. Rend. Acad. Sci. **161**:796–799. 1915.

28. ———, Sur les gamétophytes de deux Laminaires (*L. flexicaulis* et *L. saccharina*). Compt. Rend. Acad. Sci. **162**:601–604. 1916.

29. ———, Sur la sexualité hétérogamique d'une Laminaire (*Alaria esculenta*). Compt. Rend. Acad. Sci. **162**:840–842. 1916.

30. ———, Sur les plantules de quelques Laminaires. Compt. Rend. Acad. Sci. **163**:522–524. 1916.

31. ———, Sur une noveau type d'alternance des generations chez les algues brunes (*Dictyosiphon foeniculaceus*). Compt. Rend. Acad. Sci. **164**:829–831. 1917.

32. ———, Sur les plantules d'une Laminaire à prothalle parasite (*Phyllaria reniformis* Rostof.). Compt. Rend. Acad. Sci. **166**:787–789. 1918.

33. ———, Nouvelles observations sur l'*Ectocarpus Padinae* Sauv. Compt. Rend. Acad. Sci. **171**:1041–1044. 1920.

34. Schaffner, J. H., The classification of plants. XII. Ohio Jour. Sci. **22**:129–139. 1922.

35. Williams, J. L., Studies in the Dictyotaceae. I. The cytology of the tetrasporangium and the germinating tetraspore. Ann. Botany **18**:141–160. 1904.

36. ———, Studies in the Dictyotaceae. II. The cytology of the gametophyte generation. Ann. Botany **18**:183–204. 1904.

37. ———, The zoospores of the Laminariaceae and their germination. Rept. 82d Meet. Brit. Assoc. Adv. Sci. 685–686. 1912.

38. ———, The gametophytes and fertilization in *Laminaria* and *Chorda*. Ann. Botany **35**:603–607. 1921.

39. Yamanouchi, S., Mitosis in *Fucus*. Bot. Gaz. **47**:173–196. 1909.

40. ———, Cytology of *Cutleria* and *Aglaozonia;* a preliminary paper. Bot. Gaz. **48**:380–386. 1909.

41. ———, The life history of *Cutleria*. Bot. Gaz. **54**:441–502. 1912.

42. ———, The life history of *Zanardinia*. Bot. Gaz. **56**:1–35. 1913.

43. Yendo, K., The germination and development of some marine algae. II. Bot. Mag. Tokyo **33**:171–184. 1919.

A METHOD FOR ESTIMATING HYDROPHILIC COLLOID CONTENT OF EXPRESSED PLANT TISSUE FLUIDS[1]

ROBERT NEWTON AND ROSS AIKEN GORTNER

(WITH ONE FIGURE)

In the preceding paper by GORTNER and HOFFMAN[2] it was pointed out that studies of the physico-chemical properties of plant saps which include only measurements of the osmotic pressure, electrical conductivity, and H-ion concentration, leave out of account the very important influence on physical properties exerted by sap colloids. By the introduction of the refractometer as a part of the field laboratory equipment, these workers have shown it possible to make rapid and accurate determinations of the moisture content of the plant saps. Utilizing the additional data thus made available, a simple method has been devised which appears to give a relative measure of the content of hydrophilic colloids.

The freezing point depression of the freshly expressed plant juice is first obtained. Then, having determined the total solids by the refractometric method, a quantity of sucrose just sufficient to make a molar solution in the total water present is added. The freezing point depression is again determined, and is usually found to have increased more than the theoretical amount.

The values for the excess depression recorded in this paper have been based on the assumption that sucrose forms the hexahydrate in solution. The evidence for this has recently been discussed by SCATCHARD,[3] who also contributed additional data. In preliminary experiments with pure sucrose dissolved in distilled water, freezing point depressions were obtained slightly in excess of those

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[1] Published with the approval of the Director, as Paper no. 323, Journal Series, Minnesota Agricultural Experiment Station.

[2] GORTNER, R. A., and HOFFMAN, W. F., Determination of moisture content of expressed plant tissue fluids. BOT. GAZ. 74:308–313. 1922.

[3] SCATCHARD, G., The hydration of sucrose in water solution as calculated from vapor-pressure measurements. Jour. Amer. Chem. Soc. 43:2406–2418. 1921.

expected under this assumption, but it is preferred to use the theoretical value until further data on this point are available.

It is assumed that the magnitude of the excess depression is a measure of the quantity of water held in such a way as to be unavailable for the solution of the sugar. The values obtained may be calculated to percentage "bound" water. This represents the total water of hydration of all the substances in the sap, but has been found to correspond so regularly with the content of hydrophilic colloid as to indicate a close relationship. It seems probable that in most cases the water bound by substances other than colloids is of minor importance.

In table I are reported the data for a number of the samples of expressed juice included in table I of the preceding paper, and in addition for a series of gum acacia sols prepared by weighing out the necessary quantities of highly purified gum acacia and distilled water. The percentage of total solids, as read directly by the refractometer, is given in column 2. The values for viscosity, recorded in column 3, were determined by a viscosimeter of the Ostwald type, in a constant temperature bath at 25° C.; the figures are the number of seconds required for 3 cc. to flow through a capillary tube, through which the same quantity of distilled water flowed in 204 seconds. In column 4 is given Δ, the freezing point depression of the freshly expressed juice; in column 5 Δ_a, the freezing point depression after the addition of the sugar; in column 6 $\Delta_a-\Delta$, the actual additional depression due to the added sugar; in column 7 $\Delta_a-(\Delta+K_m)$, the amount by which the depression found on addition of the sugar is in excess of that expected on theoretical grounds. As previously noted, it has been assumed that sucrose forms sucrose hexahydrate in solution, and therefore K_m, the molecular constant for the depression of the freezing point, has been taken as 2.085° C. instead of the usual 1.86° C. The percentage "bound" water, given in the last column of the table, is conveniently calculated from the value for actual additional depression due to the added sugar ($\Delta_a-\Delta$). The calculations involved will be made clear in the following example, using the first item in the table.

1.86° = Δ due to 1 mole dissolved in 1000 gm. water, but 1 mole sucrose combines with 6 moles water. Thus 1 mole sucrose dissolved in 1000 gm. water = 1 mole sucrose hexahydrate dissolved in $1000-(18\times 6)$, or 892 gm. water, and 2.085° $(K_m)=\Delta$ due to 1 mole dissolved in 892 gm. water.

But in sample 13 the increase in Δ on addition of 1 mole sucrose $(\Delta_a-\Delta)$ was 2.339°, and $2.339^\circ=\Delta$ due to 1 mole dissolved in $\frac{1.86\times 1000}{2.339}=795$ gm. water; therefore the water bound per liter = $892-795=97$ gm., $=9.7$ per cent.

In laboratory practice it is most convenient to weigh out a fresh portion of the sap containing 10.0 gm. of water, add 3.422 gm. of sucrose, and redetermine the freezing point. The percentage of bound water is then given by the formula: $\frac{\text{Excess}\quad\Delta}{\text{observed}\,\Delta-\text{sap}\,\Delta}\times 892$ or bound water $=\frac{\Delta_a-(\Delta+K_m)}{\Delta_a-\Delta}\times 892.$

Comparing sample 13 with sample 16, it will be seen that whereas the percentages of total solids vary widely, the percentages of bound water are not greatly different. A reference to the values for viscosity and Δ will indicate at once the marked difference in the physical properties of these two saps, due to the large content of colloidal material in sample 16, a fact which is strikingly reflected in the percentage of bound water. Again, dialysis showed that material similar to sample 17 contained approximately twice the quantity of colloids as was contained in material similar to sample 20, and these two samples differ widely in percentage bound water. These examples are cited to illustrate the application of the method. Discussion of the significance of the variations observed in the wheat varieties is reserved for a later paper on another subject.

The percentages of bound water obtained with gum acacia sols, as shown in the table, increase regularly with concentration. In fig. 1 the percentage concentration has been plotted against the percentage bound water. The logarithms of these values have also been plotted in the same figure. It will be seen that both of these graphs suggest an adsorption curve.

The advantages of sucrose as the solute in this method are as follows: (1) it is easily obtained in a high degree of purity; (2) the large molecular weight reduces errors in weighing; (3) its behavior

TABLE I

Materials used: leaves of	Total solids	Viscosity (water = 204)	Δ	Δ_a	$\Delta_a-\Delta$	$\Delta_a-(\Delta+K_m)$	Bound water
	Per cent	seconds					Per cent
13. Triticum vulgare var. Turkey	13.5	360	1.273	3.612	2.339	0.254	9.7
15. Bryophyllum calycinum	5.9	235	0.474	2.555	2.081	−0.004	0.0
16. Cereus sp.	4.9	637	0.505	2.803	2.298	0.213	8.3
17. Triticum vulgare var. Buffum	17.8	419	1.719	4.158	2.439	0.354	13.0
18. Triticum vulgare var. Minhardi	8.5	285	1.147	3.824	2.137	0.052	2.2
19. Triticum vulgare var. Super	7.1	267	1.000	3.106	2.106	0.021	0.9
20. Triticum vulgare var. Super	9.7	292	1.085	3.279	2.194	0.109	4.4
Solutions of gum acacia							
1 per cent	1.0	309	0.005	2.147	2.142	0.057	2.37
3 per cent	3.0	487	0.013	2.186	2.171	0.086	3.53
5 per cent	5.0	684	0.025	2.221	2.196	0.111	4.50
7 per cent	7.0	932	0.034	2.254	2.220	0.135	5.42
10 per cent	10.0	1438	0.048	2.294	2.246	0.161	6.39

in solution is fairly well known; (4) its effect on the swelling of colloids is probably negligible. In objection it may be stated that plant saps probably contain invertase, but the plan has been followed of grinding the sucrose to a fine powder which dissolves rapidly with shaking, and the sap has been maintained continuously at low temperature. Under these conditions no increased depression of the freezing point which could be attributed to invertase action has been observed in a somewhat extended series of determinations on the same sample.

Conditions of equilibrium, and possible errors due to adsorption of the sugar by colloids, have not yet been investigated. The data already secured, however, appear to justify the proposal of the method for the estimation of the relative (not absolute) content of hydrophilic colloids in expressed plant tissue fluids. It seems probable that the method may be applied also to other biological fluids.

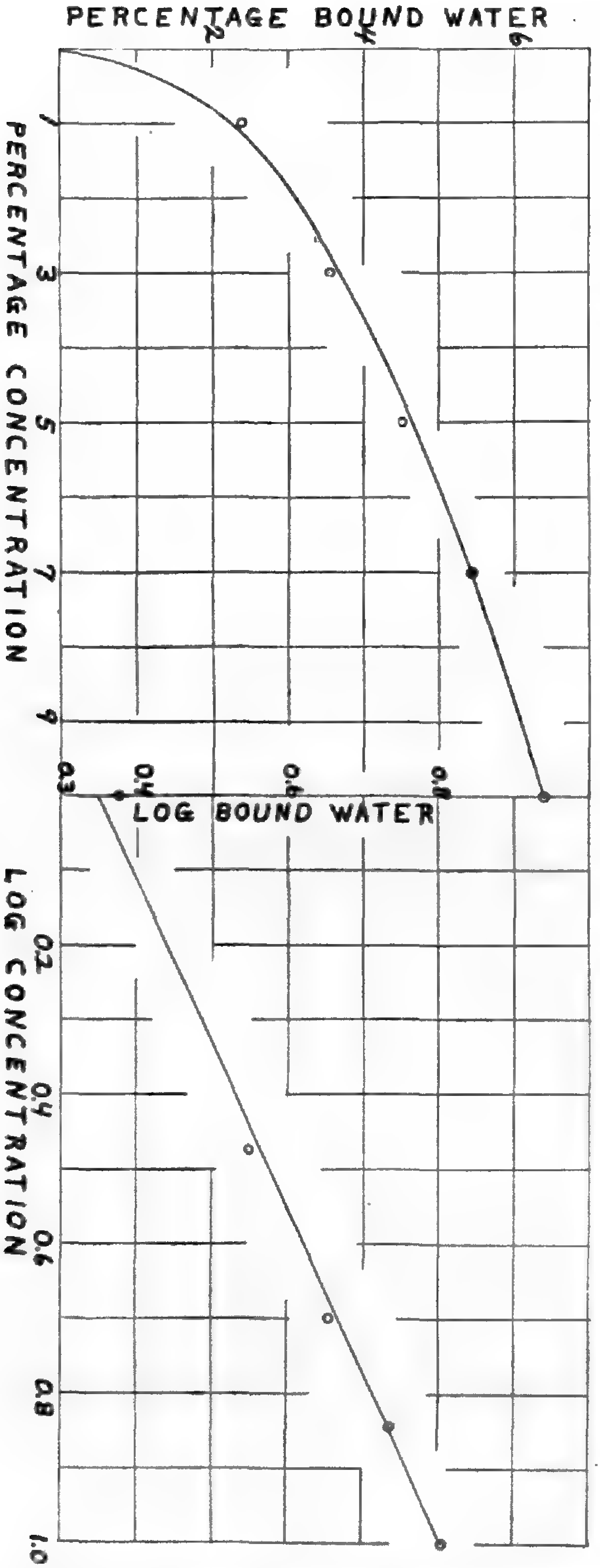

FIG. 1.—Relation between concentration of gum acacia sols and water bound by the colloid.

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GROWTH OF PLANTS IN ARTIFICIAL LIGHT

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(WITH TWO FIGURES)

The growth of plants in artificial light has been of interest in plant physiology because it offers the possibility of controlling the quality and intensity of the light, and the duration of the exposure. No reliance can be placed upon sunlight, and its quality and intensity vary greatly. The writer attacked the problem of growing plants in artificial light to gain some idea of the relation of light intensity to the formation and equilibrium concentrations of the carbohydrates formed in leaves, and to the translocation of these substances in light and darkness.

The problem of producing plants in artificial light is of some economic interest in Minnesota and other such localities, where the sunlight is of short duration and low intensity in winter. It is difficult to raise plants in the greenhouse in winter in this latitude when the sun is at a low angle. Some analyses made on cabbage leaves grown in a greenhouse at the University of Minnesota in the winter of 1920 show a practical absence of sugars from the leaves. Very little growth is produced under such conditions; the plants are weak and easily attacked by fungi.

The ability to grow plants in this climate by substituting artificial light for sunlight, or by using artificial light to supplement sunlight on dark days, will be of considerable value for plant breeders. The progeny of valuable crosses can be carried through one or more generations during the winter, and thereby decrease the time required to produce a new strain. In breeding for rust resistance at Minnesota it has been found difficult to carry biological forms of black stem rust of wheat in culture during winter, owing to the weakness of the host plants.

The writer has succeeded in raising a great variety of plants from seed to maturity, using artificial illumination entirely. Light was obtained from nitrogen-filled tungsten filament (Mazda) lamps. The lamps were mounted on the ceiling of basement rooms about five feet from the plants. Ordinary enamelled reflectors of the deep bowl type were used to throw the light downward. The lamps used were of the 200 watt and 1000 watt sizes; the latter is considerably more economical in operation than the smaller size. These lamps are rated to burn 1000 hours, but average about 3000 hours. They were burned twenty-four hours per day, so that one set of lamps was sufficient for four months or more. Breakage of the lamps occurred the most frequently when the current was turned off and the filaments allowed to cool. The continuous burning seemed to greatly increase the life of the lamps.

Plants grew well, set good seed or produced tubers in the continuous illumination. It seems unnecessary to have a period of darkness to allow translocation of the assimilate from the leaves. Several intensities of light were used and the plants grew in each. The lowest intensities of about 25 foot candles in dark corners produced much better growth than was obtained in the greenhouse during the winter. Analyses of the carbohydrate and protein contents of the plants are being made.

In one room 8×11 feet a great variety of cereals was grown, including twenty varieties of wheat, fifteen varieties of oats, eighteen varieties of barley, eight varieties of rye, six varieties of flax, several hybrids of wheat and of oats, and a few other test plants. The room was lighted by lamps with a total capacity of 3200 watts and distributed uniformly. The lamps required about 0.6 watt to produce one spherical candle. Then $\frac{4\,\pi\times 3200}{0.6}=67{,}021$ lumens total flux. On account of the light absorption and inefficiency of the reflectors, only about 60 per cent of this light reaches the ground, so that there are 40,212 lumens spread over the area 8×11 feet, giving an intensity of about 457 lumens per square foot. In this light winter rye headed at about 24 inches; Kota wheat at 28 inches; Bluestem wheat at 23 inches; Aurora oats at 17 inches; Manchuria barley at 17 inches. These plants were grown in 5-inch pots. Usually six or eight kernels were all that set in a head of wheat, but these were plump and full of starch. The temperature was maintained automatically at about 14° C. by cooling with outside air. It was unnecessary to use any heat in this room other than that produced by the lights. The light is doubly efficient, for all that is not used by the plants goes to heat. Since sunlight is not required, the rooms can be insulated much better against heat loss than a greenhouse.

In a room 6×11 feet, lighted by 10–200 watt lamps, several vegetables were grown. The temperature was about 25°–30° C., not well controlled. The light intensity was about 380 lumens per square foot. Potatoes and tomatoes grew well, but were somewhat taller than normal. Early Ohio potatoes bloomed when the vines were 44 inches long. Boston marrow squash bloomed at 42 inches, but did not set fruit. Alaska peas bloomed at 18 inches and set good seed. *Solanum niger* set abundant fruit, beginning at 6 inches, and continued fruiting up to 28 inches. Cabbage started heads at about 18 inches and showed some effects of etiolation. Buckwheat set normal seed at 20 inches.

FIG. 1.—Cabbage, lettuce, potato, tomato, beans, peas, and a number of other plants growing in continuous artificial light; room 6×8 feet, lighted with lamps of 2600 watts capacity.

In another room 6×8 feet, lighted by lamps with a total capacity of 2600 watts, the plants appeared much more normal than in the room just mentioned (fig. 1). The light intensity here was about 680 lumens per square foot and the temperature 25°–30° C. Cabbage headed at 12 inches; lettuce set abundant seed when 36 inches high. Black wax beans set pods at 35 inches; potatoes (Early Ohio)

FIG. 2.—Rotating tables operated by electric phonograph motor to equalize light and temperature.

bloomed at 48 inches, and set tubers weighing as high as 180 gm. each. White sweet clover set some seed when 40 inches high. A number of weeds were allowed to grow, and they all set abundant seed. *Boltonia asteroides* bloomed when 54 inches high; *Chenopodium ambrosioides* set seeds profusely at 35 inches; *Silene latifolia* bloomed at 16 inches but did not set seed; *Stellaria media* bloomed and set seed at 16 inches; foxtail grass (*Alopecurus*) set abundant seed at normal height.

Under the 1000 watt lamp shown in fig. 2 the light intensity was about 4188 lumens per square foot. Plants under this light were very stocky, and showed effects of too much heat. The leaves of cabbage were very turgid and stiff. The plants were rotated to equalize light and temperature, and to decrease the heating effect of the light on surfaces directly exposed.

All of the plants in these rooms except cabbage bloomed, and many produced good seed, although the illumination was continuous. It seems then that the period of illumination is not the factor which determines whether a plant will bloom or not. The results obtained by GARNER and ALLARD[1] may have been produced by a modification of the conditions of nutrition of their plants by variation in the length of the day. It is possible to obtain seed from a great variety of plants as here shown, although the illumination is continuous, and the intensity is approximately the same for all plants in one room.

Summary

A great variety of plants, including wheat, oats, barley, rye, flax, buckwheat, white sweet clover, peas, beans, lettuce, and a number of common weeds were grown from seed to maturity in continuous artificial light, and all set good seed. Potatoes, tomatoes, red clover, alsike clover, squash, and *Silene* bloomed, but did not set seed. Potatoes produced tubers of good size. All of the plants tested did not require a certain period of illumination to cause them to bloom. It is possible to produce seed from plants in winter independent of sunlight, and at no very great expense.

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[1] GARNER, W. W., and ALLARD, H. A., Jour. Agric. Res. 18:553–606. 1920.

CURRENT LITERATURE

MINOR NOTICES

North American slime moulds.—A new and revised edition of MACBRIDE'S[1] *North American slime moulds* has just been published. As the subtitle states, it is "a descriptive list of all species of Myxomycetes hitherto reported from the continent of North America, with notes on some extra-limital species." The original edition was reviewed in this journal.[2] The necessity for a second edition has given the author the opportunity not only to correct certain errata, but chiefly to incorporate new information developed by the investigation of the last twenty years. The book is primarily for American students, and is certainly an adequate presentation of this interesting group, serving the same purpose in this country that LISTER'S *Mycetozoa* does in England.—J. M. C.

Some elementary texts.—An interesting textbook for Indian high schools has been prepared by KENOYER.[3] It is interesting both in presentation and material, and adapted to high school students of India. Of course it is necessarily brief, but it develops an interesting approach to the plant kingdom. It is based upon the conviction that the best way to know plants is to grow them, and therefore emphasis is placed upon the school garden as the important laboratory for beginners. The plants selected as illustrations are in general those of general occurrence in India. This little book will interest teachers of botany in other countries.

A similar text for English schools has been prepared by WOODHEAD.[4] This is an abbreviation and simplification of the author's *The study of plants*. It also emphasizes the study of living material, experimental work, and outdoor observation. Naturally the materials are those available in England, and the details and terminology are more technical than in the Indian text. Both are doubtless adaptations to the status of high school education in the two countries.—J. M. C.

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[1] MACBRIDE, T. H., The North American slime moulds. 8vo. pp. xvii+299. *pls. 23.* New York: The Macmillan Company. 1922.

[2] BOT. GAZ. **29**:74. 1900.

[3] KENOYER, L. A., Plant studies for Indian high schools. pp. 160. *figs. 67.* The Christian Literature Society, Allahabad. 1922.

[4] WOODHEAD, T. W., Junior botany. pp. 210. *figs. 140.* Oxford: Clarendon Press. 1922.

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.*