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BOTANICAL GAZETTE

THE

BOTANICAL GAZETTE

EDITORS:

JOHN MERLE COULTER AND CHARLES REID BARNES

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P. 232, footnote 7, for 379 read 397.

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January 1908

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PEARS

The Botanical Gazette

A Monthly Journal Embracing all Departments of Botanical Science

Edited by JOHN M. COULTER and CHARLES R. BARNES, with the assistance of other members of the botanical staff of the University of Chicago.

Vol. XLV, No. 1 Issued January 16, 1908

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The Botanical Gazette is published monthly. ¶The subscription price is $5.00 per year; the price of single copies is 50 cents. ¶Postage is prepaid by the publishers on all orders from the United States, Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Hawaiian Islands, Philippine Islands, Guam, Tutuila (Samoa), Shanghai. ¶Postage is charged extra as follows: For Canada, 35 cents on annual subscriptions (total $5.35), on single copies, 3 cents (total 53 cents); for all other countries in the Postal Union, 84 cents on annual subscriptions (total $5.84), on single copies 11 cents (total 61 cents). ¶Remittances should be made payable to The University of Chicago Press, and should be in Chicago or New York exchange, postal or express money order. If local check is used, 10 cents must be added for collection.

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Contributors are requested to write scientific and proper names with particular care, to use the metric system of weights and measures, and in citations to follow the form shown in the pages of the BOTANICAL GAZETTE.

Papers in excess of thirty-two printed pages are not accepted unless the author is willing to pay the cost of the additional pages, in which case the number of pages in the volume is correspondingly increased.

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Separates, if desired, may be ordered in advance of publication. Twenty-five separates of original articles without covers will be supplied gratis. A table showing approximate cost of additional separate is printed on an order blank which accompanies the proof; a copy will be sent on request.

Entered August 21, 1895, at the Post-Office at Chicago, as second-class matter, under Act of Congress March 3, 1879.

Bulletin of Recent Publications and Winter Announcements of The University of Chicago Press

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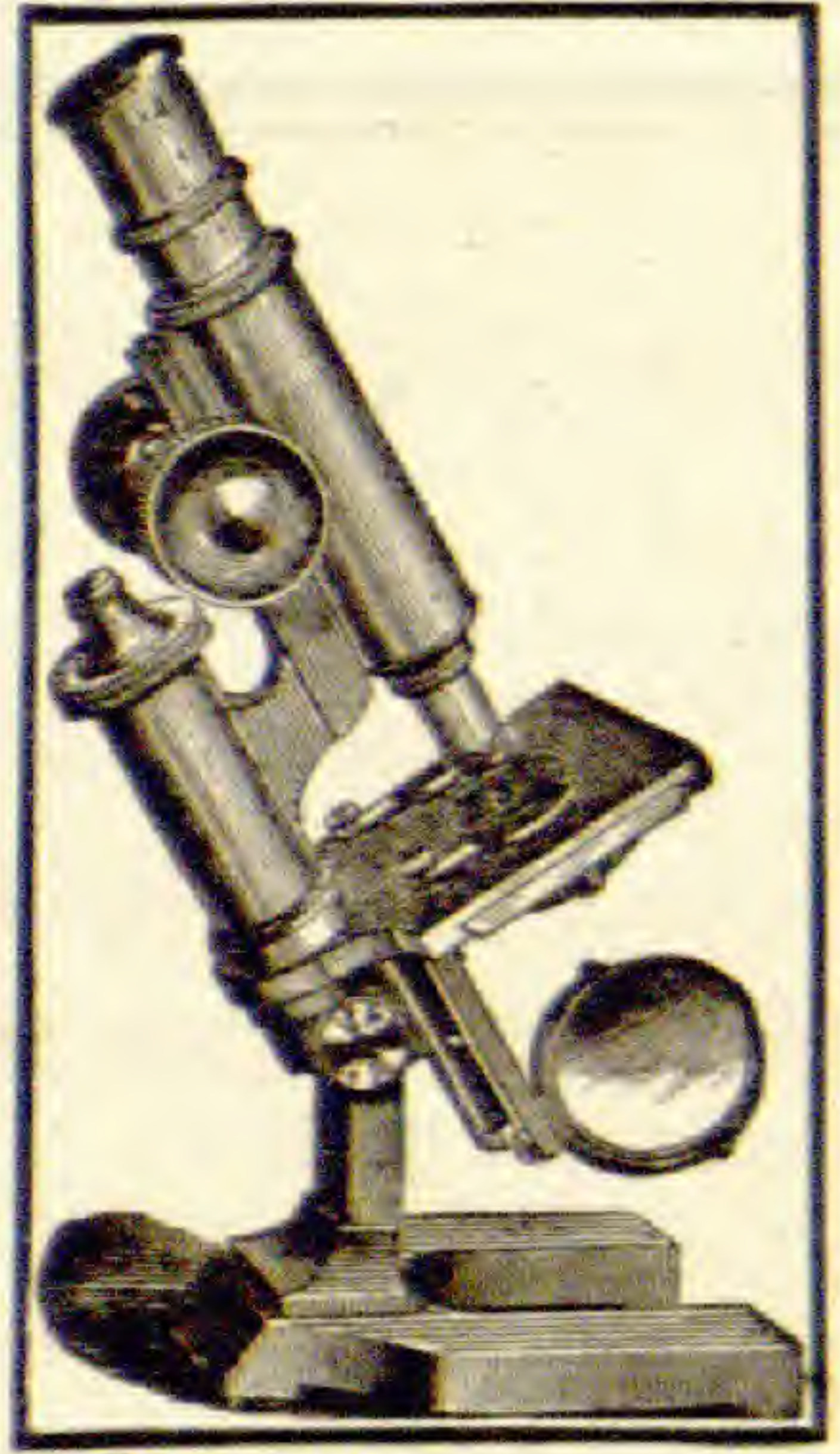

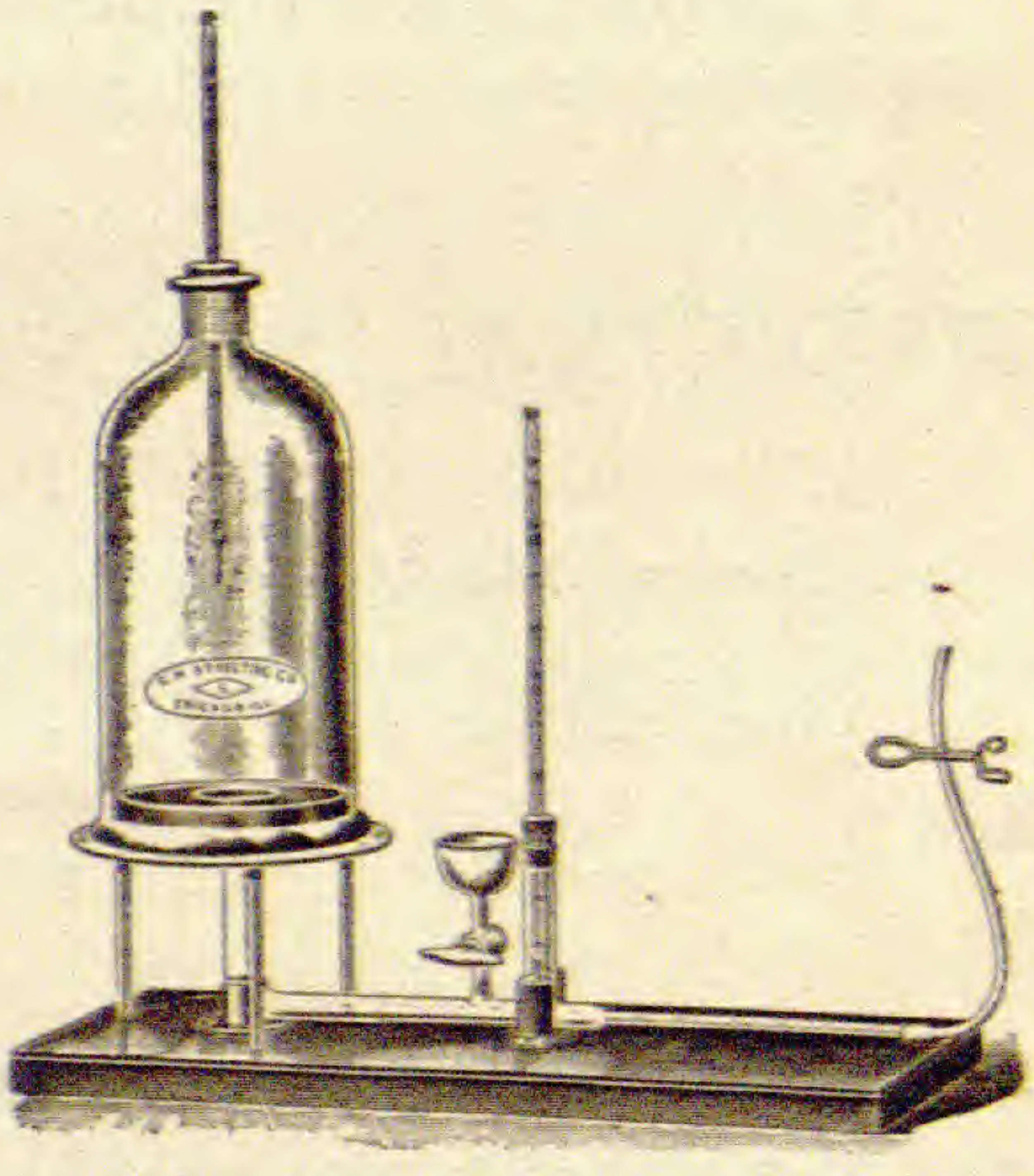

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BOTANICAL GAZETTE

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SPOROGENESIS IN NEPHRODIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 106

SHIGÉO YAMANOUCHI

(WITH PLATES I–IV)

Historical review

There have been published during the past three decades a few accounts of sporogenesis in pteridophytes. Since cytological studies have advanced rapidly in recent years, many views which were once prevalent have been proved to be erroneous, or at least to be capable of a different interpretation, and the authors themselves may have at present entirely different views from those which they held when the accounts were published. It seems worth while, therefore, to make a historical review of some of the work hitherto published.

There is first a paper by HUMPHREY (**29**) on the study of the spore mother cells of Osmunda, Psilotum, and other forms, in which the main purpose was to prove the presence of extranuclear centrosomes in plants above the bryophytes. His account of the number of chromosomes in Osmunda was taken up by STRASBURGER (**46**) in his data dealing with the periodic reduction of chromosomes in the life-history of organisms, as one of the instances which prove the constancy of the number of chromosomes in a certain period of the life-cycle.

OSTERHOUT'S (**35**) studies on Equisetum were really one of the most important contributions to our knowledge of the spore mother cells of higher plants. He found that the spindle at the outset is multipolar polyarch, according to the terminology of STRASBURGER (**47**), and that the numerous cones become grouped around the common axis, but the poles of the spindle at metaphase hold their composite nature with entire absence of the focal center or centrosome. This account, which did much to dispose of the current idea that the development of the spindle in higher plants is controlled by centrosomes, is noteworthy.

Then appeared two papers with detailed description of the method of division and distribution of chromosomes: one by CALKINS (11) on *Pteris tremula* and *Adiantum cuneatum;* and the other by STEVENS (44) on *Pteris aquilina*, *Cystopteris fragilis*, and *Scolopendrium vulgare.* CALKINS believed that there was a tetrad formation of chromosomes in the prophase of the spore mother cell of ferns, and that the process of sporogenesis in those forms followed the same course as was observed in the maturation division of sexual cells in animals, with a transverse division and consequently a qualitative reduction in WEISMANN'S sense. STEVENS' studies on the forms named above disagree with CALKINS' view in several particulars, the main point being that there is present no transverse division of chromosomes in the spore mother cell, but two longitudinal divisions, and therefore there is merely a quantitative reduction. STEVENS' observation on the formation of daughter chromosomes in the second division was criticized by STRASBURGER (47).

SMITH (42) investigated the sporogenesis of *Osmunda regalis.* His results agree with those of OSTERHOUT in regard to the absence of centrosomes, and differ as to the origin and development of the spindle; he held that the spindle was bipolar from the beginning.

In 1904 GREGORY'S paper (26) was published. His account covers the sporogenesis of eight genera of ferns, i. e., Pteris, Scolopendrium, Asplenium, Onoclea, Davallia, Fadyenia, and two other forms among Cytheaceae, and seems to confirm FARMER and MOORE'S interpretation (18) of the formation of chromosomes at the heterotypic division, indicating the occurrence of a transverse division.

The next year STEVENS (46) published his results on the spore formation of *Botrychium virginianum.* His account begins with his so-called early prophase of the spore mother cell; the first appearance of the spindle of the first and second divisions, he finds, is within the nucleus. As regards the heterotypic mitosis, he states that the chromosomes in metaphase separate in the line of transverse fission and that he agrees with the views maintained by FARMER and MOORE (**18**), GREGORY (**26**), and STRASBURGER (**49**).

CARDIFF'S (**12**) studies of synapsis and reduction in several forms of flowering plants and in *Botrychium obliquum* paid particular attention to the formation of the spirem and its behavior up to the formation of bivalent chromosomes in the heterotypic mitosis. According to him, paired threads, which appear in the presynaptic stage of the nucleus, fuse during synapsis and again split longitudinally after synapsis, probably along the line of the previous fusion.

In 1907 a paper entitled "Studies in apospory and apogamy in ferns" appeared under the joint authorship of FARMER and DIGBY (**16**), whose two preliminary notes were published in 1903 and 1905. This paper deals directly with the matter indicated by the title, but some stages in the division of the spore mother cell are described in three forms of Lastrea and one species of Scolopendrium. The paper is accompanied by many important figures, but only three of the spore mother cells with heterotypic chromosomes are given, so far as sporogenesis is concerned.

Recently there appeared two papers, one by BURLINGAME (**10**) on Ophioglossales, and the other by GRÉGOIRE (**24**), with the title "La formation des gemini hétérotypiques dans les végétaux." BURLINGAME'S paper is chiefly devoted to a study of the sporangium. From the cytological standpoint GRÉGOIRE'S paper is very important. His views, which were published in 1904 (**25**), concerning the structure of the resting nucleus of the spore mother cell, interpretation of synapsis, formation of chromosomes of the heterotypic mitosis, and distribution of the daughter chromosomes in the first and second divisions, were based chiefly upon BERGH'S studies (**6, 7**) of Allium, Lilium, and other forms; but in this recent paper GRÉGOIRE records the results of his own examination of *Osmunda regalis* and four monocotyledons, which confirm his previous views.

Nephrodium

My present investigation of *Nephrodium molle* Desv. was carried on in order to understand the characteristics of the nuclear behavior of this species, and to obtain the knowledge prerequisite for a study of apogamy in the same species. Consequently this paper should be regarded as introductory to a paper entitled "A study of apogamy," which will appear later. Much attention has been paid to the behavior of chromosomes, combining observations with theoretical views, with a hope of securing an explanation of the phenomena observed, so that the chromatin substance will be the chief object treated in the present paper.

I wish to express my gratitude to Professor JOHN M. COULTER and Dr. CHARLES J. CHAMBERLAIN for their kind advice and criticism during the progress of this work. I am also under obligation to other members of the botanical staff for courtesies extended to me.

MATERIAL AND METHODS

The material for this investigation was collected in greenhouses of the Hull Botanical Laboratory, and in Lincoln and Washington parks, Chicago, from October 1906 to March 1907.

Killing and fixation were made in various fluids, among which FLEMMING's weak solution, with a little modification in the percentage of acetic acid, proved most effective. The material was left in the fluid for two to four hours, then transferred into fluid of the same formula, but without osmic acid, ten to twenty hours. The thoroughly washed material was passed very carefully through a series of alcohols beginning at three per cent. and imbedded in 48° to 52° paraffin. The sections were cut 3–5 μ in thickness and stained with safranin gentian-violet, or iron-alum-hematoxylin, sometimes combined with counter-stains, such as safranin, orange G, eosin, or Congo red. A Zeiss apochromatic immersion 1.5^{mm}, N. A. 1.30, and compensating oculars were used for studying the preparations.

DESCRIPTION

Vegetative mitosis in the sporophyte

A leaf too young to produce sori shows in its various parts the successive stages in vegetative mitosis. The form of the mitotic figure is affected by irregularities in the size of the cell which contains it, and the abundance of plastids, oil, and other cell contents interferes somewhat with accurate observation. The mitotic figures which occur during the formation of sporogenous tissue, from the first division of the archesporial cell to the completion of spore mother cell, proved most favorable, being entirely free from such obstructions, and these mitoses will be used for the following description.

The resting nucleus in the sporogenous tissue contains a chromatin reticulum with one or several nucleoli. The general structure of the nuclear reticulum needs mentioning. It does not seem to consist of two distinct elements, chromatin and linin, with chromatin granules distributed irregularly upon or imbedded in the linin ground-substance, but it seems to be composed entirely of chromatin material. The appearance which indicates that there may exist a mixture of two substances—the dark-stained masses and light-colored strands—does not seem to be due necessarily to a different affinity for stains, but to the fact but that the more compact regions of chromatin material are more affected by stains than the more loosely aggregated regions, which are lightly stained.

The number of nucleoli is variable and there seems no rule for their position in the nuclear cavity, for sometimes they lie very close to the membrane and sometimes they are centrally located. The form is rounded in most cases, but often they are aggregated into one or two irregular masses. Even in the resting nucleus they show a vacuolized structure.

The chromatin reticulum in the resting nucleus is irregularly distributed, often great masses being located in the center or sometimes eccentric near the periphery, leaving the center vacant. Approaching the prophase, there seems to be a tendency of some of the chromatin material to form a continuous spirem, i. e., some fine strands of the chromatin may break apart entirely or may become thicker by getting the material from some ragged chromatin clumps which consequently diminish in size. The spirem, uneven at first, becomes gradually uniform in thickness and distributed throughout the nuclear cavity (*fig. 1*). Whether there is present a single spirem or not was not determined. Close examination of this stage in a number of preparations showed that it was hardly possible to make out free ends, and this may be taken as an evidence that the spirem is a continuous one. Nucleoli persist in positions distinctly isolated from masses of the chromatin reticulum, so that they do not seem to contribute to the establishment of the spirem through the migration of material. Usually at this stage they seem to unite into one mass.

While these events are going on within the nucleus, there are formed kinoplasmic caps at two poles outside. The kinoplasmic cap at first consists of a few fibers arranged in a cone, the fibers evidently running in a longitudinal line, one end focusing at one pole, and the other end diverging toward the nucleus, reaching nearly to the nuclear membrane or falling short of it. Since no change ever occurs in the nuclear membrane, these fibers certainly originate from the rearrangement of the cytoplasm where the cone arises. The whole cytoplasm, except where the kinoplasmic caps arise, shows at this time a fine fibrillar structure. Now the nuclear membrane begins to disappear at the basal region of the kinoplasmic caps, the rest of it still clearly limiting the nuclear contents from the cytoplasm outside. There seemed to be no regularity in the course of the spirem until this stage. The entangled spirem was distributed throughout the cavity, the bent parts of the loops being directed toward the membrane; but when the nuclear membrane begins to disappear near the kinoplasmic caps, some parts of the spirem seem to move out in the direction of the caps.

Then follows the entire disappearance of the nuclear membrane, the segmentation of the spirem into a certain number of slender chromosomes, and the intrusion of spindle fibers which become attached to the chromosomes at their ends (*fig. 2*). At this time there is first observed the longitudinal splitting of the chromosomes, the two parts resulting from the splitting always remaining closely associated. The chromosomes, in general, are directed along the axis of the two poles; one end reaches near the pole, and about the equatorial region lies the other end, to which spindle fibers from the two opposite poles are attached; so that the distance of the attached ends from the poles is approximately the length of two chromosomes. This stage passes into the condition shown in *fig. 3*, where the chromosomes become much less curved. The line associating the two chromosomes which resulted from the longitudinal splitting is still visible. The chromosomes which were so slender and peculiarly curved, as stated above, from the first segmentation to this period, now begin to shorten; the equatorial ends, to which the spindle fibers from the two poles are attached, remain stationary, while the ends directed to the poles move toward the equator as the chromosomes shorten. In the course of shortening, a stage such as that represented in *fig. 4* has been described by some authors as being a typical figure of vegetative mitosis in ferns.

However, the shortening of the chromosomes proceeds still farther, until the bent ends lying at the equatorial region become straightened, and the chromosomes become rod-shaped; thus there is established the stage in which the chromosomes are arranged in an equatorial plate (*fig. 5*). The two daughter chromosomes formed by the longitudinal splitting of a single one at prophase have remained side by side and have been affected equally by the process of shortening, so that now they are almost similar in size and form. When this stage of the equatorial plate is attained, the two daughter chromosomes seem to begin to separate at once. This stage is less frequently met than the earlier or later stages, even in material favorably fixed and showing an abundance of stages.

The separation of many daughter chromosomes generally begins and proceeds simultaneously, but rather rare instances are observed of several tardy ones, which separate after their associates are already on the way toward the poles. The chromosomes arranged in the equatorial plate are perfectly straight; the separation of the two halves begins at the ends where the spindle fibers are attached and proceeds toward the other end, necessarily causing a curving, as represented in *fig. 6;* but soon after their complete separation they resume their straight form and so persist. When the two sets of daughter chromosomes reach nearly to the poles, they become perfectly parallel, without overlapping (*fig. 8*), and in the more favorable cases they are as regularly placed as if they stood in one plane, a stage which is the most satisfactory for counting. The polar view of this stage (*fig. 9*) shows that the number of chromosomes is 128 or 132. During prophase and even metaphase the number of chromosomes could sometimes be estimated, especially in the polar view at the equatorial plate stage, but not with such exactness as in the early telophase.

The chromosomes grouped at the pole are drawn so tightly together that the ends toward the pole become closer than the ends directed toward the equatorial region. This aggregation process goes on farther and there results an extremely dense mass, in which it is difficult to trace the outlines of each chromosome. The chromosomes begin to be vacuolized, i. e., chromatin materials which constituted the chromosomes in their compact condition become localized in an irregular way, in some places becoming quite loose, and in others still remaining as a dense aggregation. All the chromosomes undergoing this change are associated, the ultimate result being a vacuolized mass of chromatin material from all the chromosomes (*fig. 10*). By the interaction between the cytoplasm which surrounds these vacuolized masses and nuclear sap which may be secreted within the vacuolized region of the chromosomes, there is formed a nuclear membrane. As the nuclear sap increases in amount, the nucleus grows in size (*fig. 11*).

The polar end of the daughter nucleus at the time of the formation of the membrance is always concave (*fig. 12*), very likely due to the fact that before the vacuolization the face ends of daughter chromosomes pointed to the pole are drawn together toward the pole so as to come nearer, and that in this position vacuolization has proceeded. It seems probable that the vacuolization is more pronounced in that concave region than elsewhere, so that in the young daughter nucleus the chromatin material is rather scanty at the region of the previous pole, where immediately after the organization of the daughter nucleus one or two new nucleoli appear (*fig. 13*). It may turn out that the daughter nucleus thus reconstructed shows polarity.

Coincident with the construction of the daughter nuclei there appear granules in an equatorial plane upon fibers of the central spindle. The granules first make their appearance upon the fibers in the central part of the equatorial plane and then toward the periphery. It seems reasonable that the fiber substance has contributed the material for the formation of the granules.

Contemporary with the completion of the cell wall, growth of the daughter nuclei continues and the chromatin material assumes again a ragged structure on account of further continuation of vacuolization. The general outline of the daughter nucleus becomes nearly spherical and there is established the resting nucleus of the succeeding mitosis (*fig. 14*).

The processes of nuclear division from the reticulum of the resting condition to the reconstruction of the next resting nucleus are carried on as described above in any vegetative mitosis in Nephrodium, and the sequence of mitosis preceding the formation of the nucleus of the spore mother cell is exactly the same.

Sporogenesis

PRESYNAPTIC STAGE.—The chromatin reticulum in the resting nucleus of the spore mother cell has the same structure as was just described for the nucleus of the sporogenous cell; the size of the nucleus, after its full growth, is greater than that of any nuclei of the sporogenous cells, and not infrequently it occupies almost all of the cell. No regularity could be found in the massing of the chromatin material; at certain points there are dense masses and at others almost none at all. Nucleoli, one to several in number, are always present in various locations, without any physical connection with the chromatin.

The exact structure of the chromatin reticulum needs description. It is an anastomosing complex of ragged clumps and irregularly branched strands (*fig. 15*); the former always stain deeply and the latter lightly, simply because of the difference in compactness of massing. They originated from a single source—the chromosomes of the previous mitosis—by the vacuolization process described above.

The clumps of the reticulum lose gradually their ragged look, because of the disappearance of the fine strands, the elongation of the clumps in certain directions, and the migration of material from the clumps to some of the fine strands so as to thicken them (*fig. 15a*). All these changes tend to transform the ragged structure, but they do not occur simultaneously throughout the reticulum, so the whole is still plainly a reticulate structure, as seen in *fig. 16*. Nevertheless, in regions where the reticulum is being converted into thread structure there are visible parts of threads running closely side by side (*fig. 16a*).

SYNAPSIS.—The transformation of the reticulum into a thread structure now proceeds rapidly. When the transformation is nearly completed (*fig. 17*), parts of the thread are not smooth and uniform in appearance and thickness, and in its course it is greatly tangled. These tangled masses of thread begin to contract and become localized on one side of the nuclear cavity, a process which marks the initiation of the synaptic state. Preparations stained with iron-alum-hematoxylin and extracted to a certain degree of differentiation of the stains, show that this loosely tangled mass consists of lightly colored threads and deeply stained granules; undoubtedly the thread in this tangled and contracted condition is not homogeneous throughout; on the contrary, there are present not only irregularities in thickness in parts of the thread, but also the chromatin material is more compact in certain parts and more loosely aggregated in other parts.

That the parts of the thread in this stage are double is fairly discernible (*fig. 17a*) in tangential sections of the nucleus; undoubtedly this doubling has come from the changes observed in the earlier stage represented in *fig. 16* as originating from the transformation of the reticulum; therefore this double part of the thread cannot be recognized as an indication of a new longitudinal splitting, but as two threads independent from the start.

These double threads run side by side very closely, as if they were fusing. Whether they are completely fused or not could not be determined, but their association is very close. These masses of threads become more tightly drawn together and contracted into a ball which seems to be the climax of the synaptic stage (*fig. 18*).

FORMATION OF HETEROTYPIC CHROMOSOMES.—The course of the thread or spirem is irregular in the synaptic stage; it is twisted and generally much curled, but there are present several parts of the spirem which run comparatively straight across the contracted ball of the spirem from near the nuclear wall with which it lies in contact. The loops happen to be formed near the periphery of this contracted mass, but they have nothing special to do with the formation of chromosomes.

In the majority of cases the nucleoli seem to unite into one, which becomes caught in the tangled mass in the early synaptic stage, generally far from the nuclear membrane, and its spherical form persists.

The spirem of this stage shows a relatively smooth structure and it is not easy to distinguish its double nature throughout, probably on account of the close association of the two independent parts; but tangential sections reveal that the parts of the spirem are unmistakably composed of two threads running side by side, often diverging rather widely (*fig. 19*). It seems highly probable that we see here an indication of the separation of two threads which were first observed in the presynaptic stage (*figs. 16, 16a*) and entered into synapsis, as already described. Now the tangled mass of the spirem begins to uncoil (*fig. 20*) slowly; superficial parts of the spirem in the synaptic mass at first become widely distributed, while the unraveling of the inner parts of it are naturally delayed, and the longitudinal separation of the two independent parts is observable in the portions of the spirem which are widely uncoiled (*fig. 20*).

The spirem becomes considerably thickened, certainly due to shortening, and becomes distributed loosely through the cavity; while the line of the longitudinal splitting or separation of the spirem into two elements seems obliterated, but its presence is evident in some places (*fig. 21*).

The course of the spirem again becomes irregular, and in some parts there are present complicated tangled masses of the spirem on account of the twisting of the spirem itself, but double threads, the constituents of the spirem, run mostly in close association (*fig. 22*). Shortening and thickening of the spirem proceeds until it segments into chromosomes. As shown in *figs. 23a* and *23b*, the segmentation is not simultaneous throughout the entire spirem, but finally there are formed 64 or 66 chromosomes, each bivalent in nature (*figs. 24a, b, c*).

The two halves of the bivalent chromosomes lose their close association after their segmentation, and show every possible position and connection with each other, such as H, I, J, K, L, O, T, U, V, X, Y, and so forth, and even give an impression of being of different length and size, mostly due to the point of view. The chromatin material which constitutes the surface region of the chromosomes seems loosely aggregated, but it is compactly massed in the center, and there are observed faintly stained substances of fibrillar form attached at the ends of chromosomes, which seem to be remnants of the material of the spirem left over at the time of segmentation, probably on account of the viscid nature of the spirem. The loosely aggregated chromatin material is gradually drawn and condensed toward the central part, but the condensation process takes place irregularly, so that for a while the chromosomes appear spiny. The chromosomes become shortened, and the loose fibrillar appendages are also shortened by contributing material to the main body of the chromosome, yet some can still be seen (*figs. 25a, b, c*).

A short description of the chromatic substance seems necessary. Early in the resting stage of the nucleus, the cytoplasm shows a fine fibrillar structure, almost homogeneously distributed. When the nuclear structure begins to enter into synapsis, long kinoplasmic fibers appear from the rearrangment of the cytoplasm surrounding the nucleus, but far apart from the nuclear membrane. Between these kinoplasmic fibers and the nuclear membrane there is present a clear region free from any fine fibrillar structure, which was the main feature of the other parts of the cytoplasm before the appearance of the kinoplasm (*fig. 21*). Some of the stages showing such a process are represented in *figs. 21–25*. The nuclear membrane, which has stained distinctly, now becomes less and less affected by stains and seems to be dissolved entirely, but for a short time the encroaching and anastomosing kinoplasmic fibers preserve the previous nuclear outline (*figs. 25a, b, c*). The kinoplasm, as a rule, surrounds the nuclear membrane, but some of the threads run in somewhat radial directions. No distinct polar formation was observed.

When the kinoplasmic fibers push into the nuclear cavity, some of them are seen to be attached to the chromosomes, while others pass through, and a multipolar structure becomes noticeable (*figs. 26a, b*); from the fibers of the multipolar spindle there is worked out the bipolar spindle, as indicated in *figs. 27a, b*. Shortening of the chromosomes continues always up to this time, so that now there result very short bivalent chromosomes, different in aspect from those in the early prophase. There is no regularity in the position of the chromosomes, which are found on almost all parts of the spindle, yet it is not hard to notice from successive stages, as *figs. 26a, b* and *27a, b*, that there is a tendency to move toward an equatorial region until 64 or 66 chromosomes are arranged in the equatorial plate.

The two parts of the bivalent chromosome in the equatorial plate are applied to each other throughout their entire length; ends in which spindle fibers are attached become directed toward the poles, while the other ends remain in contact. The polar view of the equatorial plate or a little earlier stage is favorable for counting the chromosomes, which showed always 64 or 66, each bivalent in nature (*fig. 29*).

SEPARATION OF THE DAUGHTER CHROMOSOMES.—Now the daughter chromosomes begin to separate; some of them, on the way toward the pole, show a V-shape, the angle being directed toward the pole. This does not seem to have resulted from the bending of one daughter chromosome, because it always remains straight after separation; hence the V-shape may be regarded as resulting from a new longitudinal fission of the daughter chromosome, providing for the two granddaughter chromosomes of the second division. This longitudinal fission occurs only at the end of the chromosome directed toward the equatorial region, without proceeding to the other end. The two arms of the V come into contact again as the daughter chromosome reaches the pole.

As a rule, the sets of daughter chromosomes pass to the poles simultaneously, but there were observed some exceptional cases where some were much delayed (*figs. 31, 32*); and rarely the irregularity is so extreme that tardy chromosomes are left behind, while their associates are already in an aggregated condition at the pole (*fig. 34*).

RECONSTRUCTION OF THE DAUGHTER NUCLEI.—When the group of daughter chromosomes reaches the pole, each chromosome for a short time remains fairly straight, which is favorable for counting the chromosomes from the polar view (*fig. 35*). Then the vacuolization process begins (*fig. 36*). There is a certain point of difference in detail between this process and that described in the reconstruction of daughter nuclei in vegetative mitosis; the polar ends of the daughter chromosomes do not seem to be drawn together so as to give a concave form, but begin to be vacuolized at once, while in a state of loose association. The vacuolization is prominent along the line of the longitudinal fission of the daughter chromosomes, a line which became obliterated when the chromosomes reached the pole. After the formation of the nuclear membrane it is not impossible to recognize a certain part of the chromatin substance as representing a certain chromosome (*fig. 38*); in other words, the vacuolization process in the telophase of the heterotypic mitosis does not cause the chromosomes to return into the ragged chromatin reticulum from which they are formed, but the reconstruction stops before the individual outline of the main body of the chromosome is lost entirely. Therefore, the daughter nucleus does not pass into the resting stage with ragged chromatin reticulum in the telophase of the first division, but always shows the main body of individual chromosomes recognized in V-shape, notwithstanding the nucleus grows considerably in size after the formation of the membrane. No nucleolus was observed in the daughter nucleus (*figs. 40, 41*). The nucleus in such a condition is ready for the second mitosis.

GRANULAR ZONE DIVIDING THE SPORE MOTHER CELL.—During the nuclear division described above, the cytoplasm generally has a fine fibrillar structure, with vacuoles here and there near the cell wall. When the chromosomes are arranged at the equatorial plate, fibers running from the poles cross in the equatorial region near the cell wall, where the cytoplasm begins to be transformed into the granules of this stage. After the organization of the daughter nucleus, the granules begin to increase and proceed rapidly toward the center along an equatorial plane of uniform thickness (*fig. 38*), at first avoiding only the space which was occupied by the central spindle (*fig. 39*). At last even the middle parts of the central spindle become replaced with or transformed into granules, so that finally the protoplast of the spore mother cell is entirely separated by a zone of these granules into two hemispheres, each with its daughter nucleus. The greater portion of the central spindle, which contributes to the formation of the granules, returns into the fibrillar structure of the cytoplasm at telophase.

CHROMOSOMES OF HOMOTYPIC DIVISION.—As described above, the daughter chromosomes which entered into the reconstruction of the daughter nuclei do not lose their individuality, but it is possible to recognize their identity even after they are combined and anastomosed together into a reticular structure (*fig. 41*). The recognizable V elements in the nucleus of the second division are what undoubtedly resulted from the influence of the vacuolization on the line of the longitudinal fission of the daughter chromosomes in the first mitosis during the last telophase; however, the arrangement of V's which they had when vacuolization began at the last telophase does not seem to have been maintained, for the closed angles of the V's are now visible on both upper and lower sides of the nucleus from such a side view as shown in *fig. 42*. Such a change in the situation of V's during the course of the vacuolization process and nuclear growth might easily occur because of the bending and curving due to their being gathered into the round cavity of the daughter nucleus.

As the disappearance of the nuclear membrane approaches, V elements become more distinctly pronounced (*fig. 43*), some of the fine strands which branched out from V's disappear, but irregularity in thickness persists and the arms of the V's end in very slender threads. The general outline of the nucleus up to this time is always flattened, so that the nucleus shows two different aspects according to whether the view is from the pole or equator of the spore mother cell; hence the same mitotic figure of this nucleus, no matter what stage it may be, will always present two different side views, one being always narrower than the other.

As the nuclear membrane breaks down, the cytoplasmic fibers enter into the nuclear cavity, where the chromosomes are crowded (*fig. 44*). The figure of this stage recalls the combined features of both the heterotypic and the typical forms: the chromosomes, long, slender, and curved, with daughter chromosomes in pairs, resemble those of the vegetative mitosis; the achromatic fibers do not construct a cap, but appear multipolar as in the heterotypic case, although they are from the start observed near the nuclear membrane instead of at a distance from it. The two arms of the V's, which now provide for the two daughter chromosomes of this division, are still hanging together at the angle (*fig. 44*) at which spindle fibers seem to become attached. When the multipolar condition has passed into the bipolar spindle, the angle ends of the V's, or the ends where spindle fibers are attached, occupy the position near the pole, while the other ends diverge toward the equatorial region (*figs. 45, 46*). The stage resembles that represented in *fig. 3* as one of the transient conditions in passing to the stage of the equatorial plate.

Gradually the divergent arms of the V's, or the daughter pair of the bivalent chromosomes in this division, draw near to each other until finally they come into close contact. Simultaneously, the bivalent chromosomes, each consisting of two daughter chromosomes, move toward the equatorial region; as the ultimate result, the ends of chromosomes to which fibers are attached are arranged in an equatorial plane, while the other free ends are directed toward the pole (*fig. 47*). The figure of this stage is also comparable with that of the vegetative mitosis shown in *fig. 4*. The shortening and thickening of the bivalent chromosomes begin and proceed in similar rate and degree in each member of the pairs, which is always in contact with its fellow (*figs. 48, 49*). These processes are exactly similar to those which have already been described in the vegetative mitosis. The bivalent chromosomes are finally shortened into straight, rod-shaped chromosomes and become arranged in an equatorial plate (*fig. 50*). Some of the bivalent chromosomes in this stage of the second division, unlike that of the first division, are slightly above or below the plane of the rest, so that the polar view is not satisfactory for accurate counting (*fig. 51*).

The separation of the daughter chromosomes which constitute the bivalent chromosomes begins at once. Generally daughter chromosomes pass in a group toward the pole, but occasionally the separation of the two halves of certain chromosomes is more delayed than the rest (*figs. 52, 53*). In any event, the daughter chromosomes which are on the way toward the pole have a straight rod shape, a little more elongated than they were when arranged in the equatorial plate. When the daughter chromosomes reach the pole they keep for a while a regular and almost parallel arrangement (*figs. 54, 55*), which was advantageous for counting. The number is 64 or 66 (*fig. 56*).

The group of chromosomes becomes more and more closely aggregated (*figs. 58, 59*). When the vacuolization begins, the individual outlines of the chromosomes are gradually lost, and after the organization of the nuclear membrane, which is formed as in the vegetative mitosis, the growth of the nucleus follows (*fig. 60*).

Simultaneous with the reconstruction of granddaughter nuclei the cell plate is laid down between them, exactly as in the vegetative mitosis (*figs. 57–60*). The second division forming two granddaughter nuclei takes place in the daughter nuclei of both hemispheres, separated already by the granular zone in the first mitosis. From the cytoplasm which filled the space between two granddaughter nuclei new spindle fibers now arise connecting them. These fibers necessarily pass the granular zone, in the middle plane of which is established the cell plate that really divides the two daughter protoplasts of the heterotypic mitosis (*fig. 61*). As a very rare case, the granular zone is not in a plane, and the fibers which connect the granddaughter nuclei of different hemispheres arise before cell plates are laid down between them, so that the protoplast of the spore mother cell in this case becomes divided simultaneously into four tetrahedral spores.

Discussion of cytological phenomena

NUCLEAR RETICULUM.—Since the terms "chromatin" and "linin" were given by FLEMMING (**21**) and by SCHWARZ (**42**) respectively to the substance of the nuclear network which stains deeply and to the substance of the achromatic reticulum, these two substances have been regarded as essential constituents of the nucleus in its resting stage, and it became the general formula in describing the resting nucleus to state that it contains a fine network of linin fibers in which are imbedded chromatin granules.

To determine the real nature of chromatin and linin, and to find out the origin and differentiation of these two elements needs special investigation, which is not, of course, the object of the present work; however, the observation of events that occur between the telophase of one mitosis and the prophase of the next gave the writer the following notion regarding the nature of chromatin and linin, similar to the conclusion drawn by GRÉGOIRE and WYGAERTS (**25**).

When a group of daughter chromosomes reaches the pole, they are first drawn tightly together, so as to lose, apparently, their individual outlines. Vacuolization then takes place in this mass, and with the appearance of vacuoles parts of the mass of chromatin substance become irregularly separated and distributed so as to become transformed into a ragged reticular structure consisting of anastomosing, irregular clumps and finer, branched strands. The membrane, which may be formed by the contact of the surrounding cytoplasm with the nuclear sap that appears in the vacuoles, separates the mass of chromatin substance from the cytoplasm outside at a very early period, so that it is highly probable that no achromatic structures are included within the nucleus. The nuclear reticulum of the resting stage is nothing but the result of further advancement of the vacuolization. The new spirem of the ensuing division is formed again by the rearrangement of the ragged chromatin reticulum.

From the foregoing it seems quite probable that the nuclear reticulum is derived entirely from the chromosomes, the lightly staining threadlike strands in the resting nucleus being only a part of the chromatin substance that has assumed such a structure.

REDUCTION OF CHROMOSOMES AND SYNAPSIS.—Since tetrads were first figured by HENKING (**28**) in the maturation division of an insect, several authors have reported the tetrad formation in various animals in connection with the reduction of chromosomes. From studies of the plant cell, the presence of the tetrads in the spore mother cell was independently described by OSTERHOUT (**35**) and CALKINS (**11**). OSTERHOUT figured the tetrads in the prophase of sporogenesis in Equisetum. CALKINS found in Pteris and Adiantum that the tetrads arise from rings or double rods, parallel or crossed, which resulted from the longitudinal splitting of primary chromatin rods or segments and which break up into four parts (the tetrads), followed by a transverse division. An essentially similar mode of tetrad formation was described by BELAJEFF (**5**) in Iris and by ATKINSON (**4**) in Arisaema and Trillium.

As already described, there is no tetrad formation in Nephrodium, chiefly due to the fact that both first and second divisions do not occur in rapid succession, and that the appearance of the fission providing for the second division is delayed until after the separation of the bivalent chromosomes of the first mitosis. At present most investigators believe that tetrads, in a strict sense, are not formed in the majority of cases, excepting in a few groups of animals.

The nature of these two successive divisions of chromosomes in the spore mother cell has been discussed by many authors, chief attention being paid to phases after the segmentation of the spirem into a certain number of chromosomes. Among early observers there has been every possible variation of opinion. Miss SARGANT (**39**) found in Lilium that both divisions were longitudinal. This view was soon concurred in by STRASBURGER (**47**), FARMER and MOORE (**17**), and others. Two years later STRASBURGER (**50**) and MOTTIER (**33**) concluded that the fission of the chromosomes in the second division was transverse, and essentially similar results were obtained by ISHIKAWA (**30**) in Allium, and by BELAJEFF (**5**) in Iris. DIXON (**13,14,15**) and SCHAFFNER (**40, 41**) held a different view, believing that the continuous thick spirem twists up into loops that are the inception of chromosomes, and that the chromosomes of the first division result from the loops, separating by a transverse division at the angle of the loops; while the second division is longitudinal. ATKINSON (**4**) found two different methods in Arisaema and in Trillium; in the former species the first division was transverse, as DIXON and SCHAFFNER found; while in the latter the second was transverse, as STRASBURGER and MOTTIER claimed.

Among these three divergent views, which claim respectively that there occur two longitudinal divisions, that the first division is transverse and the second longitudinal, and that the first division is longitudinal but the second is transverse, the third was of short duration. The authors (STRASBURGER and MOTTIER) who described the existence of such a case changed their opinion and returned to their former view that both divisions are longitudinal, so that there persist only two views, one claiming the presence of two longitudinal divisions, and the other claiming that the first is transverse: the former one of these was held by GUIGNARD (**27**), GRÉGOIRE (**22**), LLOYD (**31**), and others.

However, the more recent studies of synapsis have involved very detailed and accurate observations concerning the nature of the reduction division. Some of the investigators mentioned above have changed their views, while others still maintain them, but in both cases a new or modified notion concerning the origin of chromosomes and the nature of reduction division is held.

As described before, the nucleus of Nephrodium in the presynaptic stage consists of a complex, anastomosing ragged chromatin reticulum. This ragged reticulum shows a tendency to become transformed into a thread structure, but the process does not occur simultaneously in different regions. When the transformation has taken place, the two parts of the thread are observed running side by side from the first. Such a condition as is represented in *figs. 16* and *16a* is evidently what was called "leptonema" by WINIWARTER (**51**). Further transformation of the thread structure from the ragged reticulum results in a nucleus with a continuous chromatin thread or spirem whose double nature is only visible at certain parts on account of the close association. The thread becomes tangled and contracted in one side of the nuclear cavity (*figs. 17* and *17a*) until finally there results the climax of the synaptic stage (*figs. 18, 19*); these two stages may cover the "zygonema" condition of WINIWARTER.

The above brings the writer to the conclusion that the spirem is of double nature from the outset, probably derived from the paternal and maternal source, as was interpreted by ALLEN (**1, 2, 3**), GRÉGOIRE (**25**), BERGH (**6, 7, 8, 9**), ROSENBERG (**37, 38**), and CARDIFF (**12**).

In preparations stained with iron-alum-hematoxylin and extracted so as to obtain a proper differentiation, the paired chromatin threads of the spirem appear as if consisting of darkly stained granular parts and a lightly colored ground-substance of threadlike structure, but this simply means that they are not of homogeneous thickness; the more aggregated the chromatin substance, the more dark appears the stain, and therefore probably the individuality of the two halves of the spirem may not be lost entirely during the close association.

There are observed in Nephrodium a number of parts of the spirem running through the contracted mass from the nuclear membrane where the mass lies in contact (*fig. 18*). GRÉGOIRE (**24**) found that a similar condition of the spirem exists in Osmunda, and that in a later stage the chromosomes resulting from the thick spirem held a similar orientation in regard to the nuclear membrane. From this he concluded that there is no continuous thick spirem in the prophase of heterotypic division of Osmunda. Since in Nephrodium the thick spirem as it emerges from synapsis is always in contact with the nuclear membrane up to the segmentation of chromosomes, the condition shown in *fig. 18*, where the number of parts of the spirem run out from one side of the membrane, may have some significance which we are unable to interpret at present.

The thick spirem emerging from synapsis showed first, at the regions widely uncoiled from the contracted mass, longitudinal fissions which came undoubtedly from the separation of the two independent threads that entered into association during synapsis. The thick spirem simply segments into 64 or 66 bivalent chromosomes, each of the pairs being derived from the independent thread. In a certain stage before the "strepsinema" condition, appearances of looping were also observed in connection with the contracted region, but they had nothing to do with the formation of chromosomes as described by FARMER and MOORE (**17, 18, 19**), GREGORY (**26**), FARMER and SHOVE (**20**), MOTTIER (**34**), and SCHAFFNER (**41**).

Consequently, the reduced number of chromosome segments of the heterotypic mitosis in Nephrodium are not two sporophytic chromosomes joined end to end, which come to lie side by side by the approximation of the arms of the loops and break apart at the head of the structure; but they are bivalent chromosomes or pairs of sporophytic chromosomes derived from the two threads of the synaptic stage that are always in close association side by side from the beginning, as claimed by ALLEN, BERGHS, and GRÉGOIRE.

Results obtained by MIYAKE (**32**) in a number of monocotyledons and by OVERTON (**36**) in a number of dicotyledons accord in general with this description of the mode of formation and separation of heterotypic chromosomes.

Although the present account of Nephrodium differs widely from the accounts of FARMER and MOORE, GREGORY, STEVENS, and STRASBURGER in the interpretation of the synapsis and the prophase of heterotypic mitosis, yet it agrees in one fundamental point, namely, that the first division is heterotypic and separates whole sporophytic chromosomes.

HOMOTYPIC DIVISION.—That the chromosomes in the second division in Nephrodium divide longitudinally is in accordance with the vegetative mitosis; however, there exists an essential point of difference between the two cases, namely, in vegetative mitosis the resting stage is always intercalated between the chromosomes in the last telophase and those of prophase of the ensuing division, so that each mitosis is followed by a resting stage, and a line of new longitudinal fission of the chromosomes arises in the early prophase of each division; while in the homotypic mitosis no resting nucleus is formed in telophase of the preceding mitosis, and therefore the homotypic mitosis should not be regarded as a complete one, standing by itself. Really an early part of the prophase of the second division may be regarded as being initiated at the first division, because the homotypic mitosis separates two daughter halves of a single sporophytic chromosome of the first division; the partial longitudinal fission of this sporophytic chromosome was begun during the anaphase of the first mitosis, although the fission was obliterated in the last telophase.

According to the count of chromosomes which was made in the telophase of the first division, the daughter nucleus may contain 64 or 66 chromosomes, the reduced number; however, it should be remembered that this is certainly due to the obliteration of the longitudinal fission which appeared in the anaphase, and since this early part of the event of the second mitosis is actually initiated in the anaphase of the first division, the daughter chromosomes should well be considered as bivalent, consisting of two granddaughter chromosomes, although the actual separation is completed in the prophase of the second division. Therefore the homotypic division, in spite of its dependence upon the first division, should be regarded rather as a real part of the reduction division, which completes the process initiated in the heterotypic mitosis; in other words, the reduction division is initiated in the heterotypic mitosis, but the reduction of the first division is only apparent; the real reduction in the number of chromosomes is completed in the telophase of the second division. It follows that the first nucleus which contains the reduced number of chromosomes is the spore, and consequently the spore marks the starting-point of the new generation, as discussed by GRÉGOIRE (**25**).

CENTROSOME.—The presence of the centrosome among the pteridophytes was reported in the spore mother cell by HUMPHREY (**29**) in Psilotum and Osmunda, and by CALKINS (**11**) in Adiantum and Pteris. OSTERHOUT (**35**) denied the presence of the centrosome in the spindle formation of Equisetum.

In Nephrodium, the spindle always arises from the rearrangement of cytoplasm. In sporogenesis a multipolar polyarch spindle is first observed when the nuclear membrane disappears, while in typical mitosis in the vegetative cell, two kinoplasmic caps first appear at the poles of the nucleus before the disappearance of the nuclear membrane. In both cases when chromosomes are arranged in the equatorial plate, spindle fibers gather into a focal center which lacks the centrosome or centrosphere, and the gathering of the fibers into the focal center persists until the last anaphase or early telophase; however, in no stage of mitosis are there present centrosomes, radiations, centrospheres, or any kind of structure which was believed to control nuclear division. As regards the mechanism of mitosis, the writer is not prepared to enter into any discussion. The centrosome question in connection with the blepharoplast in pteridophytes will be discussed in the next paper, which will deal with gametogenesis and fertilization in Nephrodium.

UNIFORMITY IN THE NUMBER OF CHROMOSOMES.—STRASBURGER'S paper (**46**) entitled "The periodic reduction of the number of the chromosomes in the life-history of living organisms," which was the first enunciation of the significance of sporogenesis and the reduction phenomena in relation to the alternation of generations in plants, was based upon the discovery that there is a uniformity in the number of chromosomes in certain phases of the life-history of the individual. Evidence seems to be accumulating from different groups of plants confirming the notion of constancy in the number of chromosomes in individuals under normal development, although there are instances where different authors report different counting.

Generally speaking, accuracy in counting the chromosomes depends upon the number and form of the chromosomes in the individual under consideration. When the number of chromosomes is large and their form is slender and curved, accurate counting is naturally difficult. In Nephrodium the writer found this difficulty, due to the great number and curved form of the chromosomes, but it was not impossible to make reliable counting by using certain stages in which the chromosomes assumed a straight rod shape and a regular arrangement, characteristic of this species. In sporogenesis there are three conditions favorable for counting. First the prophase after the segmentation of the spirem into chromosomes, up to the stage just before the disappearance of the nuclear membrane, was favorable for counting It might well be expected that two parts of the same chromosome which happened to be cut might appear in two successive sections of the same nucleus, and this would make the counted number larger than the actual. Nevertheless, after carefully examining the mitosis in this stage, it was possible to find out the sections with the least number of cut chromosomes, sometimes only two, as was given in *figs. 24a, b, c* and *25a, b, c, d.* The second condition good for accurate counting is the polar view in the early metaphase; and the third case is the polar view at a certain stage of the late anaphase, in which the chromosomes are arranged near the pole.

In the typical mitosis of the vegetative cell, on the other hand, the prophase previous to the disappearance of the membrane is not good for counting, because of the curved and twisted nature of the chromosomes. The polar view of the late prophase or early metaphase is favorable for gross estimation, but in a certain critical stage of late anaphase, when the chromosomes are in irregular arrangement at the pole, accurate counting is always possible.

From the counting made in the stages mentioned above, the writer believes that there is a constant number of chromosomes in Nephrodium, i. e., 128 or 132 in typical mitosis and 64 or 66 in the reduction division; the result is evidently not in harmony with the conclusion drawn by FARMER and DIGBY (**16**) in their recent paper.

The reason for the presence of two sets of numbers of chromosomes (such as 64 or 66) has not been determined.

Conclusion

Since this study of sporogenesis was carried through as preparatory for the study of apogamy in Nephrodium, the general conclusions will appear in the final paper. Two points, however, may be summarized here: (1) there is uniformity in the number of chromosomes in the sporophyte; and (2) in the normal life-history there is a reduction of chromosomes in sporogenesis.

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EXPLANATION OF PLATES I–IV

All figures were drawn with the aid of an Abbé camera lucida, using Zeiss's apochromatic objective 1.5^{mm}, N. A. 1.30, combined with compensating ocular 18, except *figs. 15a, 16a, 17a,* and *19,* which were drawn with same combination of objective and ocular under higher magnification obtained when the tube was extended to the farthest point.

The plates are reduced one-half the original size, except *plate 1,* which is reduced two-fifths.

PLATE I

Vegetative mitosis in the sporogenous cell in the eight-cell stage

FIG. 1. The prophase; the spirem before segmentation into chromosomes; a kinoplasmic cap with a few fibers at each pole; the membrane beginning to disappear at those points.

FIG. 2. Spirem segmented into chromosomes; chromosomes long and curved, split longitudinally, the two halves running closely in contact.

FIG. 3. Chromosomes arranged parallel along the axis passing from pole to pole, one end of chromosomes in which spindle fibers are attached lying in an equatorial plate.

FIG. 4. The intermediate stage in passing from the previous condition to the stage of an equatorial plate; the chromosomes much shortened, one end where spindle fibers are attached being bent, while the end directed to the pole is straight.

FIG. 5. Metaphase, showing an equatorial plate; chromosomes short and rod-shaped.

FIG. 6. Late metaphase; the daughter chromosomes beginning to separate.

FIG. 7. Anaphase; two sets of the daughter chromosomes on the way toward pole; chromosomes almost straight, rod-shaped, slightly more elongated than metaphase.

FIG. 8. Late anaphase; rod-shaped chromosomes parallel at the pole.

FIG. 9. Polar view of stage given in previous figure, showing 128 chromosomes.

FIG. 10. Group of daughter chromosomes vacuolized, the upper side, formerly the pole, being concave.

FIG. 11. Polar view of stage shown in previous figure; arrangment of chromatin material resulting from vacuolization of chromosomes presents regularity.

FIG. 12. Daughter nuclei after formation of membrane, still of flattened form; cell plate has appeared in middle region of central spindles.

FIG. 13. Slightly oblique side view of daughter nucleus after formation of daughter cell; size increased; two nucleoli have appeared in the previously polar region.

FIG. 14. Polar view of daughter nucleus in later stage than that shown in previous figure; nucleoli apart from chromatin material.

PLATE II

Sporogenesis

FIG. 15. One of three sections of a resting nucleus in spore mother cell; chromatin reticulum without any regularity, looking ragged; three nucleoli are present in this section, some of them vacuolate.

FIG. 15*a*. Portion of chromatin reticulum of previous figure under higher magnification; reticulum consists of irregular clumps and slender strands, giving ragged aspect.

FIG. 16. One of three sections of a nucleus in which is shown the tendency to form the thread structure from a rearrangement of the chromatin reticulum; a single nucleus is seen in this section.

FIG. 16*a*. Portion of chromatin reticulum from *fig. 16* under higher magnification; by the disappearance of some of the strands and elongation of ragged clumps, thread structure is formed; these threads from the outset independent.

FIG. 17. Early synaptic stage; chromatin reticulum, almost transformed into continuous thread, begins to contract at one side of the nuclear cavity.

FIG. 17*a*. Portion of the parallel threads in *fig. 17* under higher magnification.

FIG. 18. Synapsis; nucleolus caught in the contracted mass of thread at a position not in contact with the nuclear membrane.

FIG. 19. Portion of the separating threads from a tangential section, the next to that shown in *fig. 18*, under higher magnification.

FIG. 20. The nucleus emerging from synapsis; uncoiled parts of the spirem show the longitudinal splitting or separation of two threads which entered into close association in synapsis.

FIG. 21. Spirem shortened, thickened, and more uncoiled; line of longitudinal splitting or separation visible; kinoplasmic fibers present surrounding the nucleus, circumscribing the comparatively clear zone near the membrane.

FIG. 22. Spirem more thickened and distribution more irregular than in the preceding stage shown in *fig. 21;* kinoplasmic fibers approaching nearer to the membrane.

FIGS. 23*a*, 23*b*. Two sections of the same nucleus when chromosomes begin to be segmented; two halves of the spirem almost equally thickened and in close association; spirem irregularly contracted.

FIGS. 24*a*, 24*b*, 24*c*. Three sections of the same nucleus after segmentation of spirem into 66 bivalent chromosomes; association of two halves of each chromosome has become somewhat loose, so that various combinations of the two daughter halves are shown, such as **H, I, J, K, L, O, U, V, X, Y**, and so forth; thin strands of chromatin material visible attached to the ends of chromosomes; two chromosomes (*a* and *b*) are cut into two, so that the two parts of the each chromosome appear in two successive sections.

FIGS. 25*a*, 25*b*, 25*c*. Four sections of the same nucleus; chromosomes show spiny structure; nuclear membrane has disappeared, the outline of nucleus being maintained by the encroaching and anastomosing of fibers; two chromosomes (*a* and *b*) are cut into two, so that the two parts of each chromosome appear in two successive sections.

PLATE III

Sporogenesis continued

FIGS. 26*a*, 26*b*. Two sections of the same nucleus, after intrusion of fibers; multipolar spindle is established.

FIGS. 27*a*, 27*b*. Two sections of the same nucleus, after the multipolar spindle has passed into the bipolar condition; bivalent chromosomes, two daughter halves always in pairs, have shortened considerably.

FIG. 28. Early metaphase, the bivalent chromosomes being regularly arranged in an equatorial plate.

FIG. 29. Polar view of early metaphase; lightly shaded chromosomes represent those which could be seen at a deeper focus; 64 bivalent chromosomes are present.

FIG. 30. Metaphase; daughter chromosomes begin to separate.

FIG. 31. A later stage than that shown in *fig. 30;* some of the daughter chromosomes which lie on the way to the pole indicate a new fission that provides for the second mitosis.

FIG. 32. Anaphase; rate of movement of daughter chromosomes toward the pole is variable.

FIG. 33. Late anaphase; some of the daughter chromosomes at the pole still show **V**-shape, due to the fission first indicated in *fig. 31*.

FIG. 34. Early telophase; daughter chromosomes are grouped at the pole, two of them being tardy; no **V**-shaped chromosome present.

FIG. 35. Polar view of stage shown in *fig. 34*, showing 64 chromosomes.

FIG. 36. Telophase viewed from the pole; chromosomes vacuolized; nuclear membrane constructed.

FIG. 37. Polar view of daughter nucleus in a later stage of telophase; vacuolization farther advanced.

FIG. 38. Daughter nucleus begins to grow in size; masses of the chromatin reticulum may represent main bodies of vacuolized chromosomes; granular structure begins to arise along periphery of equatorial region where fibers from poles meet.

PLATE IV

Sporogenesis continued

FIG. 39. Polar view of stage shown in *fig. 38;* granular zone surrounding cross-sections of central spindles.

FIG. 40. Spore mother cell separated into two hemispheres by granular zone; daughter nucleus has grown in size; V elements distributed in chromatin reticulum represent main bodies of vacuolized chromosomes.

FIG. 41. Polar view of daughter nucleus shown in *fig. 40.*

FIG. 42. Side view of nucleus of second division; a little later stage than that given in *fig. 41.*

FIG. 43. Nucleus in same stage as previous figure; only parts of chromatin reticulum visible in one focus are drawn; V elements distinct.

FIG. 44. Prophase of nucleus of second division after disappearance of nuclear membrane; spindle multipolar.

FIGS. 45, 46. Prophase after bipolar spindle is established; these two figures are in the same stage, the former being the view from the pole of the spore mother cell, the latter the view from the equator of the same.

FIG. 47. Divergent arms of V's, i. e., daughter halves of chromosomes of second division have become parallel, the ends where spindle fibers are attached lying in the equatorial region.

FIGS. 48, 49. Late prophase; the two figures represent the same stage, viewed from the equator and pole of the spore mother cell respectively.

FIG. 50. Metaphase; two daughter halves in each bivalent chromosome exactly similar in size and shape.

FIG. 51. Polar view of stage intermediate between the two conditions shown in *figs. 48* and *50,* this aspect being due to the overlapping of the chromosomes.

FIGS. 52, 53. Anaphase; two figures represent the same conditions viewed from the equator and pole of the spore mother cell respectively; two or three pairs of daughter chromosomes are tardy in separation.

FIGS. 54, 55. Late anaphase; the two figures show the same stage viewed from the equator and pole of the spore mother cell respectively.

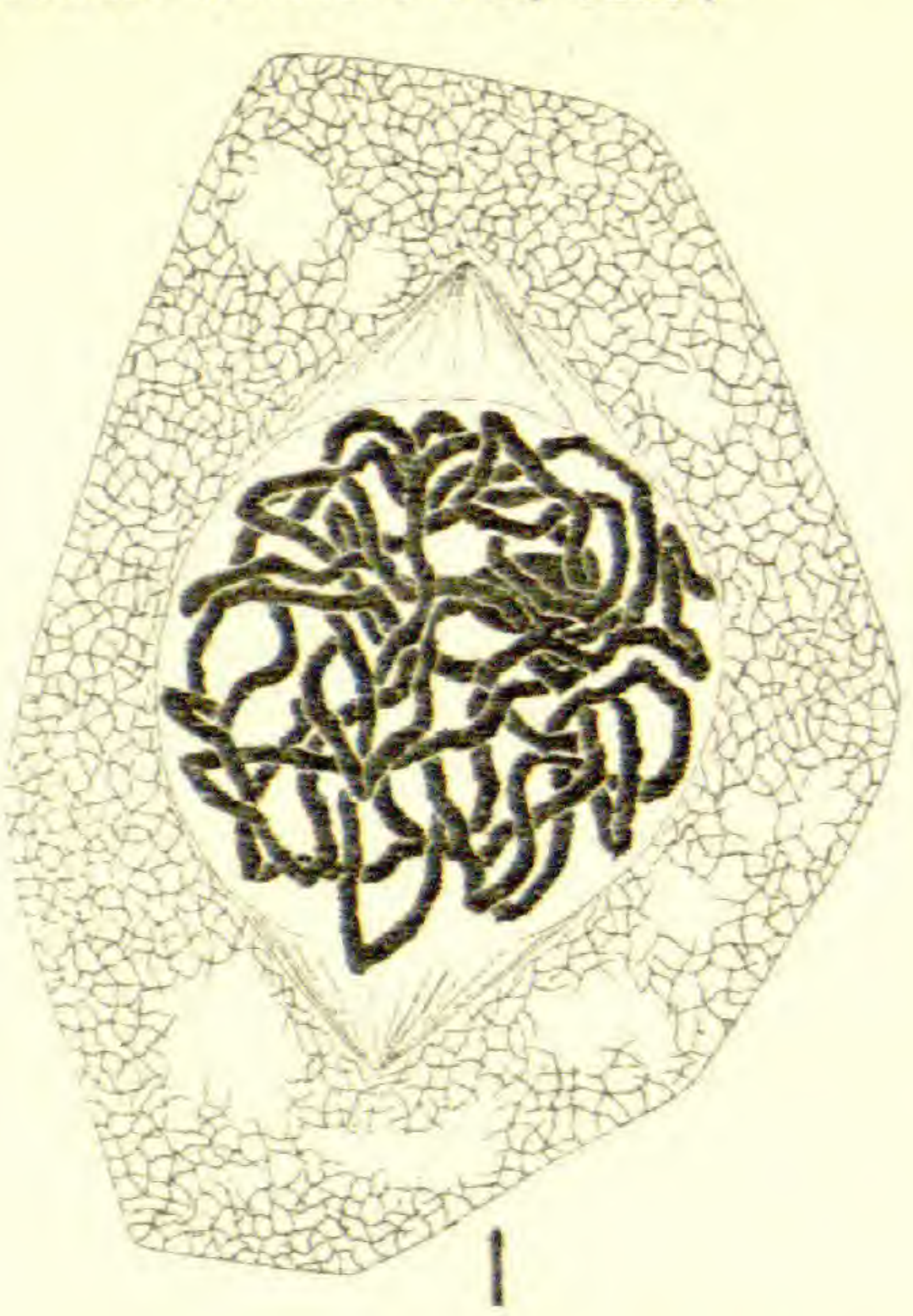
FIG. 56. Polar view of daughter chromosomes of late anaphase shown in *figs. 54* and *55;* 64 chromosomes.

FIG. 57. Early telophase; daughter chromosomes of second division at the pole and arranged regularly; cell plate laid down at middle region of central spindles.

FIGS. 58, 59. Telophase; the two figures show the same stage, viewed from equator and pole of spore mother cell; cell plate has divided the protoplast.

FIG. 60. Late telophase; membrane of the granddaughter nucleus formed; new fibers present between granddaughter nuclei of different hemispheres which were separated by granular zone in telophase of first division.

FIG. 61. Still later condition than stage shown in previous figure; cell plate completed through middle region of granular zone, thus dividing the two protoplasts separated in the first mitosis.



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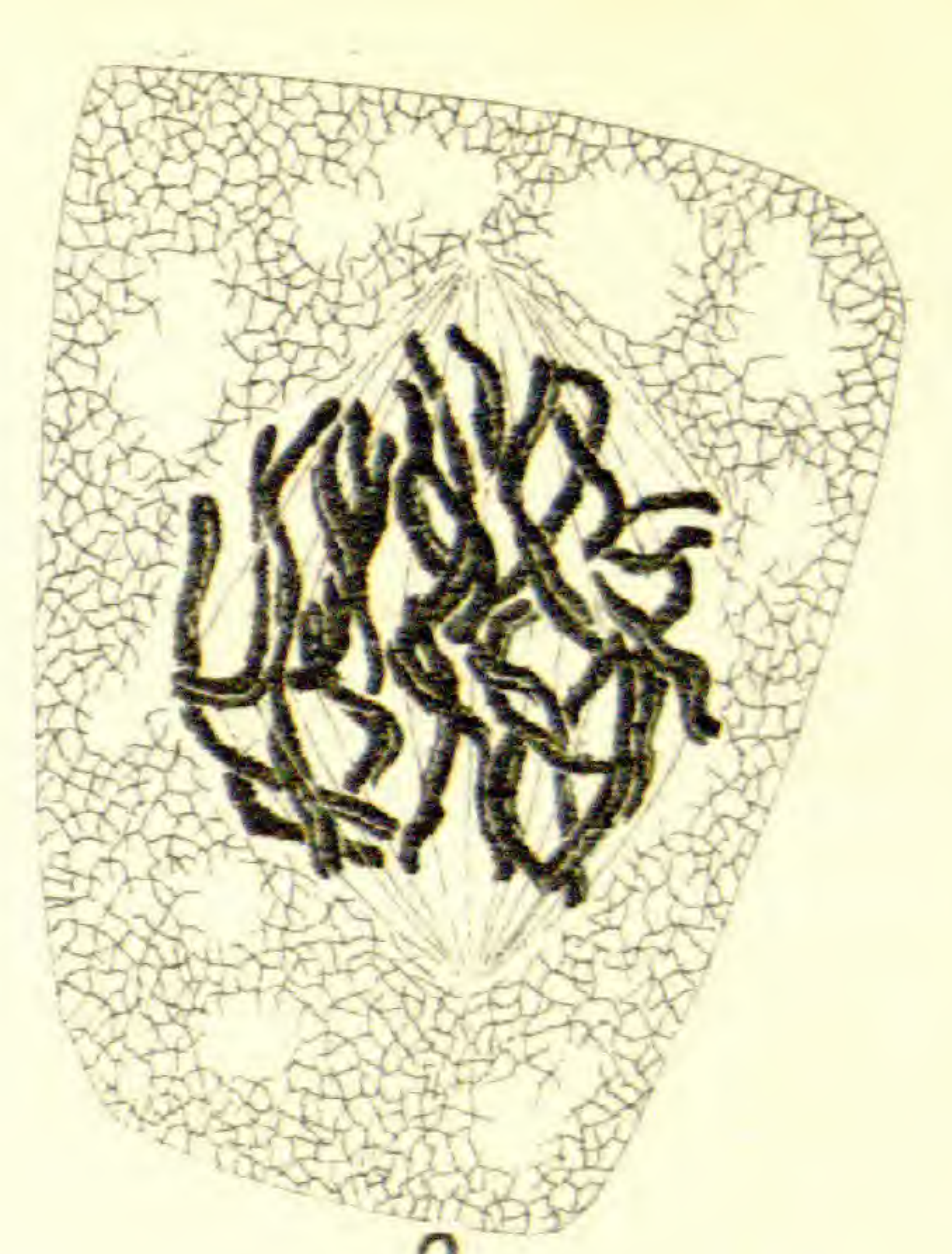

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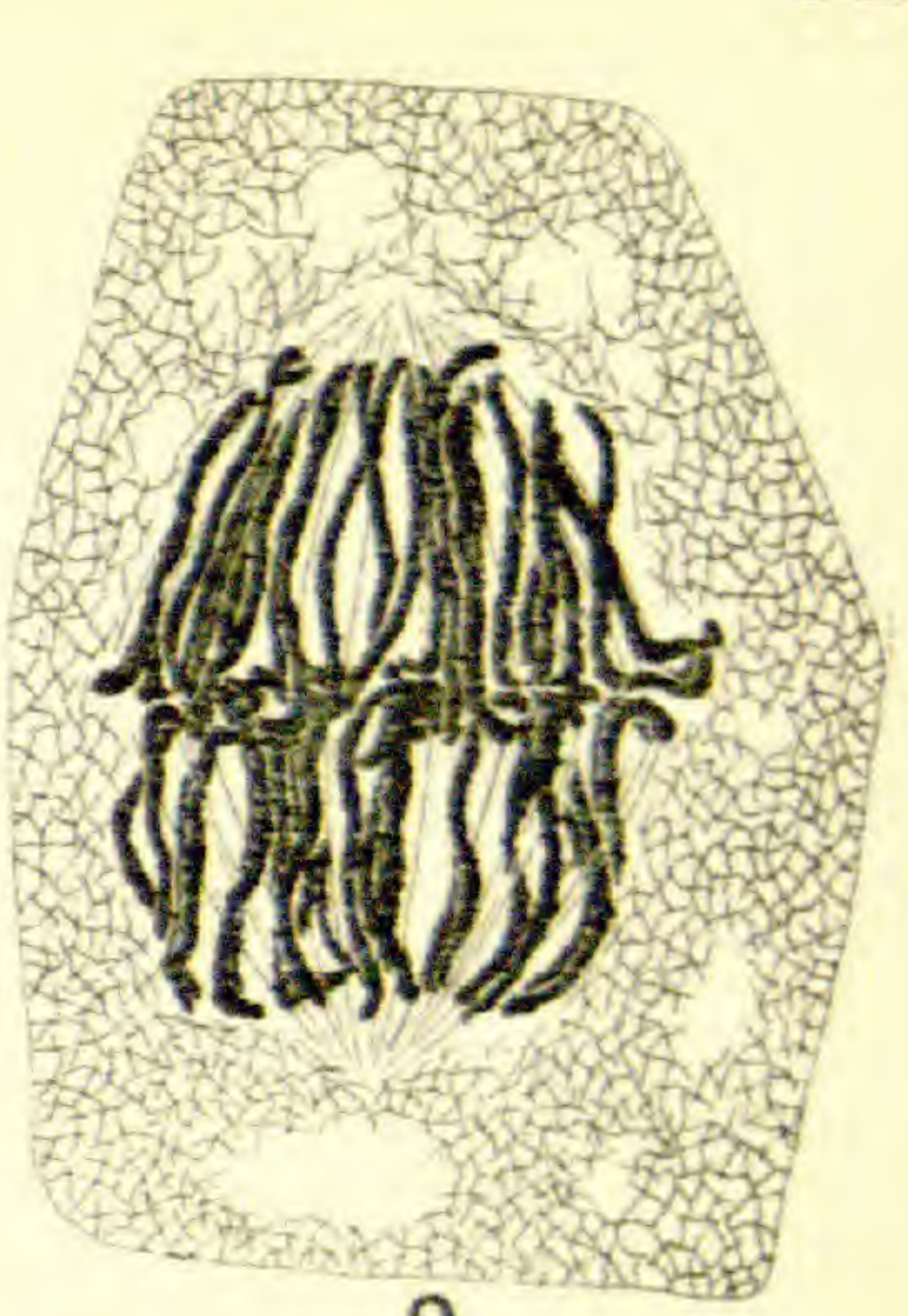

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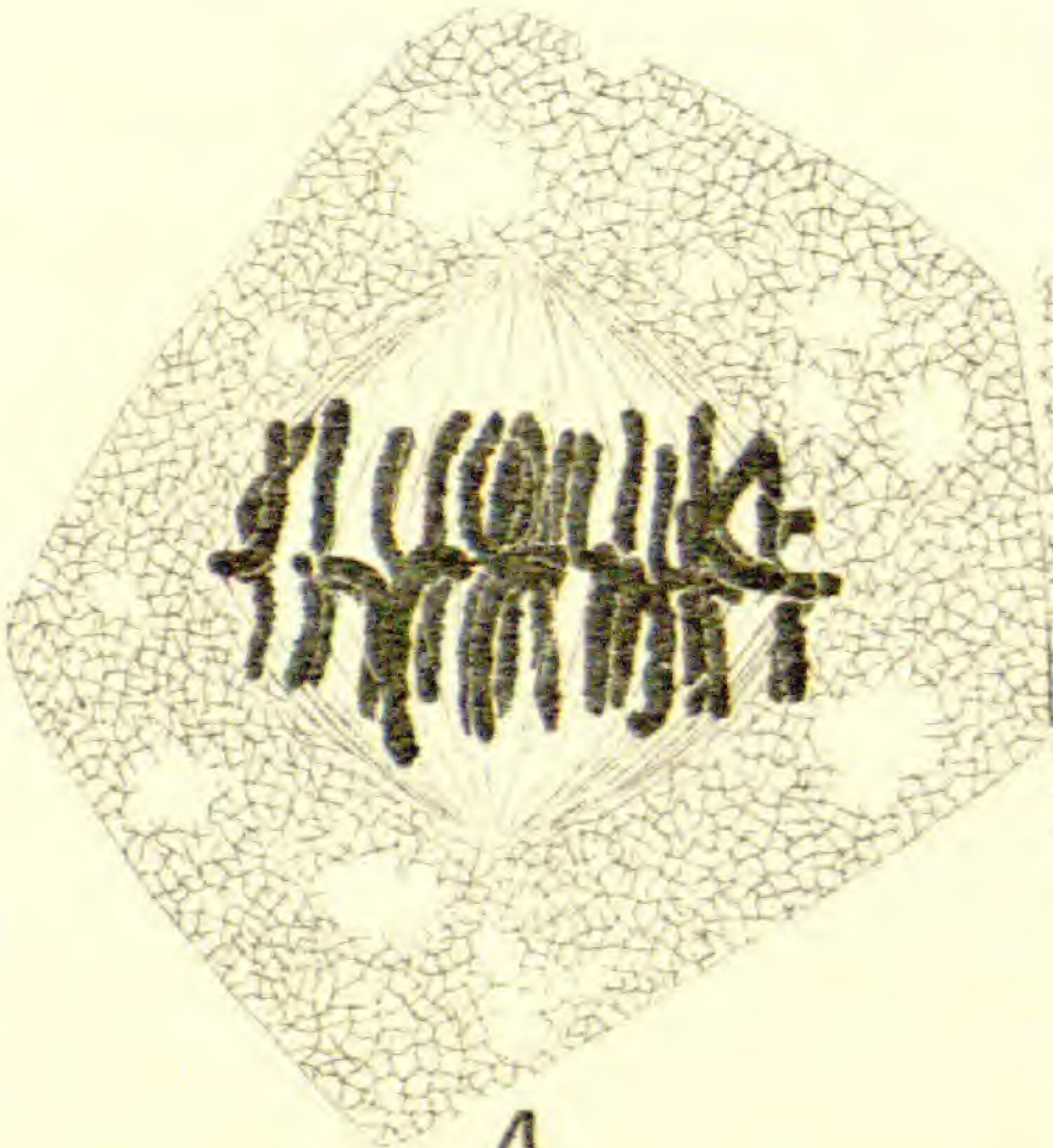

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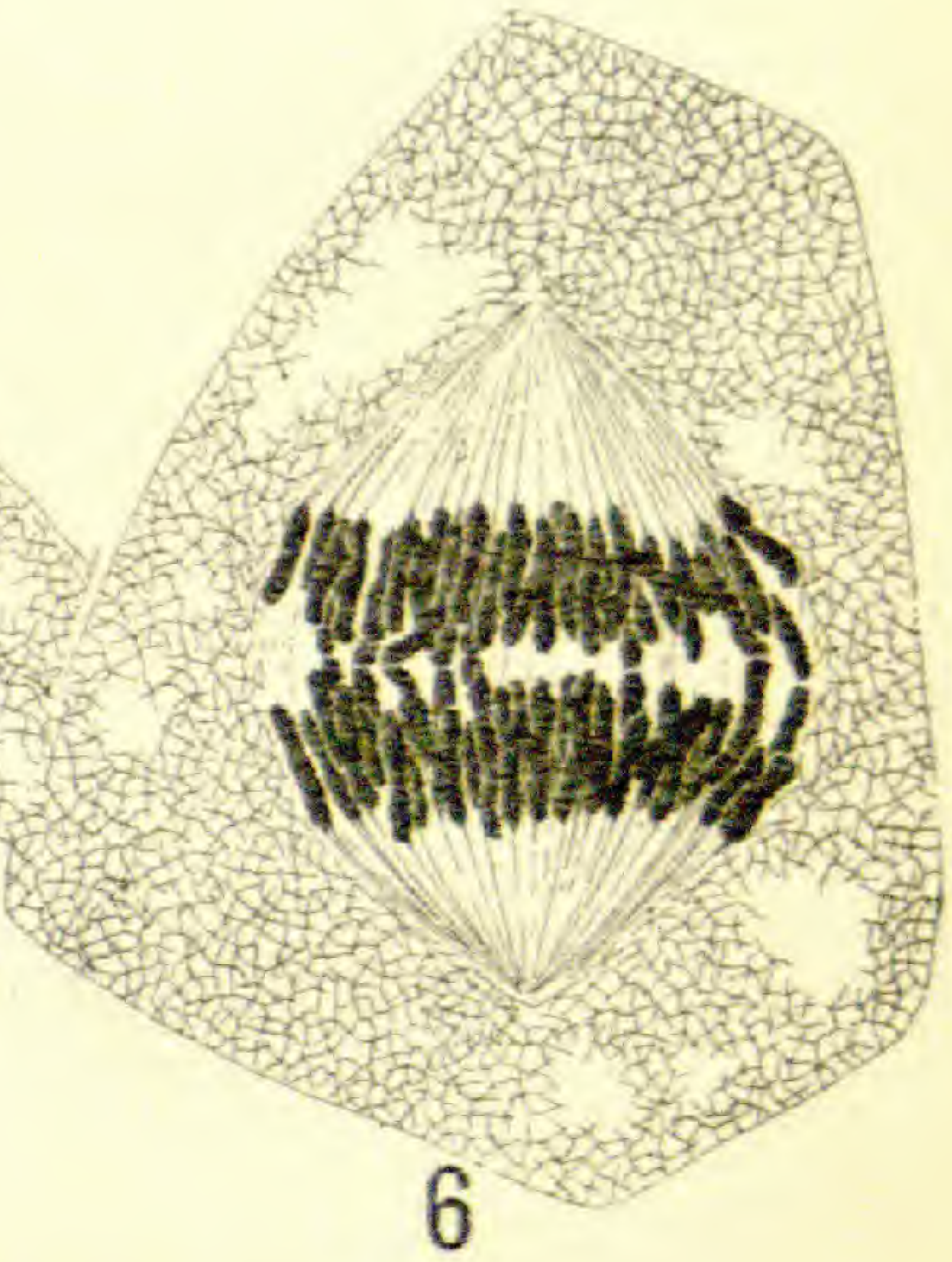

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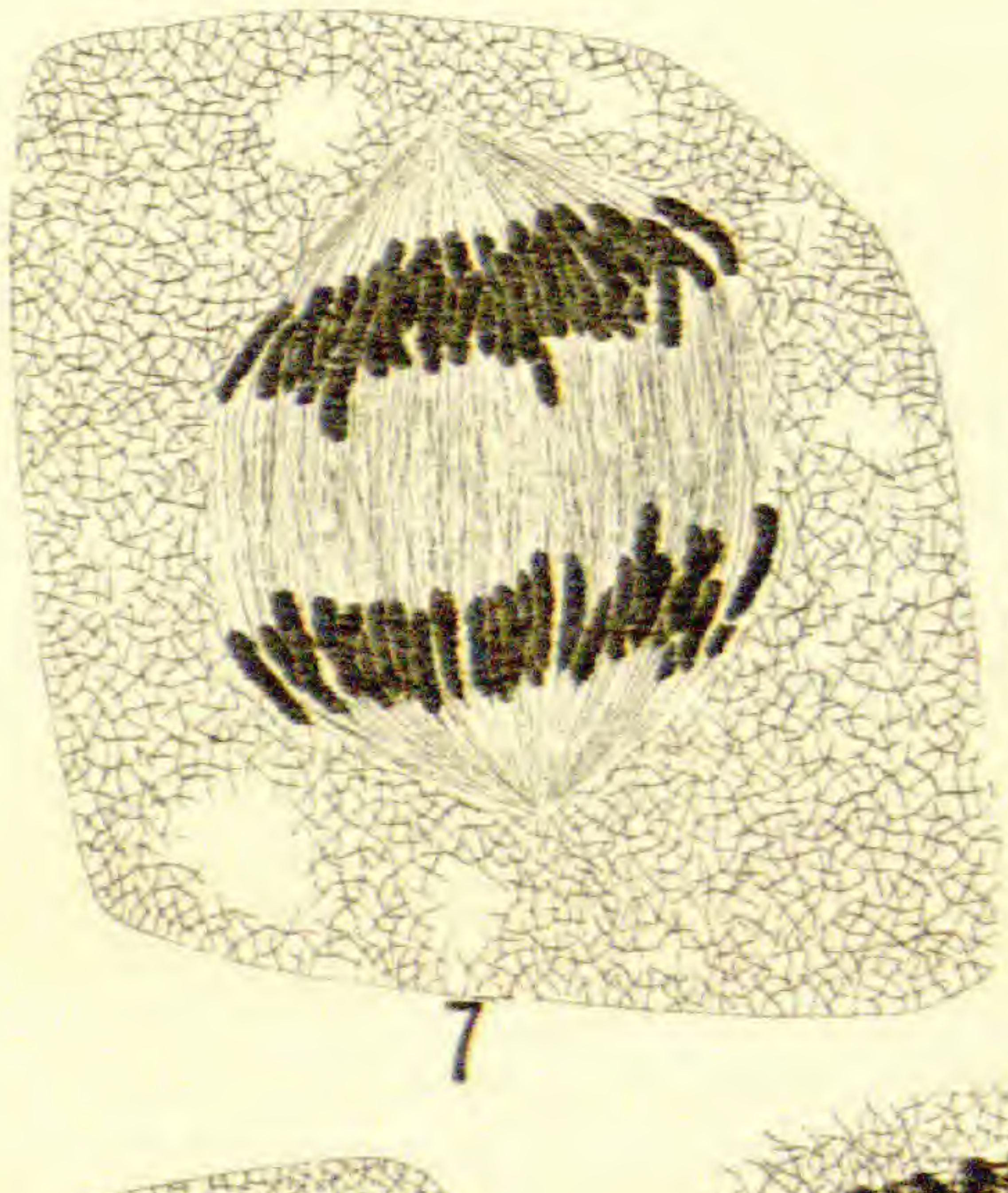

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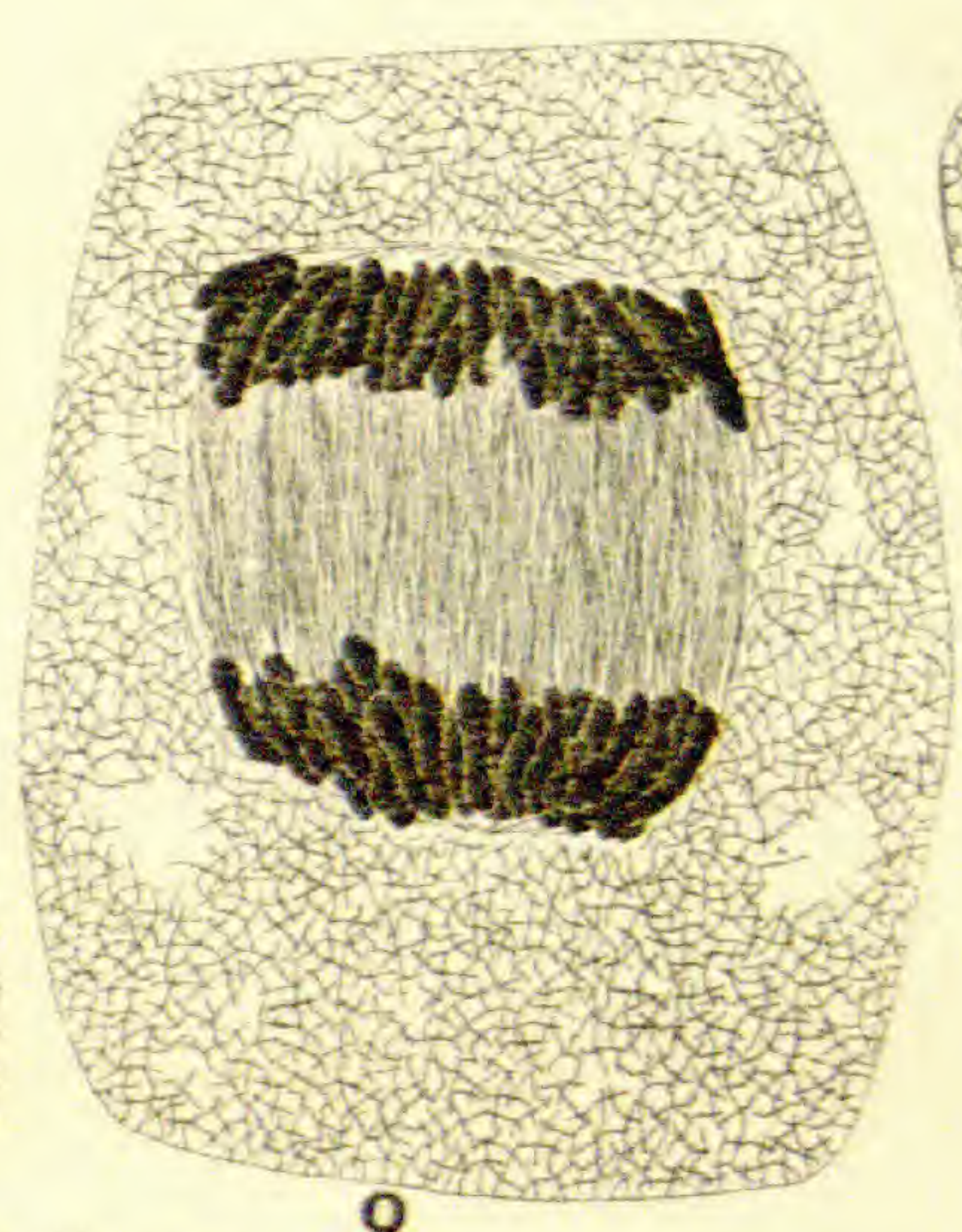

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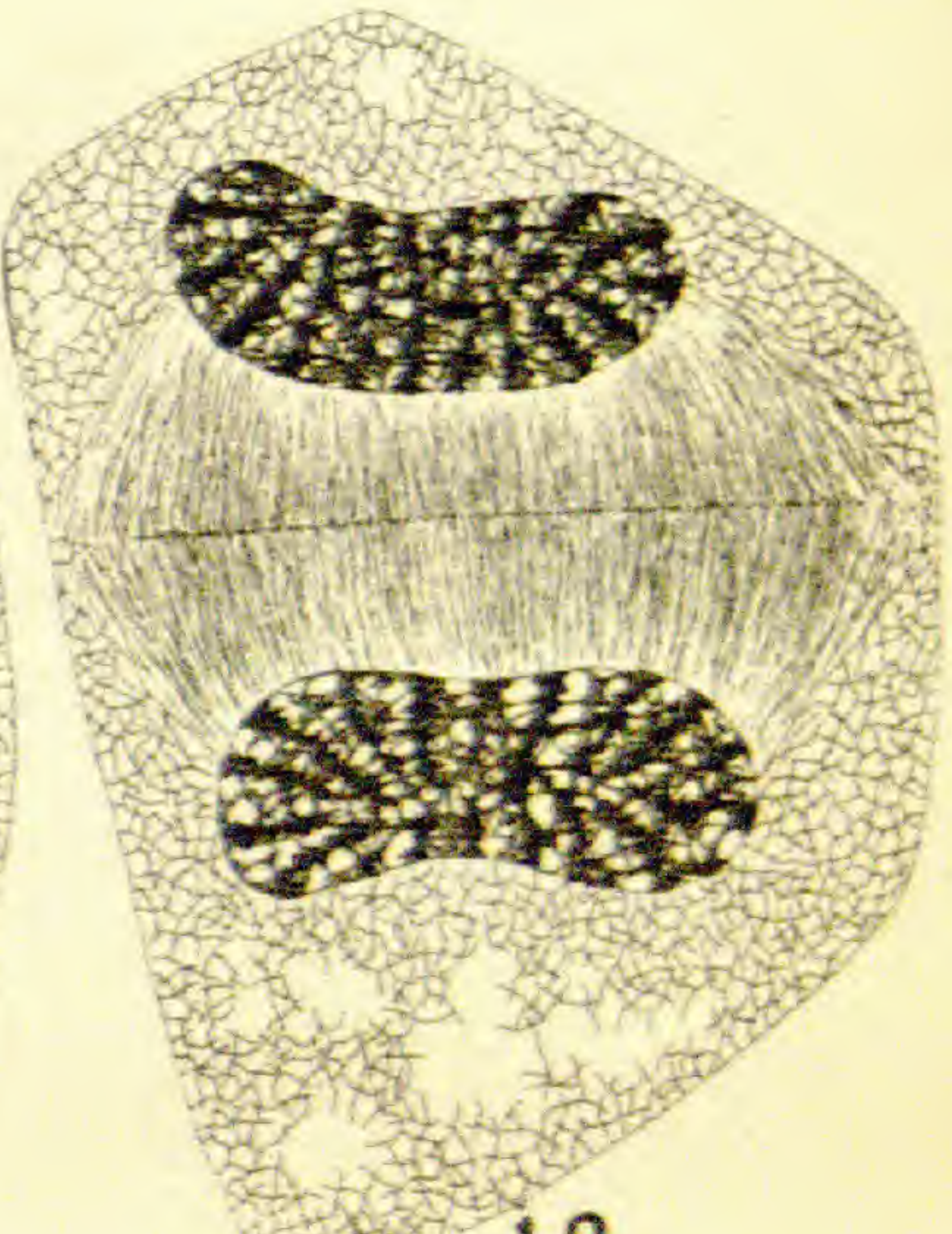

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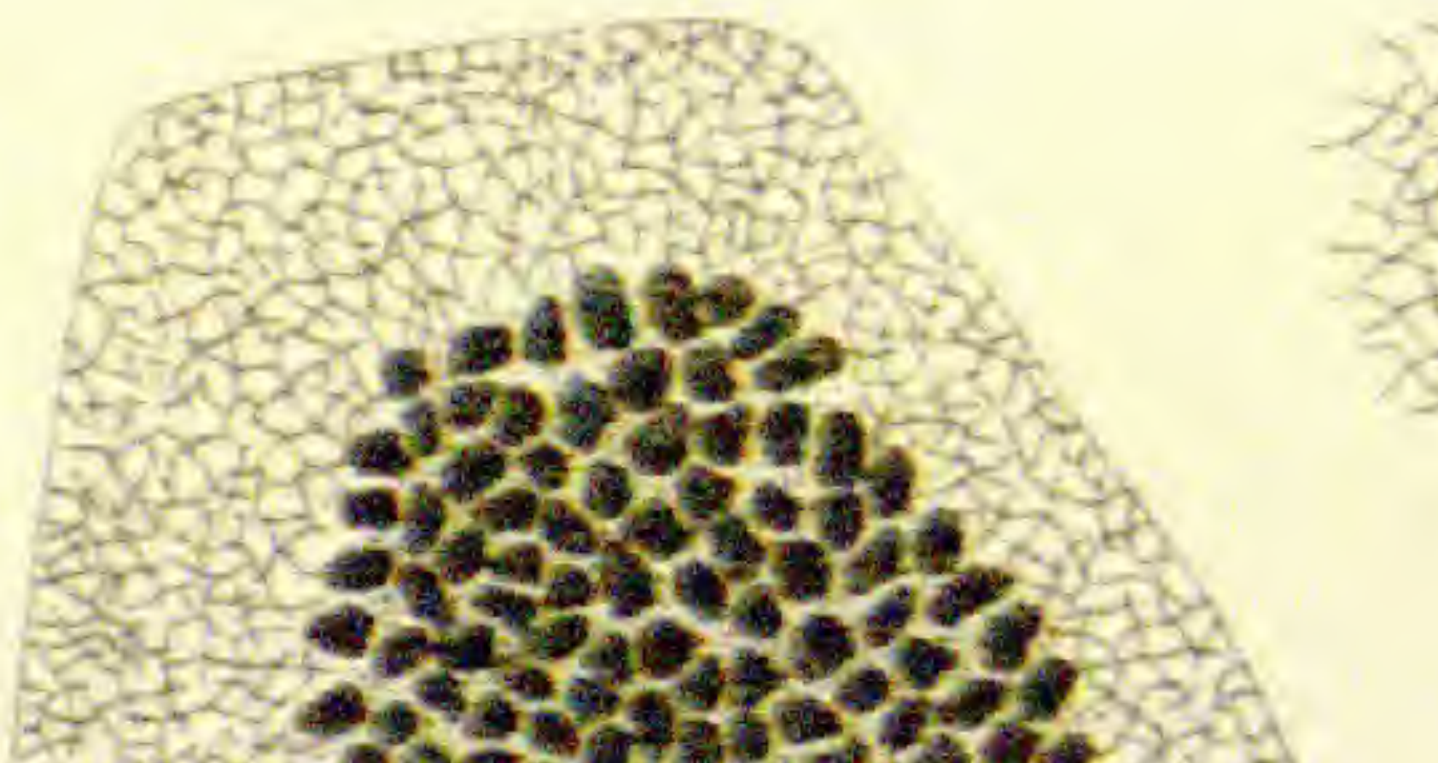

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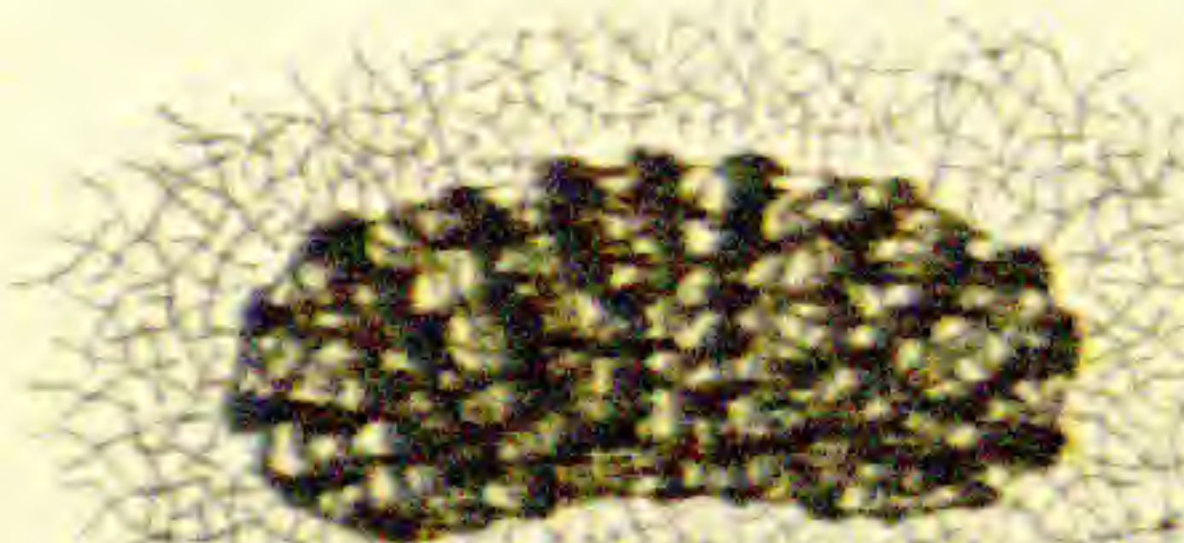

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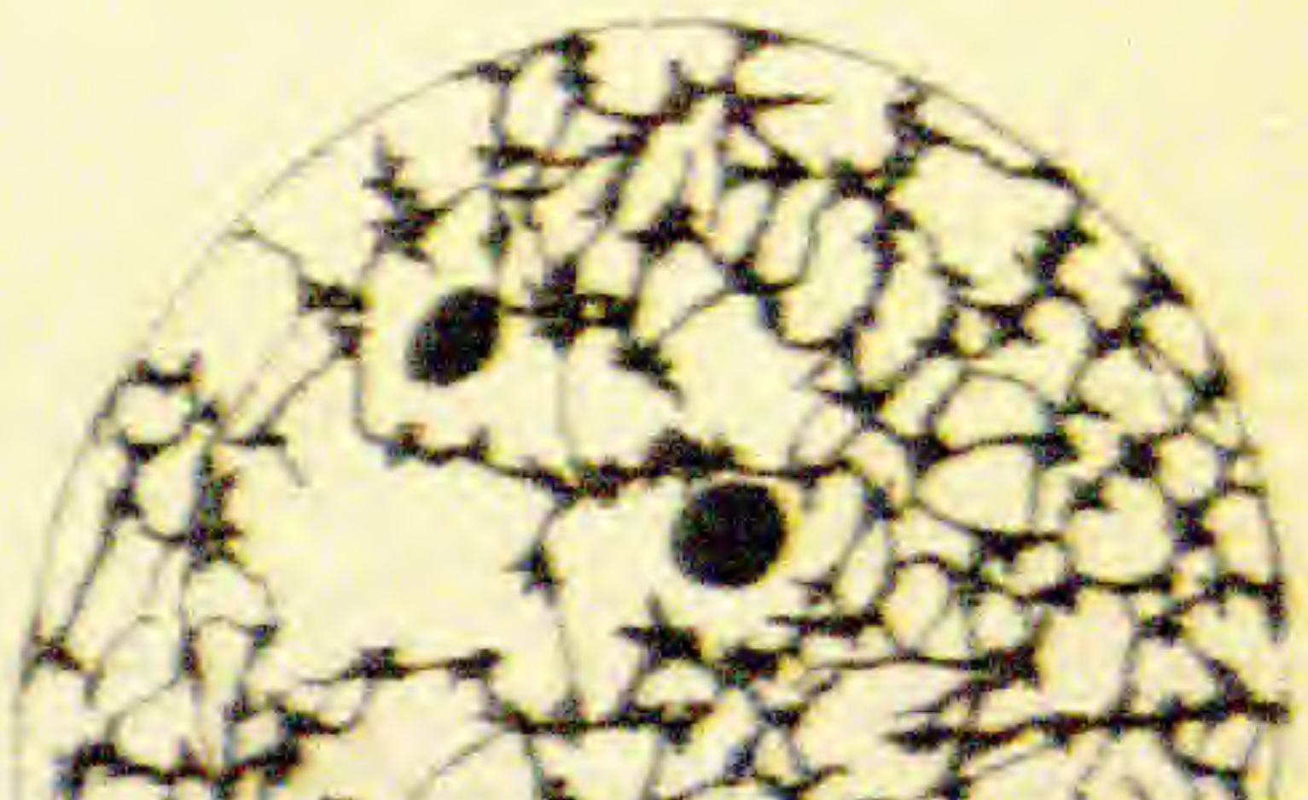

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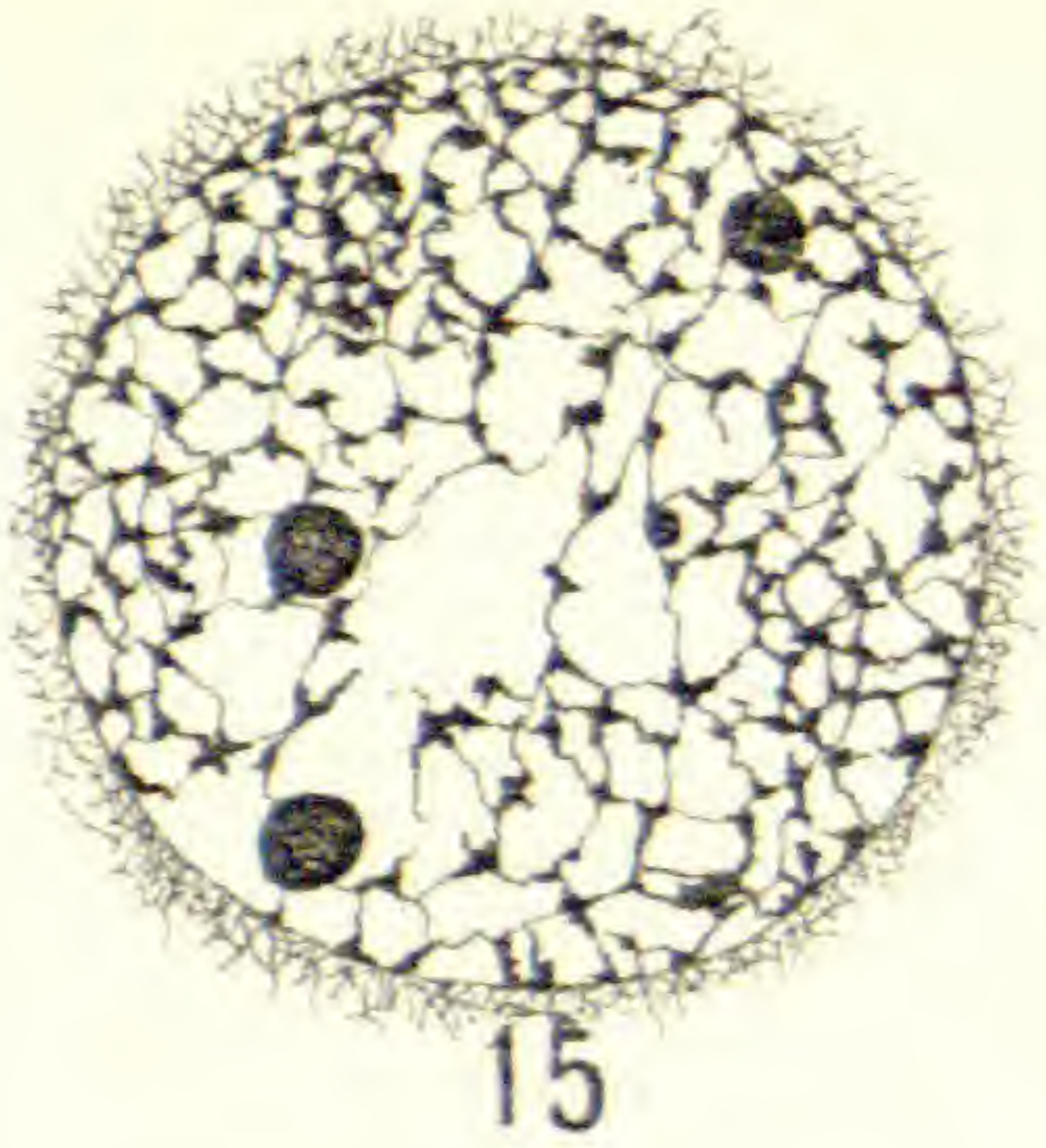
YAMANOUCHI on NEPHRODIUM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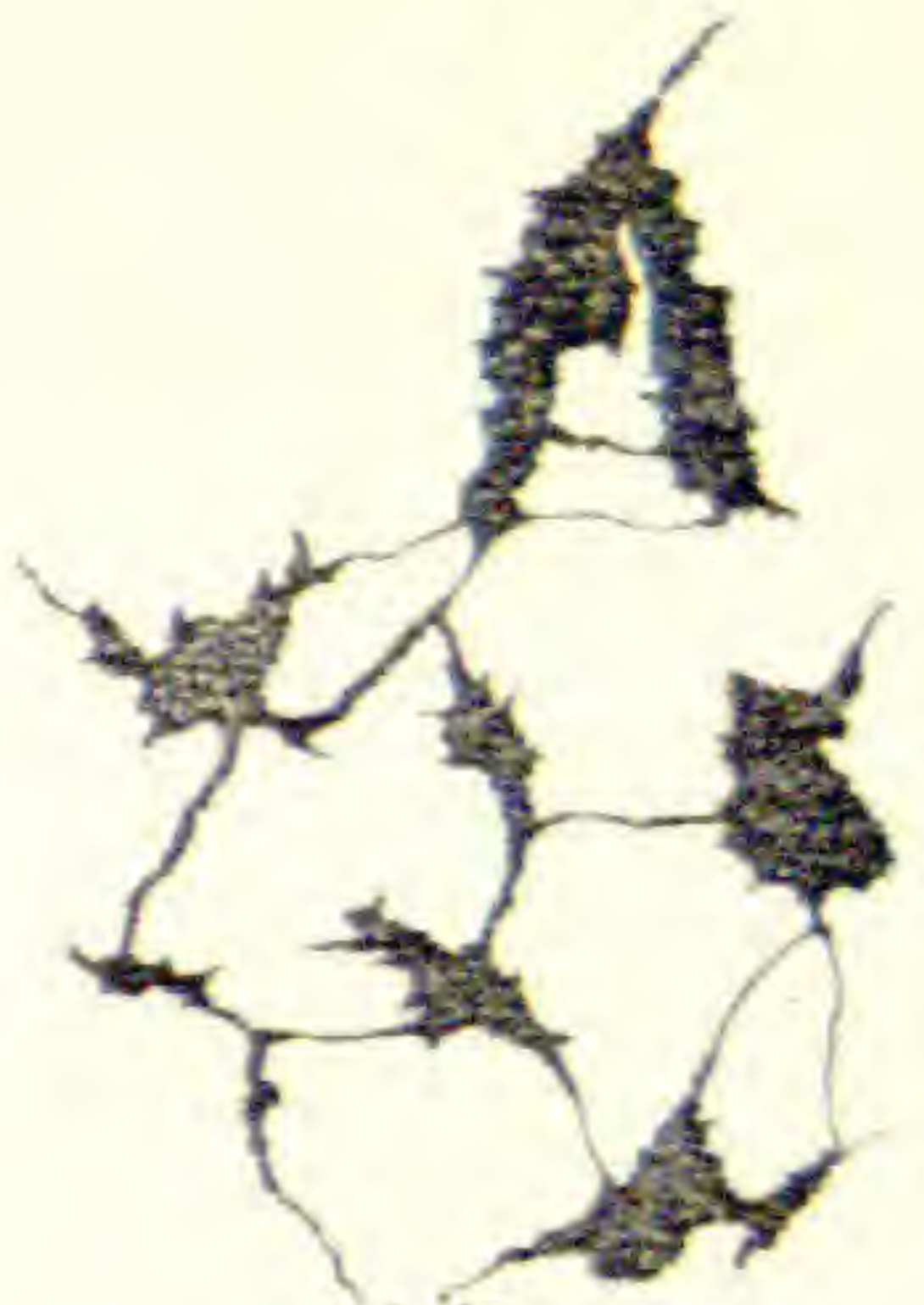
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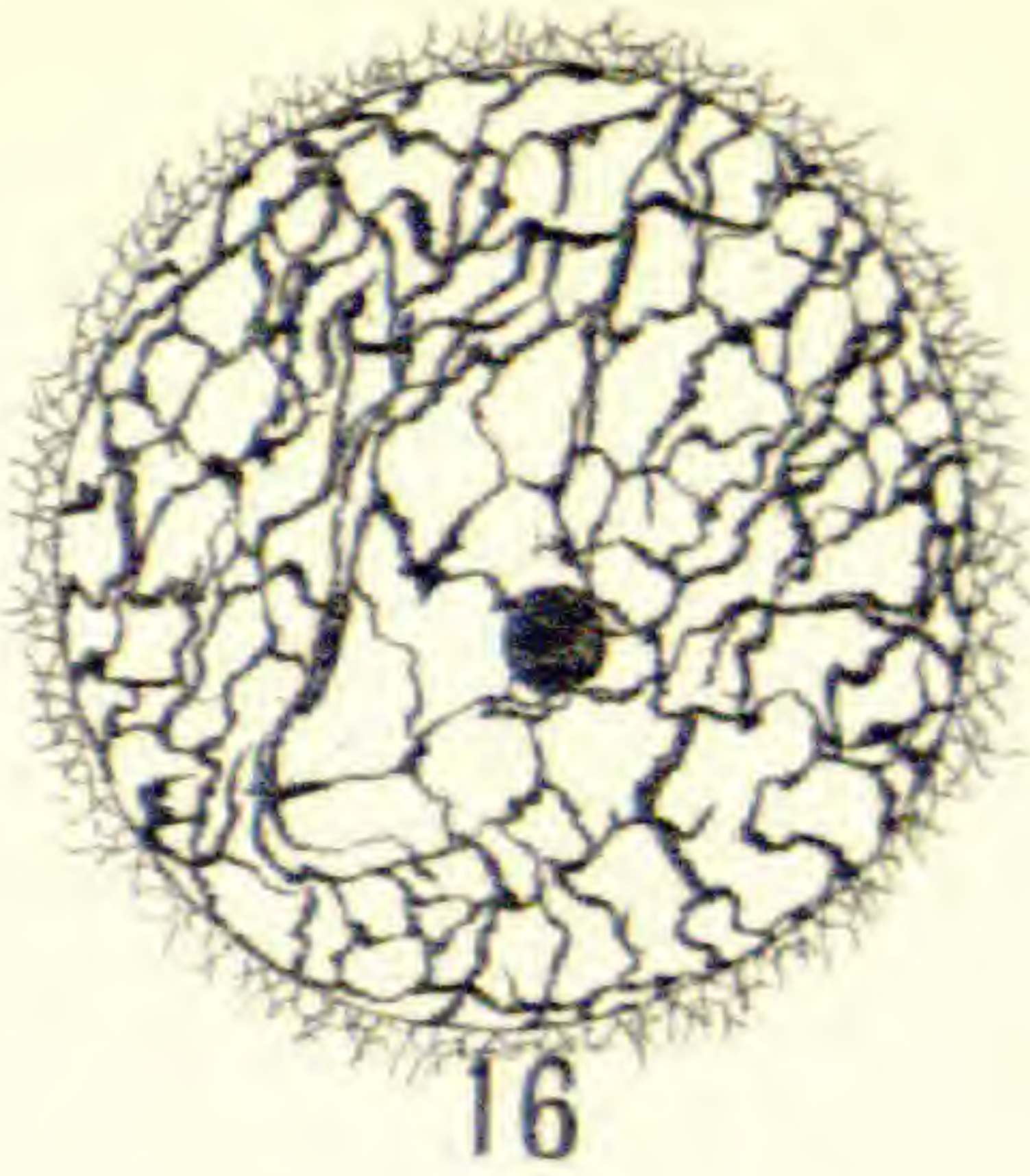
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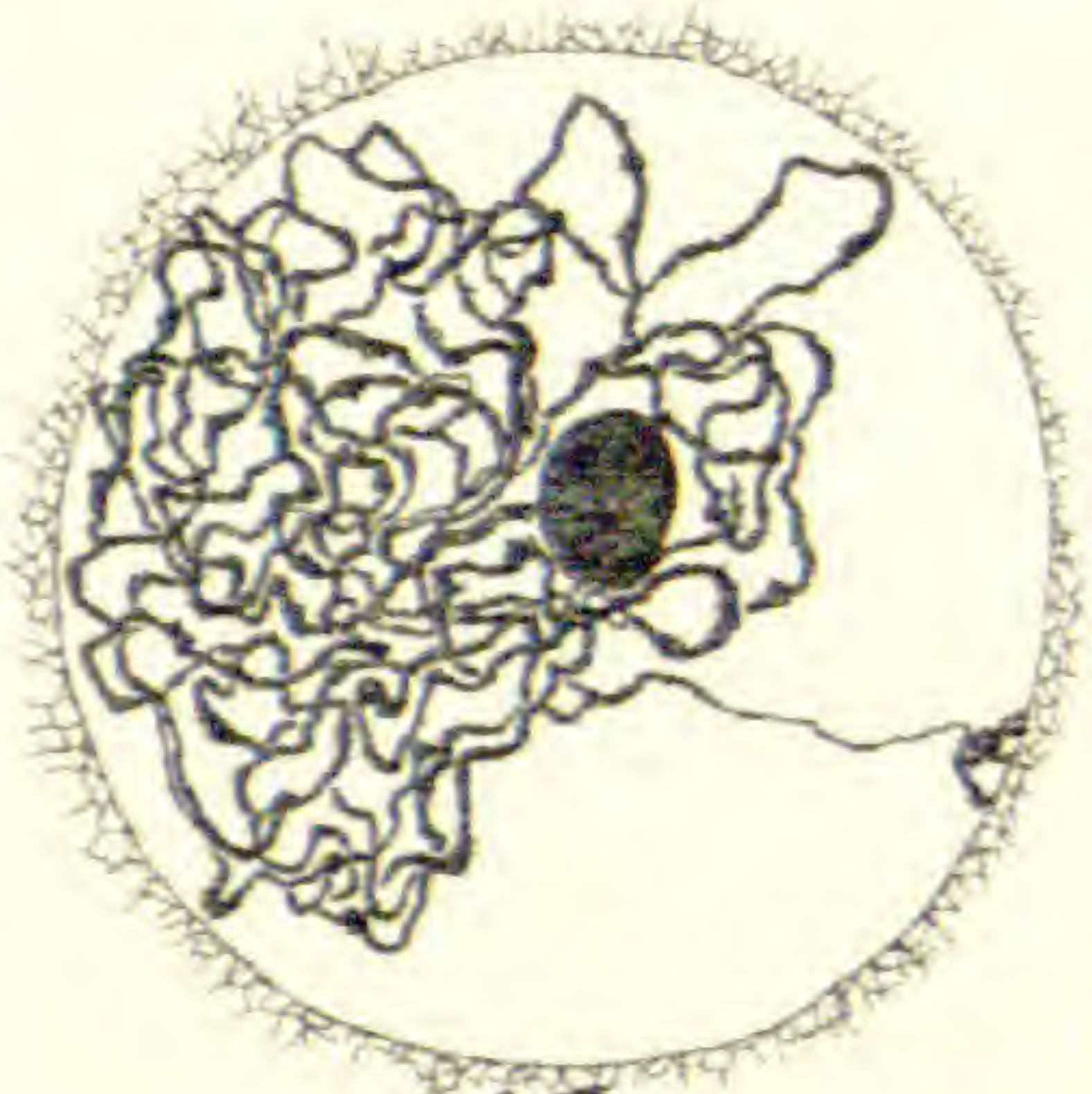
16*a*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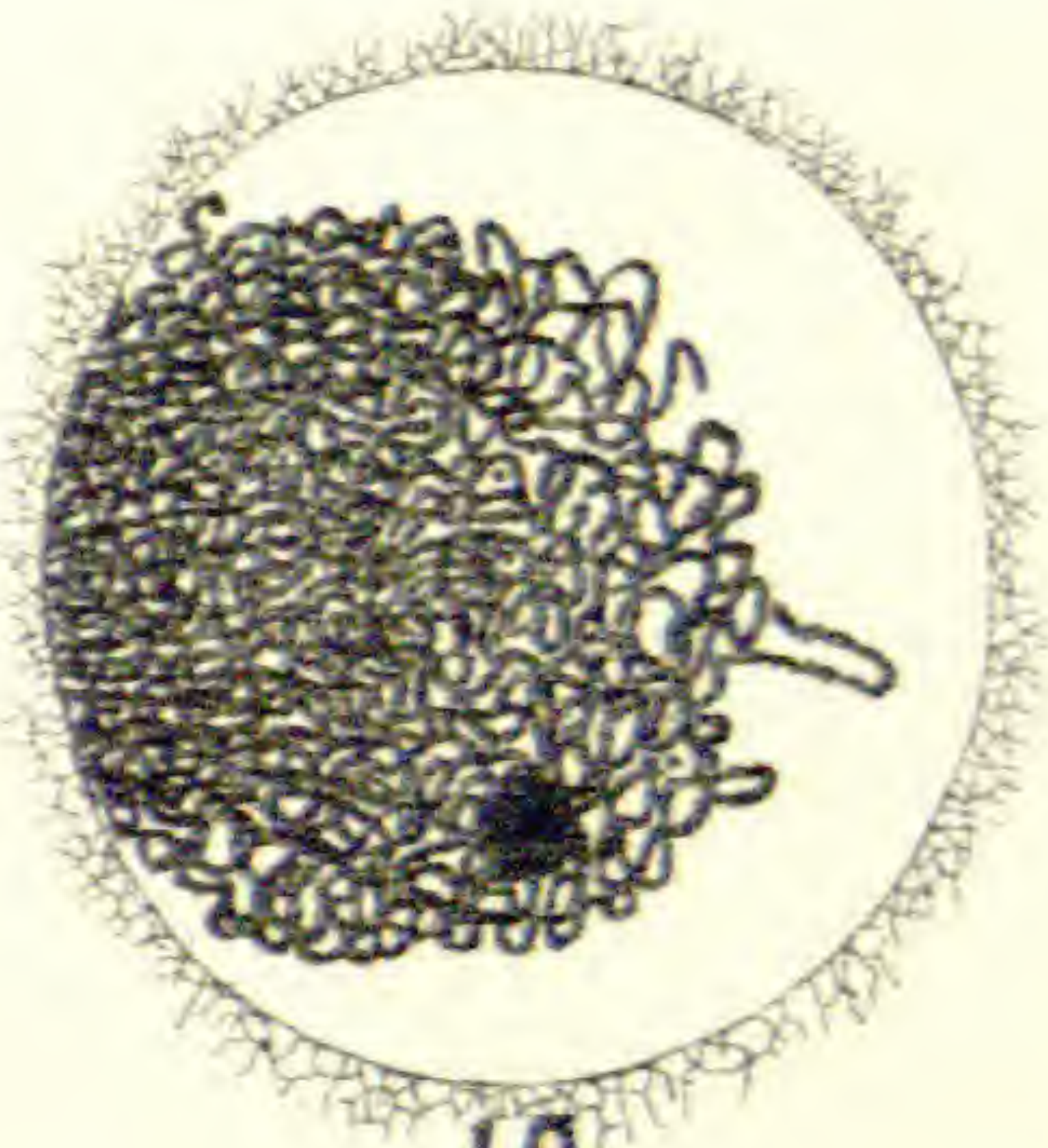
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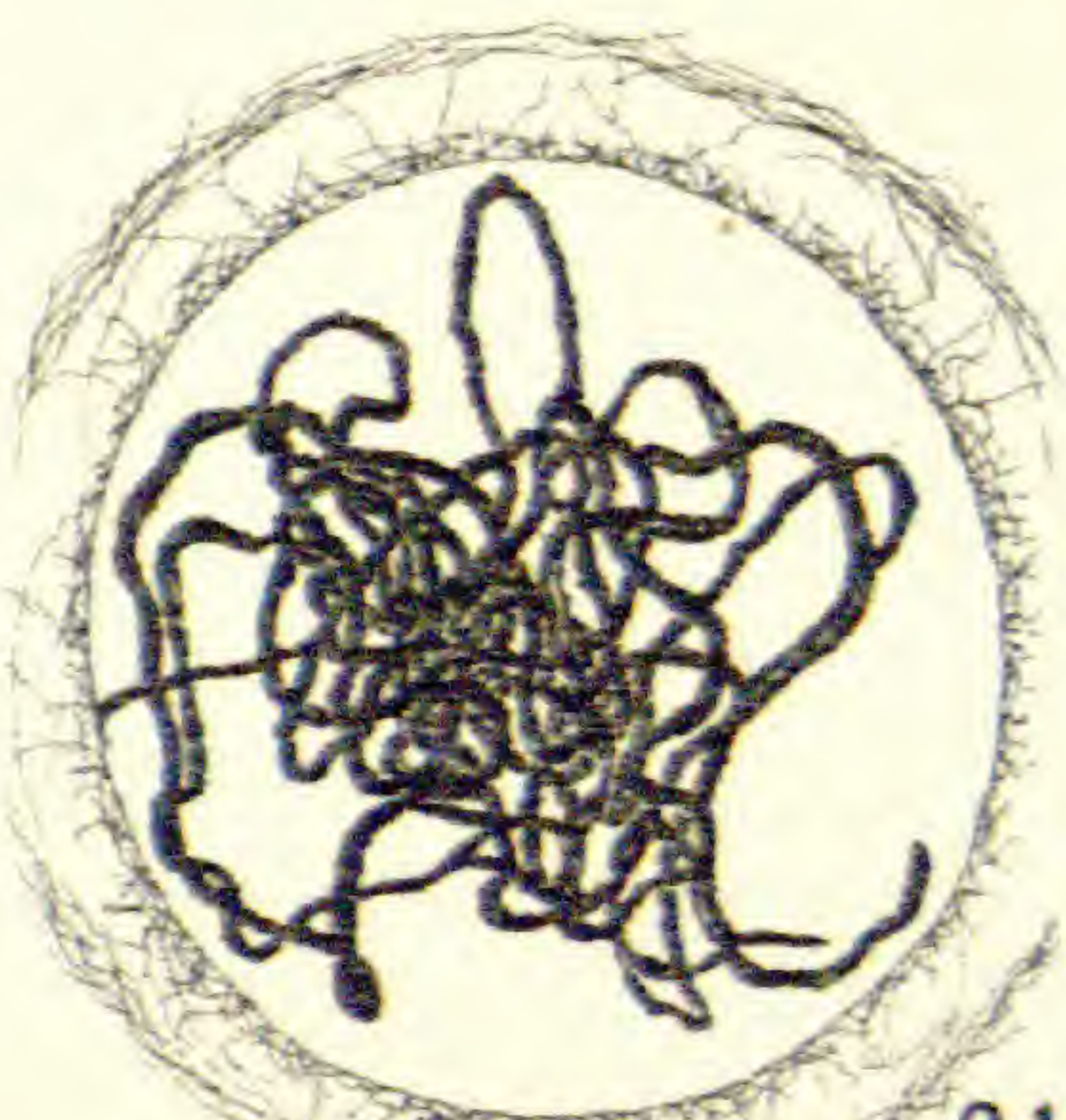
17*a*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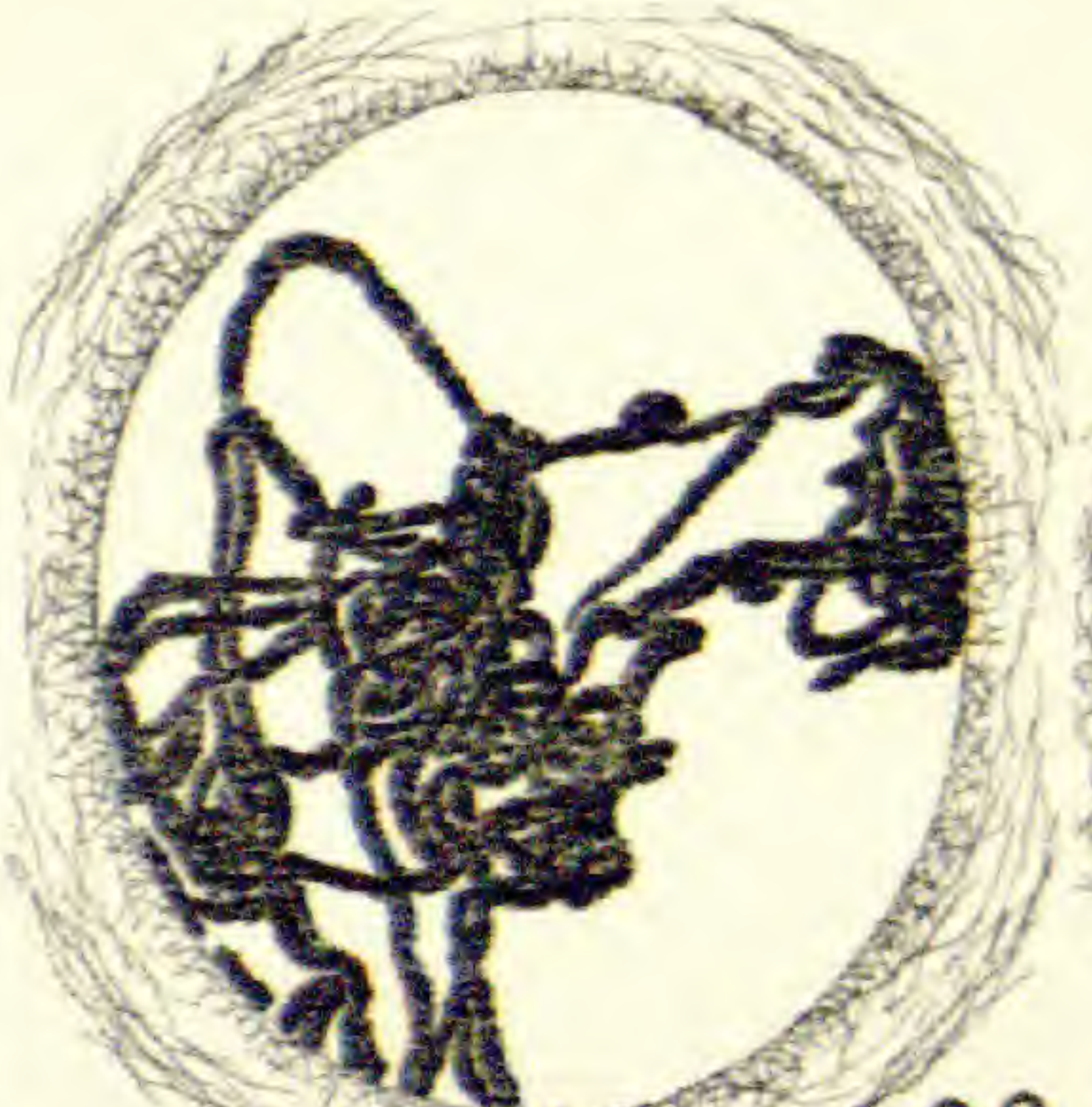
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23*a*


23*b*

24*a*

24*b*

24*c*


25*a*

25*b*

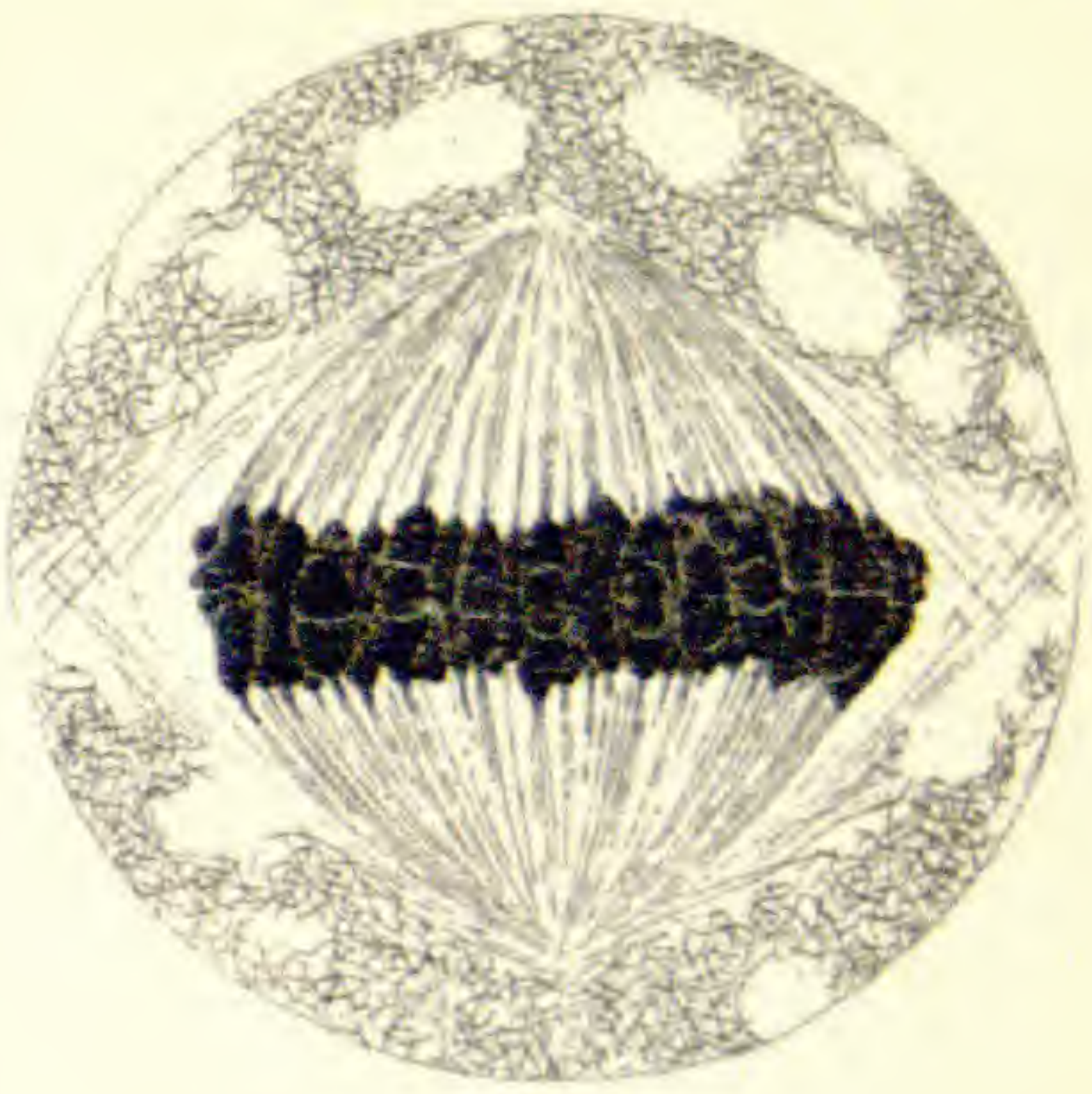
25*c*


25*d*

YAMANOUCHI on NEPHRODIUM

26*a*

26*b*



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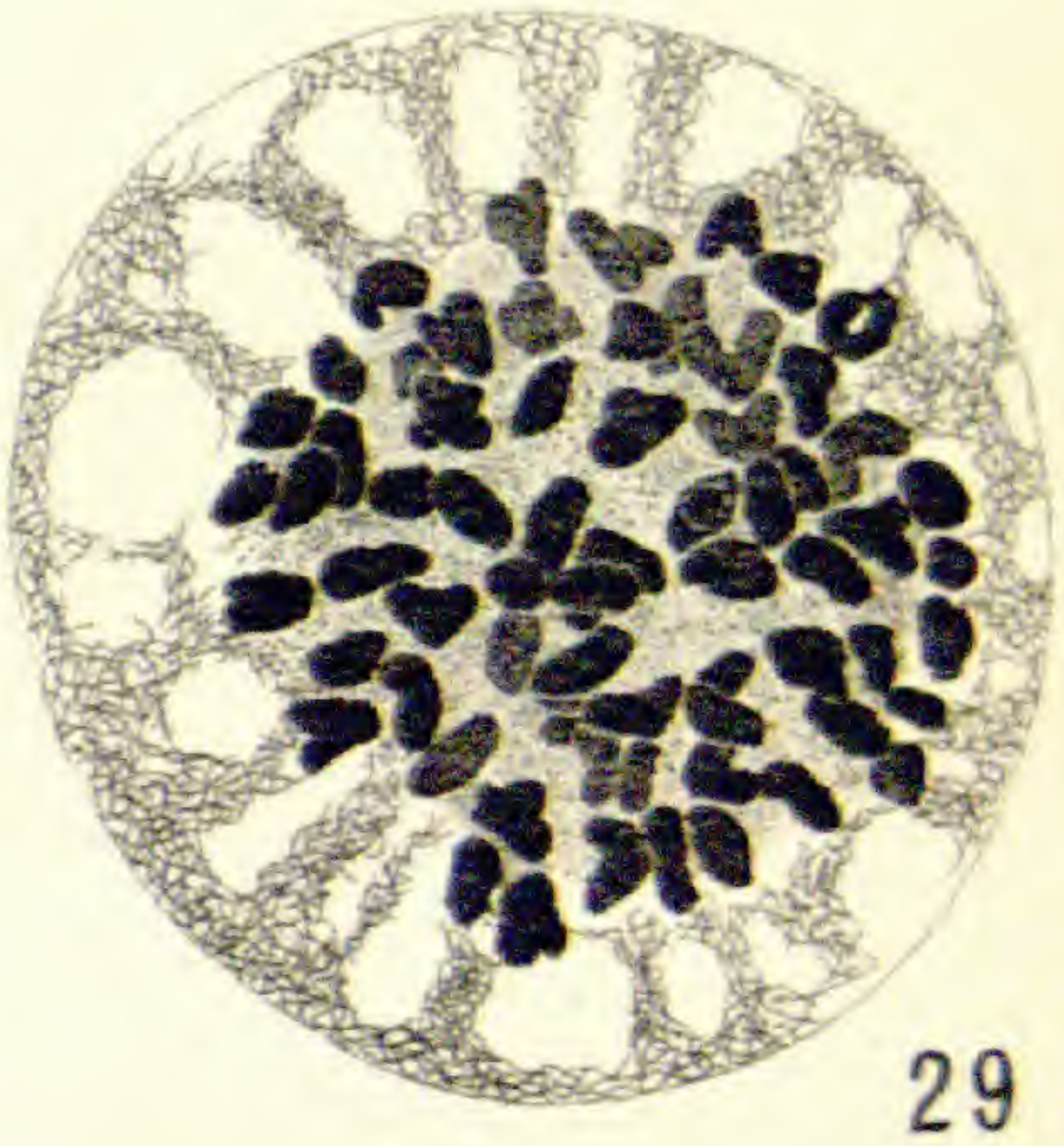
27*a*

27*b*



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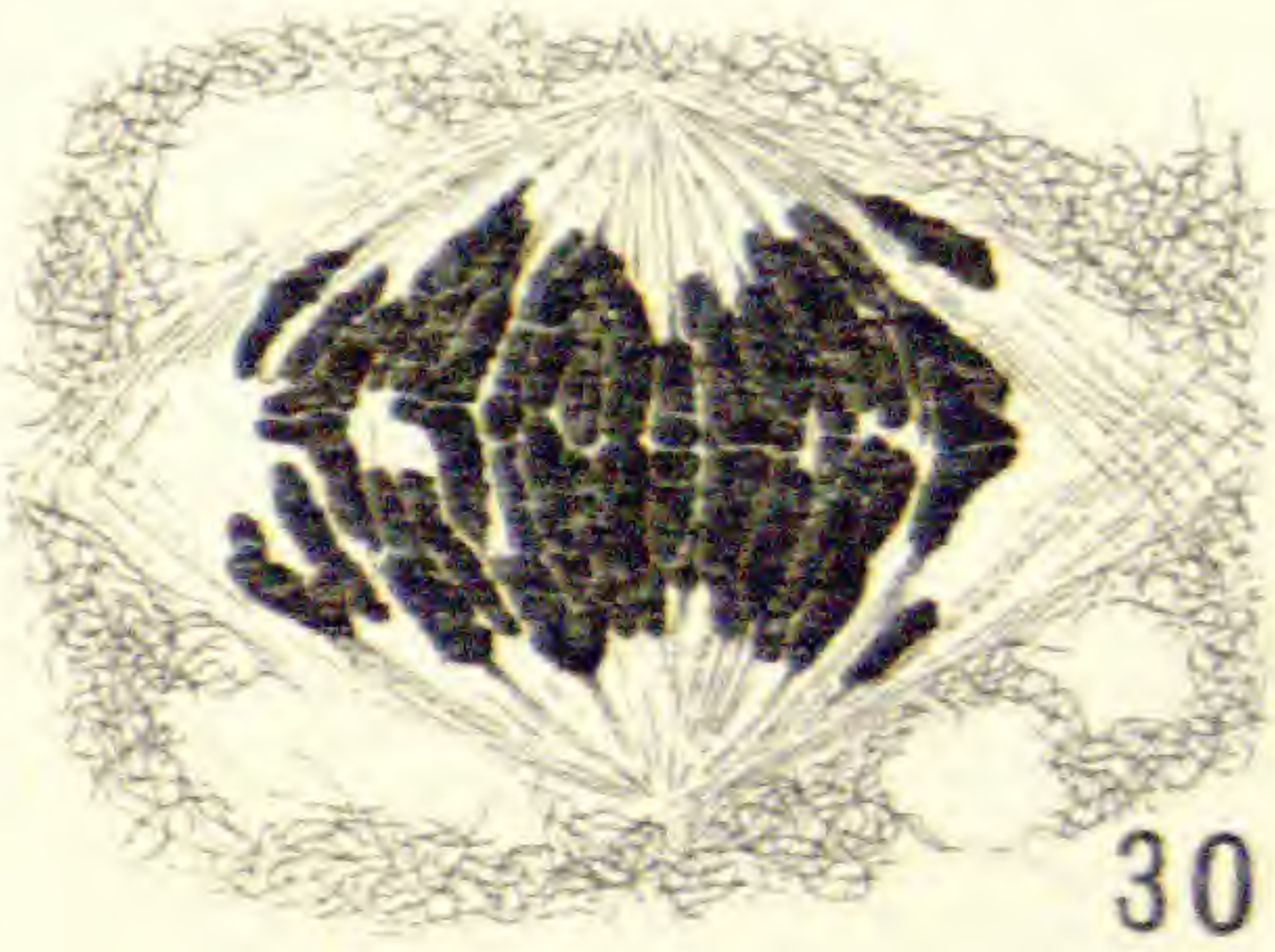

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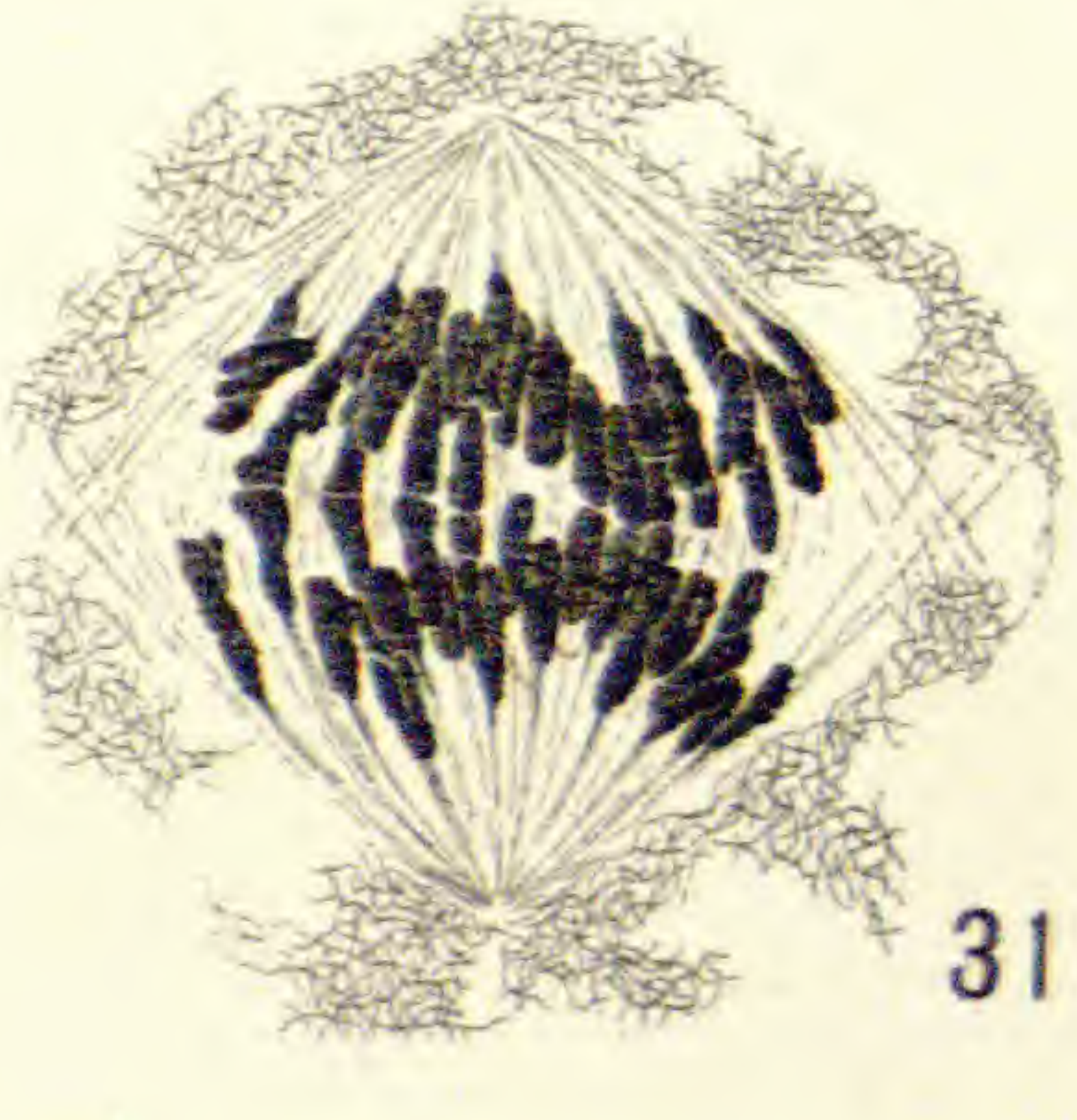

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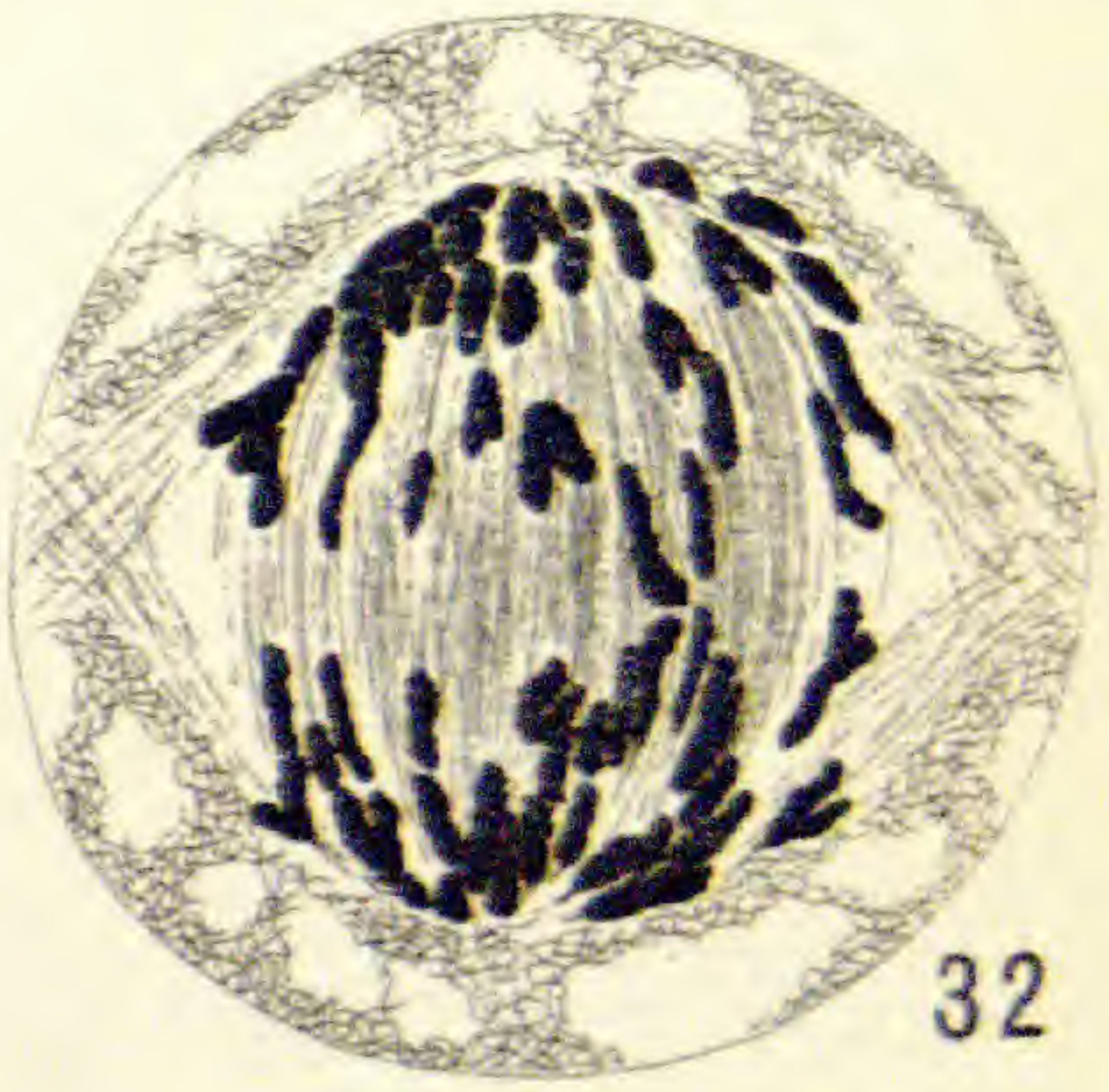

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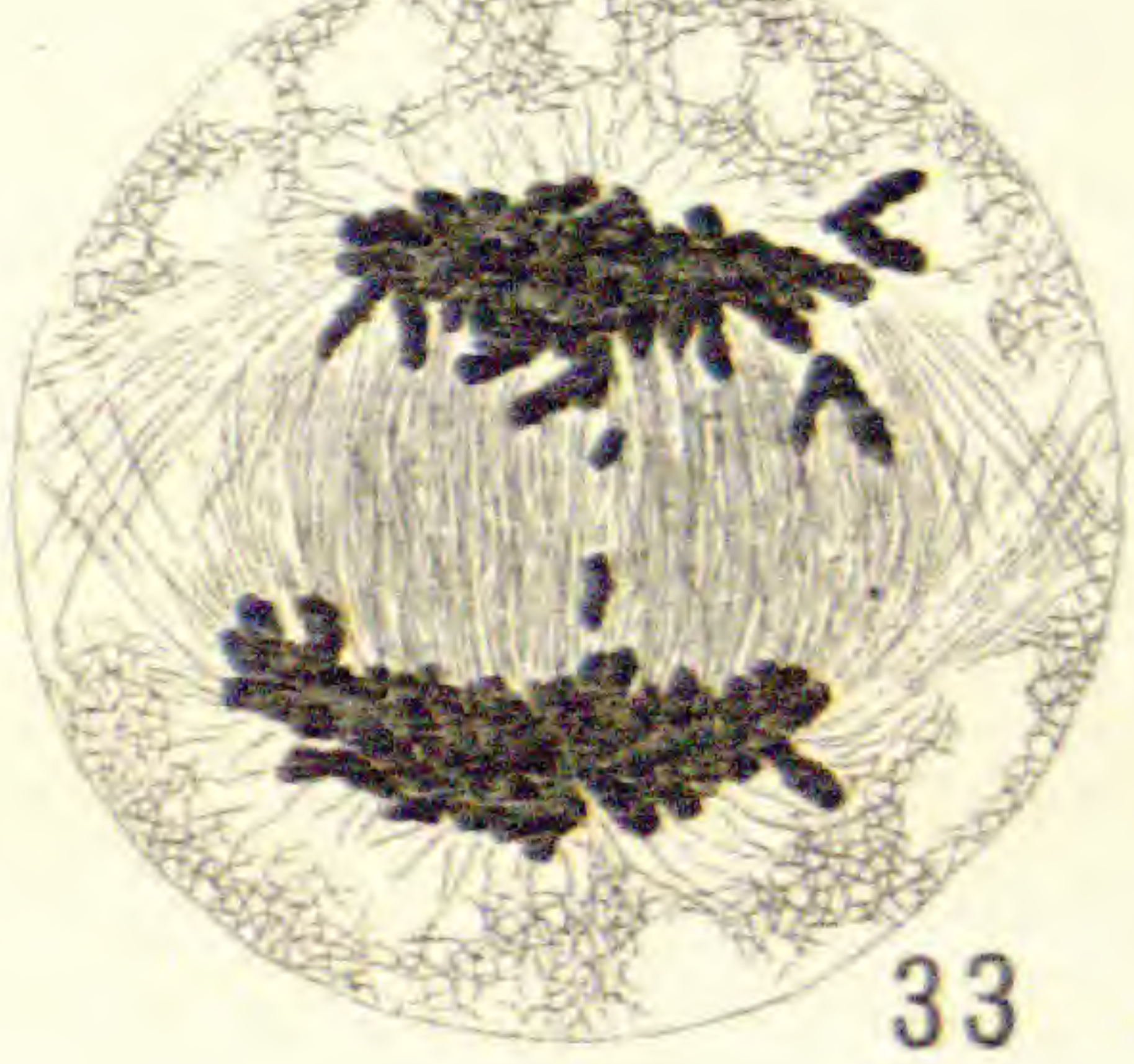

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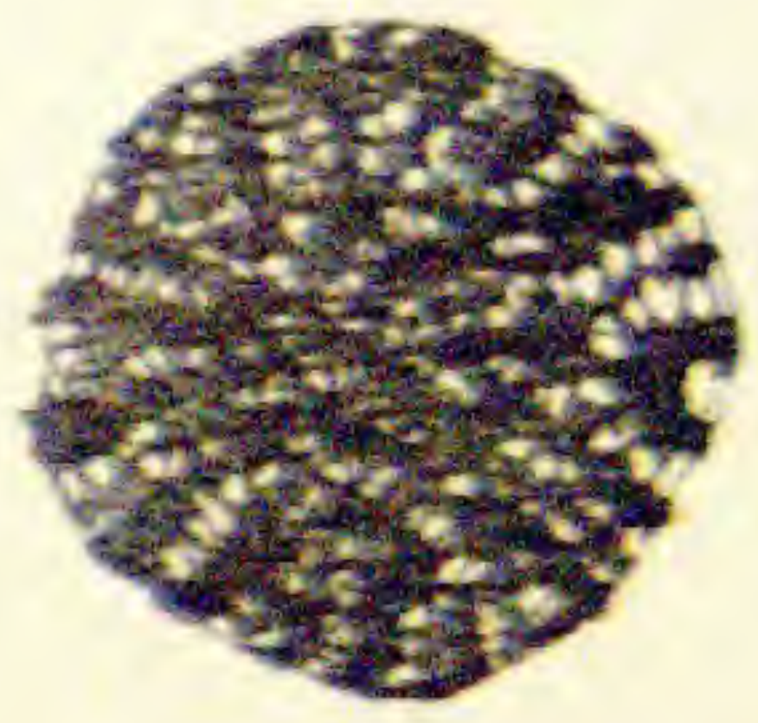

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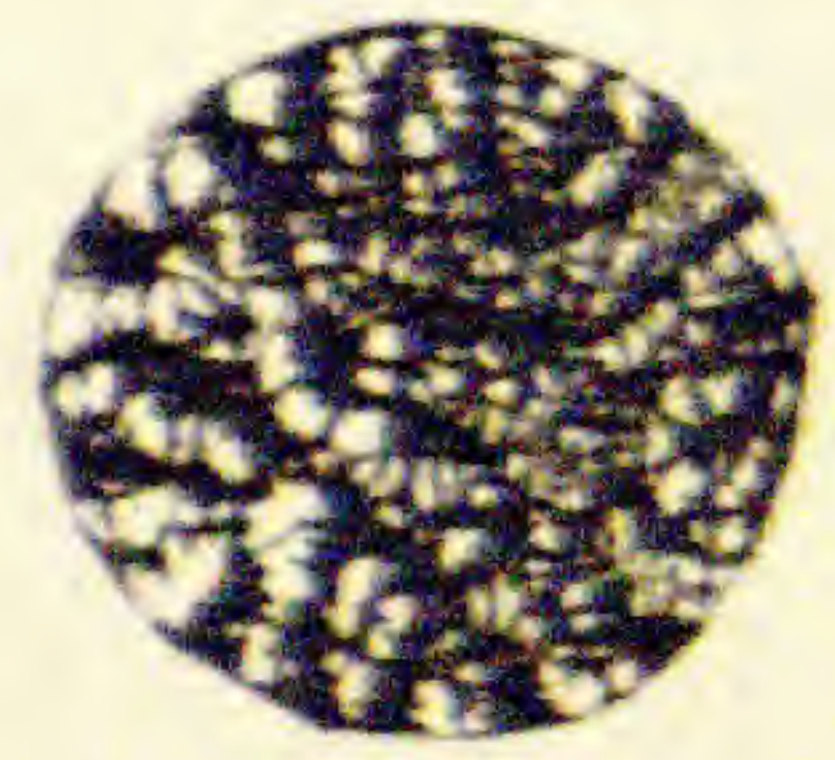

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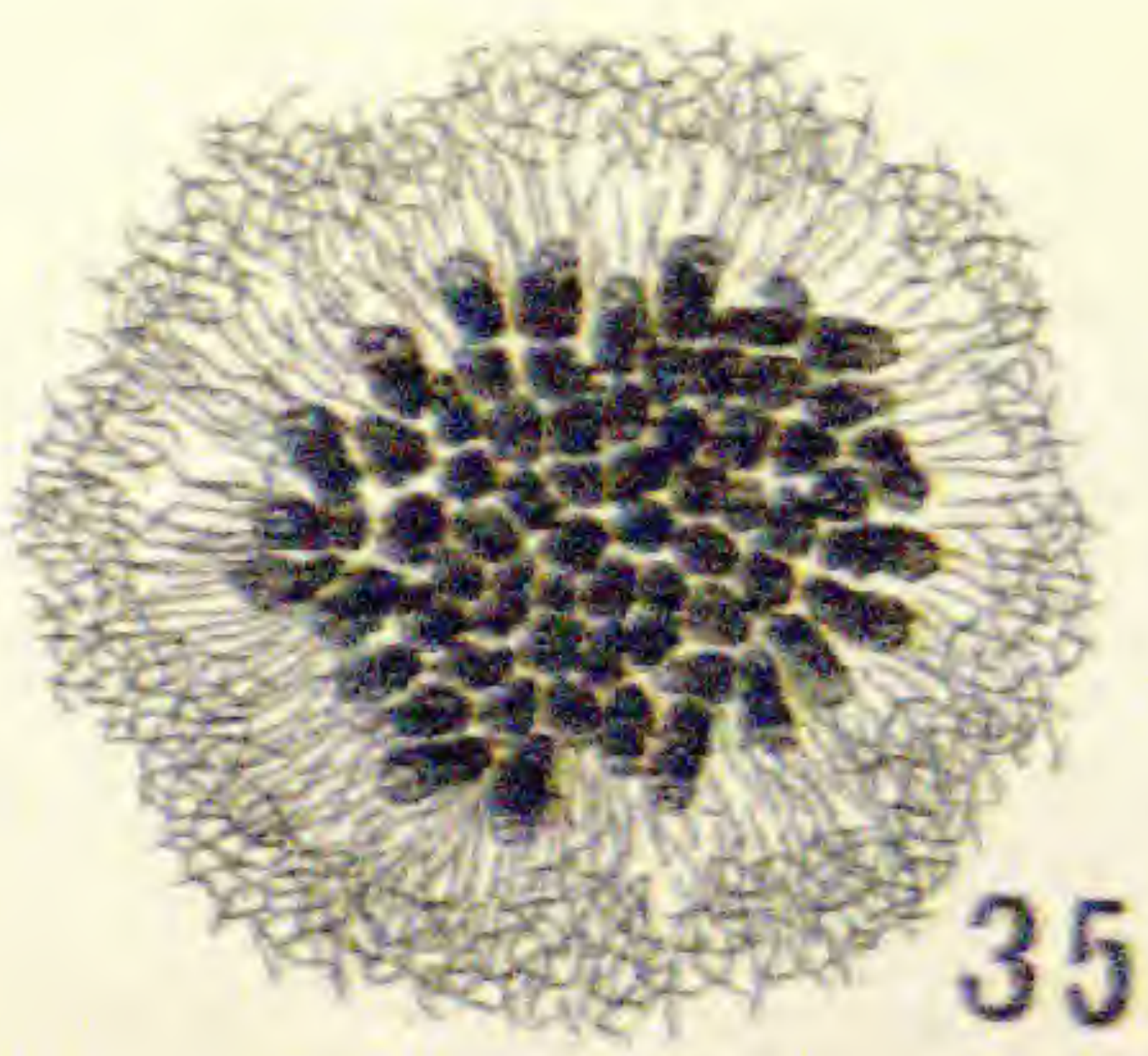

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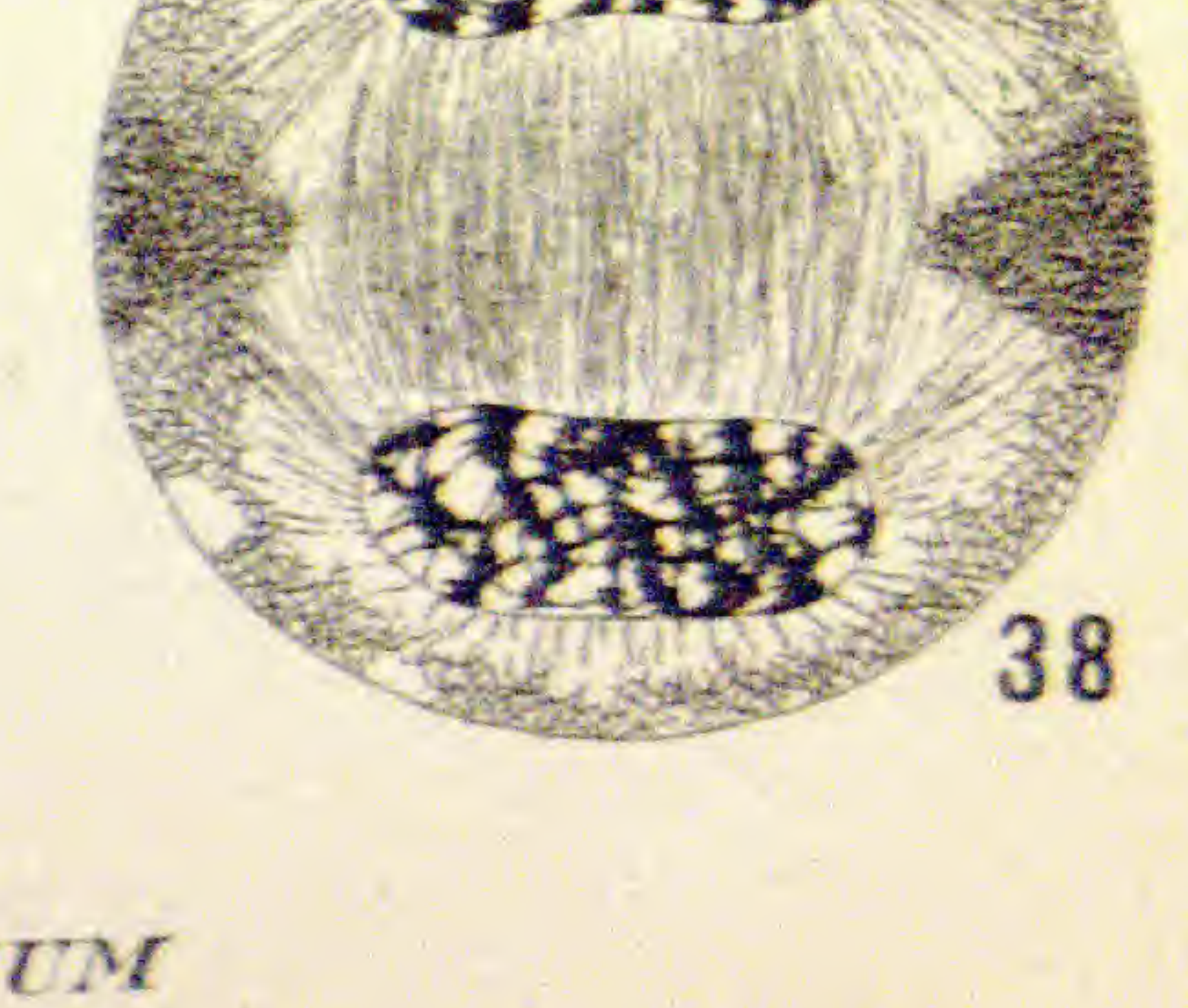

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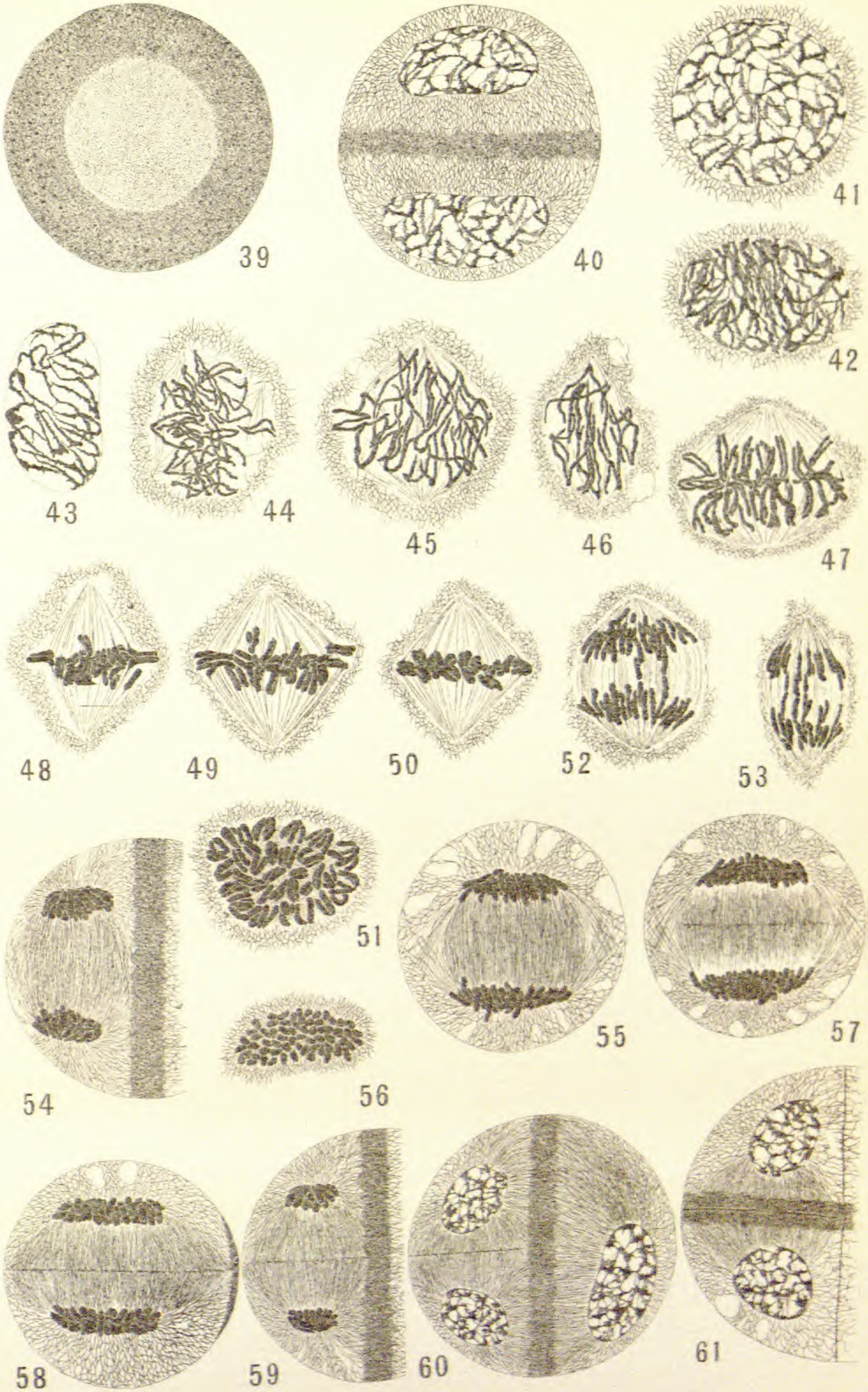
YAMANOUCHI on NEPHRODIUM



YAMANOUCHI on NEPHRODIUM

THE DEVELOPMENT OF THE POLLEN OF SARRACENIA

M. Louise Nichols

(WITH PLATE V)

During March 1907, through the courtesy of Dr. J. M. Macfarlane, I had the opportunity of gathering material for this study from the fine collection of sarracenias in the greenhouses of the University of Pennsylvania. The species examined were *S. flava*, *S. purpurea*, *S. variolaris*, *S. rubra*, and *S. psittacina*, besides the hybrids *S. flava* × *variolaris* and *S. flava* × *purpurea*. A close similarity prevails in the pollen of the different species, nor does any marked difference appear in the hybrids. Abnormalities of division among the latter are practically absent.

The material consisted of flower buds fixed in chrom-acetic acid. Thin sections of the anthers were stained by the iron-hematoxylin method and with safranin and malachite green.

The nucleus of the pollen mother cells contains an intensely staining nucleolus surrounded by a clear space. With safranin and malachite green the color of the nucleolus is red. The surrounding plasm (linin) does not stain deeply, and shows a trace of fibrillar structure (*fig. 1*). The approach of the synapsis stage is indicated by a marked and apparently rather sudden growth in size of the nucleus. The nucleolus also becomes larger, and the clear space surrounding it less well defined but wider. The linin shows more definite indications of a network (*fig. 2*). A few deeply staining granules now appear within the linin; these increase in number as the network develops (*fig. 3*). Occasionally granules can be seen in close proximity to the nucleolus (*fig. 3*), which become larger and irregular in shape, and show a tendency to accumulate in the vicinity of the nucleolus and to surround it (*figs. 4–7*). In such nuclei there is sometimes evident a similarity in size of pairs of chromatin clumps. Thus in *fig. 4* there are visible two masses of chromatin, one on each side of the nucleolus of about the same size; while farther removed from the nucleolus there are two others, smaller than the first, but approximating each other in size. Similar relations are seen in *fig. 5*.

The chromatin next becomes densely massed around the nucleolus, and the irregular clumps elongate more or less into rods. So compactly do they come to lie around the nucleolus that it is completely hidden from view, and only at the margin of the somewhat rounded mass can a threadlike appearance of the chromatin be observed. The chromatin is now gathered together at one side of the nuclear space and the synapsis is well established (*figs. 7, 8*). The nucleolus gradually emerges from the center and takes a position to one side of the chromatin. When it has completely emerged its staining capacity is seen to have diminished (*figs. 9–12*). In some cases a bipartite arrangement of the chromatin is observable (*figs. 11, 12*).

The synaptic mass in the cells at one end of the anther sac now begins to lose its power to take the stain uniformly, and an appearance of dark clumps lying in a paler matrix is produced. The nucleolus is also usually vacuolated (*figs. 13, 15, 16*). The dark bodies approach the nucleolus and it seems to sink into or to be surrounded by the paler area. Within its substance is frequently seen material staining deeply like the bodies without; often this forms a partial ring around a vacuole (*fig. 16*). The nucleolus now becomes well filled with darkly staining material, while the substance without, which takes a similar stain, decreases in amount; it finally disappears entirely. At the same time the nucleolus has become entirely black with safranin red, and lies wholly within the paler substance (*figs. 17–19*). This condition was observed in *S. purpurea* and in *S. flava*×*purpurea*. The other species did not present suitable material for this stage.

The linin next begins to extend toward the circumference of the nucleus and to be converted gradually into a network (*figs. 20–22*). Dark granules, purple with safranin and malachite green, now appear in the network, and at the same time the nucleolus again shows vacuoles (*figs. 23, 24*). It seems to go through in a reverse order its former series of changes, dark material within the nulceolus often surrounding a central vacuole (*fig. 23*). Sometimes the nucleolus presents the appearance shown in *figs. 23a* and *23b*. At one focus three darkly staining spheres can be seen, whereas at a slightly higher level these spheres are replaced by vacuoles. In one case a body closely resembling the nucleolus was observed lying just outside the nucleus, as if it had been accidentally displaced in cutting the section (*fig. 28*). If this be true, it would seem to indicate that the nucleolus is more or less independent in structure and has a resistant wall of its own. Occasionally, especially in *S. purpurea*, two nucleoli are found, one slightly smaller than the other (*fig. 24*). Sometimes (observed in *S. flava* and *S. variolaris*) the central portion of the nucleolus appears dark, the peripheral portion lighter, while from the central mass are budded off minute spheres (*figs. 22, 25*); or half the nucleolus may stain deeply, the other half faintly (*fig. 27*). The chromatic network eventually consists of fine granules distributed on delicate linin fibrils (*fig. 27*). At this stage the nucleolus, in preparations stained with safranin and malachite green, colors purple, inclosing darker purple granules, the vacuoles pinkish. The nucleoli of the tapetal cells, on the other hand, stain red.

The conversion of the network into chromosomes during the prophases of the first maturation division is illustrated in *figs. 29–31*. A longitudinal splitting of the chromatin granules and linin fibers is clearly seen, the halves thus formed often showing a tendency to spread apart (*fig. 30*). The chromosomes occur in pairs, which come to lie parallel to each other (*figs. 29–31*). They gradually shorten and thicken, so that finally little difference is evident between the length and breadth. That the spindle is formed, at least in part, from the linin fibers would appear from *fig. 32*, which represents a very late prophase. The spindle fibers when fully formed are sharply defined and show minute swellings. Two fibers may be seen attached to a chromosome in *fig. 33*. The separation of the daughter chromosomes is represented at its inception in *fig. 34*. The U-shaped chromosome would seem to correspond to the more elongated body of similar shape in *fig. 29*, and if this is true the first division separates univalent chromosomes and is reducing. The nucleolus persists at its full size until a late prophase. The total number of chromosomes in *S. variolaris* and in *S. rubra* is apparently twelve. The material did not present stages suitable for determining the number in the other species studied. SHREVE (**10**) reports twelve in *S. purpurea*.

After the first division of the chromatin, the daughter nuclei undergo no reconstruction, but arrange themselves for the succeeding mitosis (*figs. 36, 37*). The chromosomes seem to become somewhat elongated and still manifest a longitudinal split. The second division separates the chromosomes along the longitudinal split and is therefore equational (*fig. 37*). The daughter nuclei are now reconstructed. A large dark nucleolus appears in each, while the rest of the nucleus is occupied by a linin network and scattered chromatin granules. *Fig. 38a* would seem to indicate that these granules are formed by the breaking-up of larger clumps. The nucleolus stains red, the network purplish with safranin and malachite green. During mitosis the chromosomes are bright red, but after reconstruction this color is gradually lost. The cytoplasm, which has remained undivided, now constricts and the four cells of the tetrad are formed (*fig. 40*).

DISCUSSION

THE NUCLEOLUS.—This term, in its limited sense, has been applied to bodies within the nucleus more or less spherical in shape and staining unlike the chromatin (WILSON **12**). The great diversity in the observations on the behavior of this body has given rise to several theories concerning its origin and function. MONTGOMERY (**6**), in his comprehensive review of the literature on this subject in both plant and animal cells, and WAGER (**11**), in his discussion of the nucleolus in plant cells, mention a large number of authors whose observations lead them to believe that there is a connection between the nucleolus and the formation of chromatin. This view is supported, not only by the morphological relations of nucleolus and chromatin, but also by researches on their chemical relations. Another opinion is that the nucleolus has something to do with spindle formation. STRASBURGER (**9**) and NĚMEC (**7**) adopt this view. Many writers on the Protista also report kinoplasmic material arising from the nucleolus. A third theory maintains that the nucleolus contains material which is periodically expelled into the cytoplasm. By some, notably HÄCKER (**3**), this is considered an excretory function. Others believe that the phenomenon is correlated with the appearance in the cytoplasm of substances having there a definite physiological duty.

My own observations afford support to the first of the explanations outlined above. The growth in size of the nucleolus simultaneously with the appearance of darkly staining granules in the nucleus, the accumulation of this material in the nucleolus, and its expulsion, lead irresistibly to the conclusion that the formation of the chromatin is intimately connected with nucleolar activity. The relations of the nucleolus and the chromatin in the prophases of the first maturation division again plainly suggest an elaboration of chromatin by the nucleolus. The fact that at this time the nucleolus stains purple, whereas ordinarily it stains red with the safranin and malachite-green combination, indicates that a chemical change of some sort is taking place within it. The globules of material thus elaborated escape into the nuclear sap, there to be absorbed by the linin and distributed along its threads. GREGORY (2) was led to a somewhat similar view by his study of the pollen development in sweet peas. His observations indicate that "the nucleolus receives and stores the bulk of the chromatin during the resting periods which intervene between successive mitoses."

The varying observations concerning the behavior of the nucleolus give rise to the opinion that it has not the same definiteness of function as the chromosomes and centrosomes. Since there is certainly an exchange of material between cytoplasm and nucleus, and within the nucleus progressive and regressive changes in chemical constitution of its contents, it would seem that a rounded body staining unlike chromatin and therefore designated nucleolus might frequently arise, but representing at different times and in different cells a different physiological activity. The precise nature of the metabolism of the cell, it is natural to suppose, varies according to the kind of cell and the species of plant or animal, and therefore the nucleoli may be expected to vary in appearance and in their relation to the chromosomes or other constituents of the cell. That this may be the case in the ova of related species of animals has been demonstrated by MCGILL (4) in her studies of the dragon flies. In some forms apparently the nucleolus elaborates material for the use of the chromatin and thus granules and irregular masses occur within it. In others perhaps the material is not greatly transformed in the nucleolus, but passes out into the nuclear sap there to be absorbed and utilized by the chromatin. In the latter instance the connection between the nucleolus and the formation of chromatin would be difficult of demonstration. Thus such evidence as is given by DUBLIN (1) of the non-participation of the nucleolus in chromatin formation is negative in character, and does not necessarily disprove this function of the nucleolus.

SYNAPSIS AND THE INDIVIDUALITY OF THE CHROMOSOMES.—The chromatin in this stage is so densely massed together as to make it impossible to distinguish individual chromosomes and to determine whether or not there takes place a conjugation of chromosomes as required by MONTGOMERY'S hypothesis. According to the researches of MIYAKE (**5**) and OVERTON (**8**), supporting STRASBURGER'S observations, the chromosomes are preceded in the pollen mother cells by prochromosomes, centers around which the chromatin collects, and which correspond to the chromosomes in number. In Sarracenia I was unable to satisfy myself that the points around which the chromatin collects were constant in number or that the number was the same as that of the chromosomes.

The behavior of the nucleolus just described is not entirely favorable to the idea of the individuality of the chromosomes as such. If any morphological differentiation of the nucleus into areas corresponding to the chromosomes really exists at this time, its basis might possibly lie in the linin. The chromatin and nucleolus at the stage represented in *fig. 19* stain red with safranin, while the linin takes the green. This stain serves to bring out a fibrillar structure in the linin. It is well known that the chromosomes differ greatly in staining capacity at different periods in their development. At certain stages they seem to lose a part of their substance and to diminish in size as well as in staining capacity; whereas at another period they are able to absorb material, grow in size, and color more deeply. The phenomena described might therefore be explained on the assumption that the morphological basis of the chromosomes remains in the linin, while that part of their substance which causes them to color deeply is absorbed by the nucleolus.

PHILADELPHIA NORMAL SCHOOL

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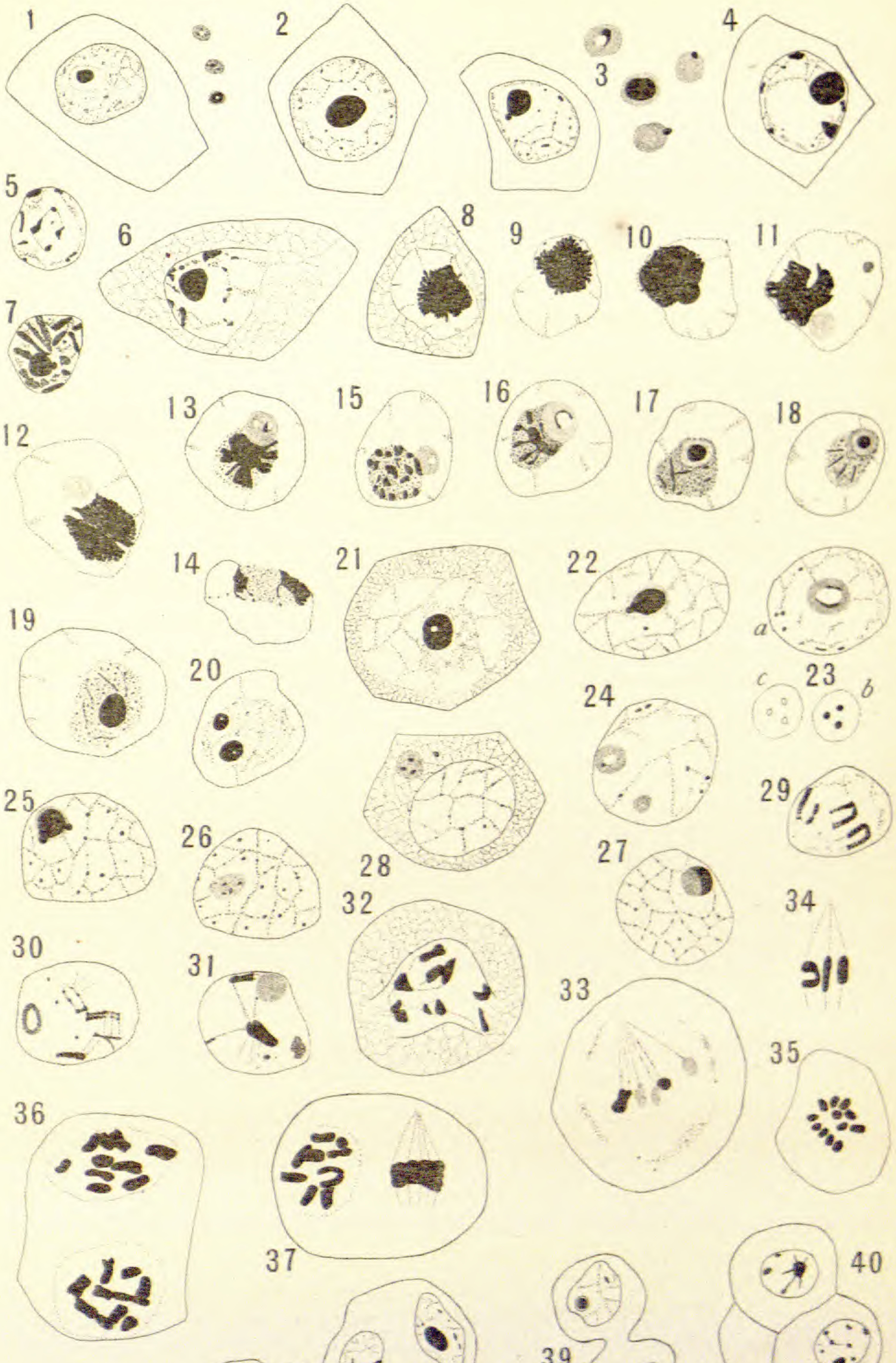

Nichols, del.

NICHOLS on POLLEN of SARRACENIA

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EXPLANATION OF PLATE V

The drawings were made with the camera lucida, ocular 6, oil immersion objective $\frac{1}{12}$, enlarged to twice the original size and reduced one-half.

Figs. 1–10, S. purpurea; 11, 12, 27, and *34, S. flava; 13–21, 23, 24, 26, 28, S. flava×S. purpurea; 22, 25, 29–32, 33, 35–40, S. variolaris*

Fig. 1. Resting pollen mother cell; *a, b, c,* nucleoli.

Figs. 2–8. Transition to synapsis; *3a, b, c, d,* nucleoli; *fig. 5,* pole view; *fig. 6,* side view of approximately the same stage.

Figs. 9–12. Synapsis; gradual emergence of the nucleolus.

Figs. 13–19. Absorption of chromatin by the nucleolus; *fig. 14,* section below the level of the nucleolus.

Fig. 20. Extension of the achromatic substance.

Figs. 21–28. Formation of the chromatic network; *figs. 23b* and *c,* nucleoli.

Figs. 29–32. Prophases of the first maturation division.

Figs. 33, 34. First maturation spindles.

Fig. 35. Pole view of the same.

Figs. 36, 37. Second maturation division.

Figs. 38–40. Completion of the cell divisions and formation of cell walls.

EMBRYOGENY OF ARISAEMA TRIPHYLLUM

JAMES ELLIS GOW

(WITH TWENTY-FOUR FIGURES)

This species is interesting as a representative of the lower monocotyledons, and as an extreme type of its family. If, as suggested by ENGLER, those aroids which possess hermaphrodite flowers are to be taken as the primitive type of the family, and the monoecious genera are to be considered as reduced forms, Arisaema must be regarded as an example of extreme reduction, since it is usually dioecious. According to this view, it should stand farthest in point of development from the original prototype of the aroid family, and although it does not show as complex a structure as do other genera, it should be considered as having probably undergone a longer course of development than they. But it seems quite as reasonable to suppose that the monoecious genera are the more primitive forms, and if this view be accepted, Arisaema must take its place at the foot of the list, as the least differentiated form. If the former hypothesis be accepted, we must regard the occasional monoecism of this species as a case of reversion; if the latter, it must be regarded as a variation which is strictly in accord with the general tendency of the family. It is plain, however, that the problem cannot be solved by merely using the monoecism or dioecism of the species as a basis for classification, but that its whole life-history must be taken into account; and even then the question cannot be answered satisfactorily until the life-histories of all the aroid genera have been worked out with far greater completeness than has yet been done.

The material here described was all gathered in the vicinity of Iowa City, Iowa, during the summer of 1901. A part of it was worked up during the following winter, but it was found necessary to drop the work, and the later phases were not undertaken until the present year. All ovaries were cut out one by one, or in groups of four or five, killed and fixed in 1 per cent. acetic acid, sectioned in paraffin to a thickness of 2–5 μ, and stained with DELAFIELD'S hematoxylin, HAIDENHAIN'S iron-alum-hematoxylin, or safranin and gentian-violet. In the latter case a saturated solution of each stain in anilin water was used, and very excellent results were obtained.

MEGASPORANGIUM

The ovary of Arisaema contains one to six erect, orthotropous ovules (*fig. 1*). The ovules in over three hundred ovaries were counted, and the average number was found to be four (*fig. 2*). They are probably lateral in origin, although at first glance they appear to be axial. The occurrence of four ovules grouped equilaterally about a slight protuberance in the middle of the base of the ovary (*fig. 1, x*) would indicate the latter as the suppressed apex of the axis. This reminds one somewhat of the suppression of the axis in Dieffenbachia,[1] except that in Arisaema the suppression is much more complete.

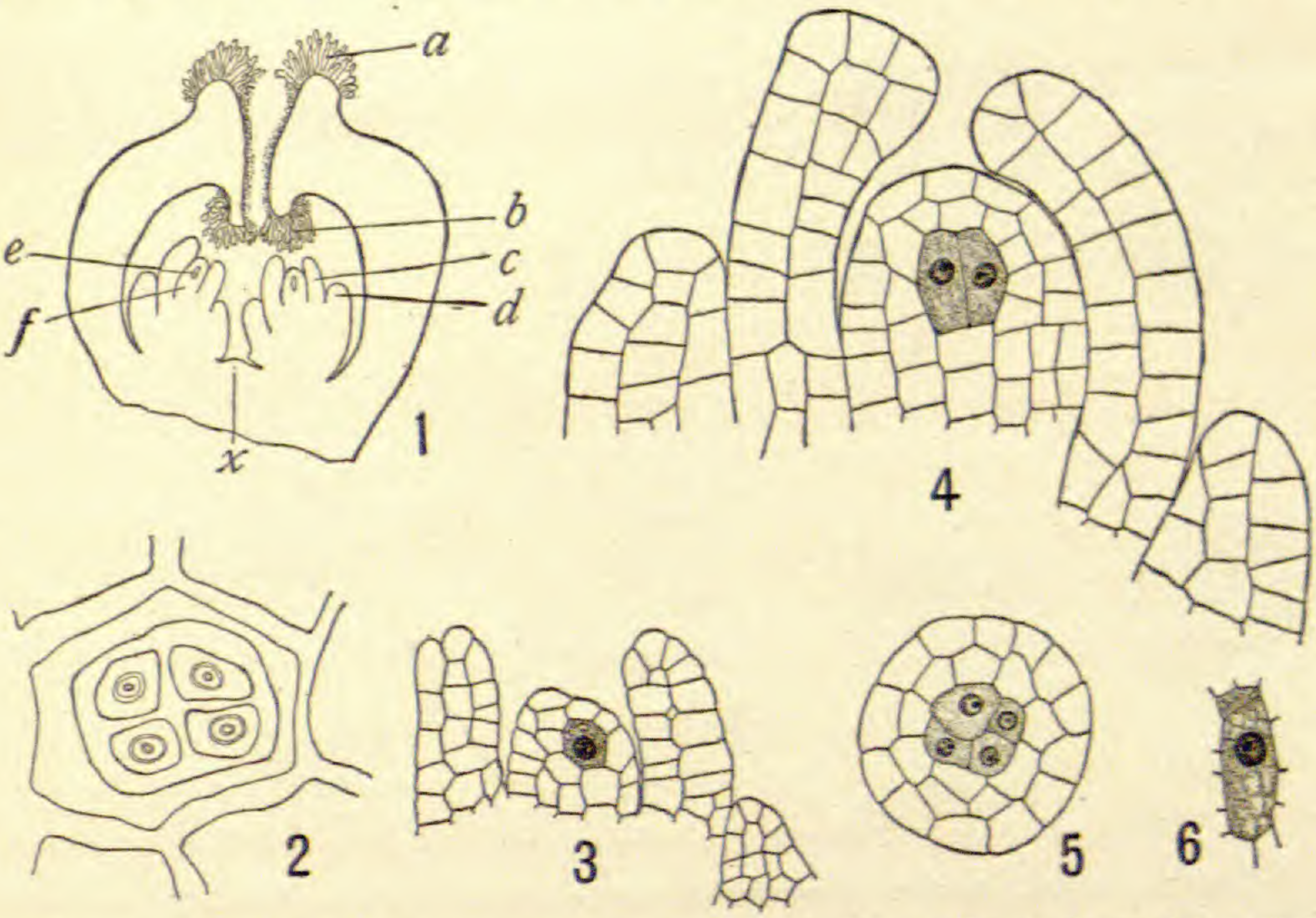

FIG. 1. Ovary of Arisaema: *a*, stigma; *b*, conducting cells; *c*, inner integument; *d*, outer integument; *e*, embryo sac; *f*, nucellus; *x*, end of axis. ×36.—FIG. 2. Transverse section of ovary. ×36.—FIG. 3. Archesporial cell; contents of surrounding cells not drawn. ×385.—FIG. 4. Two sporogenous cells resulting from vertical division of archesporium. ×400.—FIG. 5. Cross-section of nucellus showing four sporogenous cells. ×400.—FIG. 6. Division of sporogenous cell. ×400.

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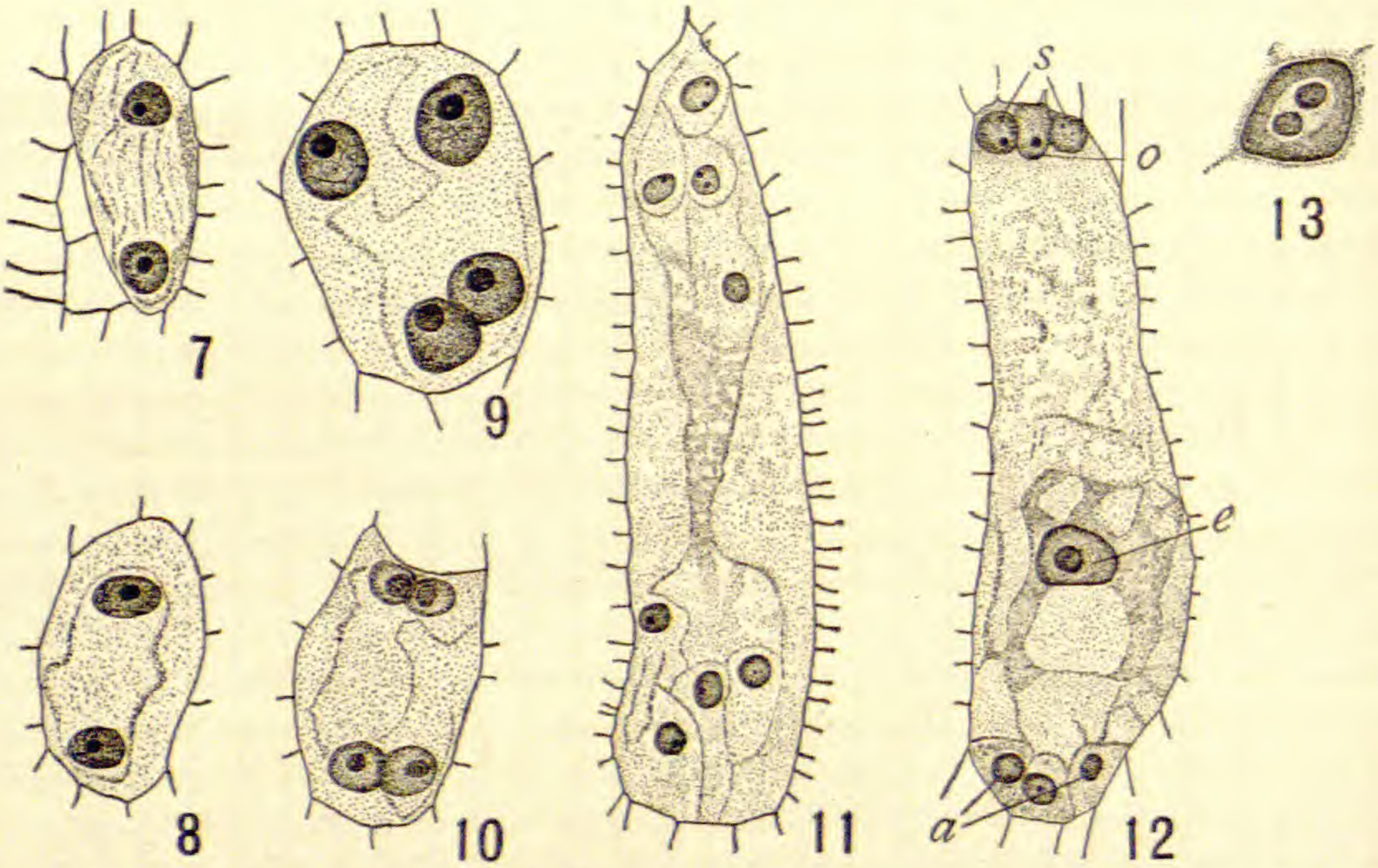
[1] CAMPBELL, D. H., Studies on the Araceae. Annals of Botany 14:1–25. *pl. 1.* 1900.

The earliest material was collected during the first week in April. At that time the ovules appear as minute protuberances from the base of the ovary, the inner integuments have just begun to show, and the cells of the nucellus are as yet undifferentiated. The ovule grows with great rapidity, the outer integument soon appears, and the inner integument passes beyond the apex of the nucellus. At the same time the nucellus itself increases greatly in size, and the archesporial cell, recognizable by its greater size and more definite reaction to stains, makes its appearance in the hypodermal layer (*fig. 3*). This is followed by a periclinal division of the epidermal cells of the nucellus. The first two divisions of the archesporial cell are by anticlinal walls at right angles to each other, thus giving rise to four sporogenous cells lying side by side (*figs. 4, 5*). One of these outstrips the others in its growth, crowding them and the surrounding cells of the nucellus. It undergoes one transverse division, by which a small cell, frequently with an ill-defined nucleus, is cut off (*fig. 6*). Whether this is a tapetal cell, or one of a "row of two" formed by a mother cell, was not determined. At this stage the



FIGS. 7, 8. First nuclear division of embryo sac. ×920.—FIGS. 9, 10. Second division in embryo sac. ×920.—FIG. 11. The eight-nucleate embryo sac. ×920.—FIG. 12. Embryo sac after fusion of polar nuclei; index letters suggest the structures. ×920.—FIG. 13. Binucleolate endosperm nucleus. ×920.

inner (functioning) cell is usually 0.1^{mm} in length, and its nucleus, by successive divisions, gives rise to the usual eight nuclei of the embryo sac (*figs. 7–11*). The oosphere, synergids, and antipodal cells are organized as usual, and the polar nuclei fuse (*fig. 12*). The fusion endosperm nucleus frequently contains two nucleoli (*fig. 13*).

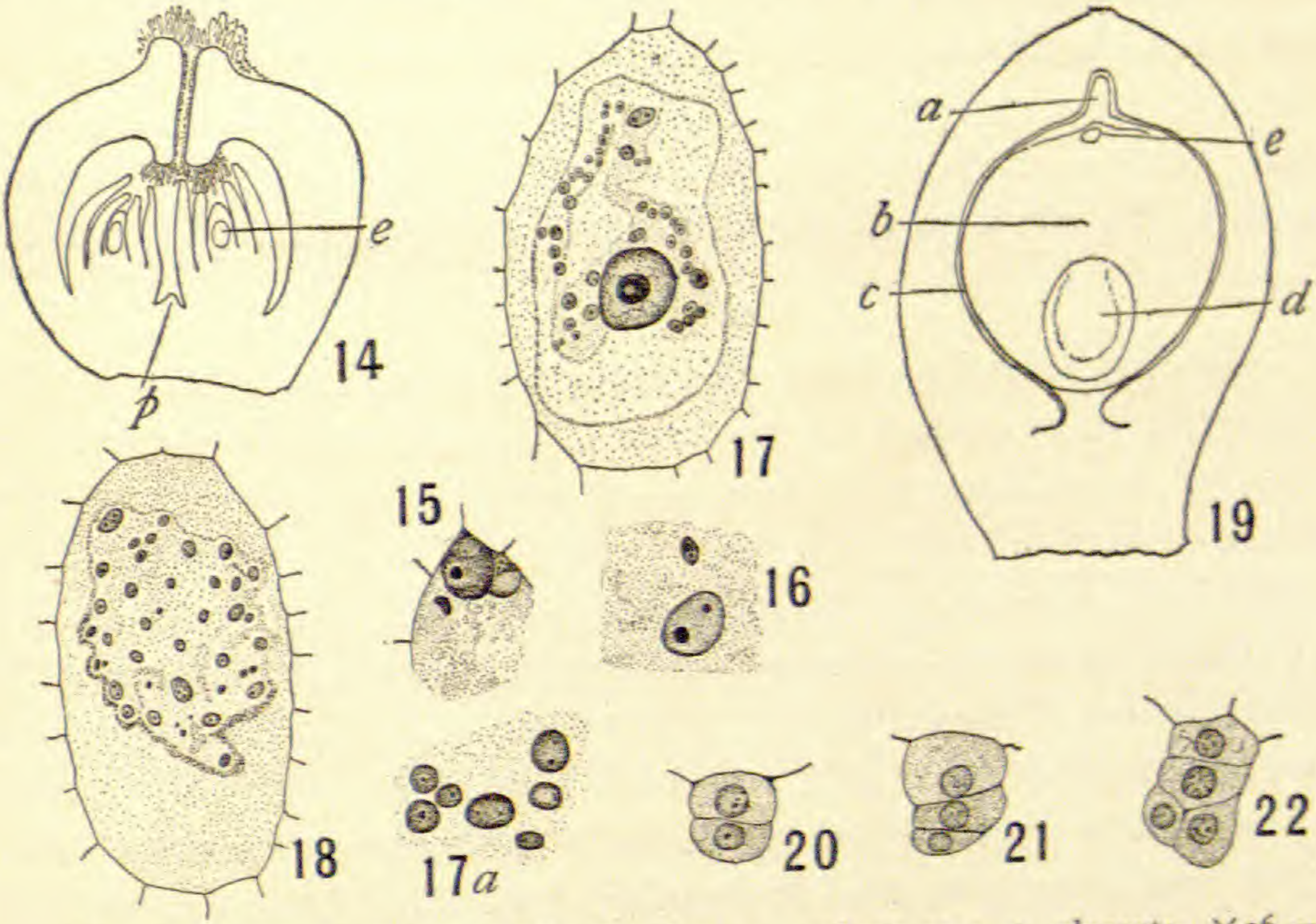


FIG. 14. Ovary at period of fertilization; *e*, embryo sac; *p*, placenta. ×36.—FIG. 15. Oosphere and male nucleus. ×920.—FIG. 16. Endosperm nucleus and second male nucleus. ×920.—FIG. 17. Diagonal section of embryo sac near chalazal extremity, showing endosperm and, probably, one antipodal dislodged by knife. ×920.—FIG. 17*a*. Endosperm nuclei enlarged. ×2050.—FIG. 18. Endosperm nuclei. ×920.—FIG. 19. Longitudinal section of endosperm (*b*), surrounded by remains of inner integument (*c*), and of nucellus (*a*), and containing embryo (*e*), and large vacuole (*d*). ×14.—FIG. 20. Two-celled embryo. ×920.—FIG. 21. Three-celled embryo. ×920.—FIG. 22. Four-celled embryo. ×920.

When the nuclear divisions are complete, the embryo sac has increased to a length of about 0.35^{mm}. The lateral cells of the nucellus have been greatly crowded, but its tip remains intact. The inner and outer integuments have grown until they press against the conducting cells in the roof of the ovary (*fig. 14*).

FERTILIZATION

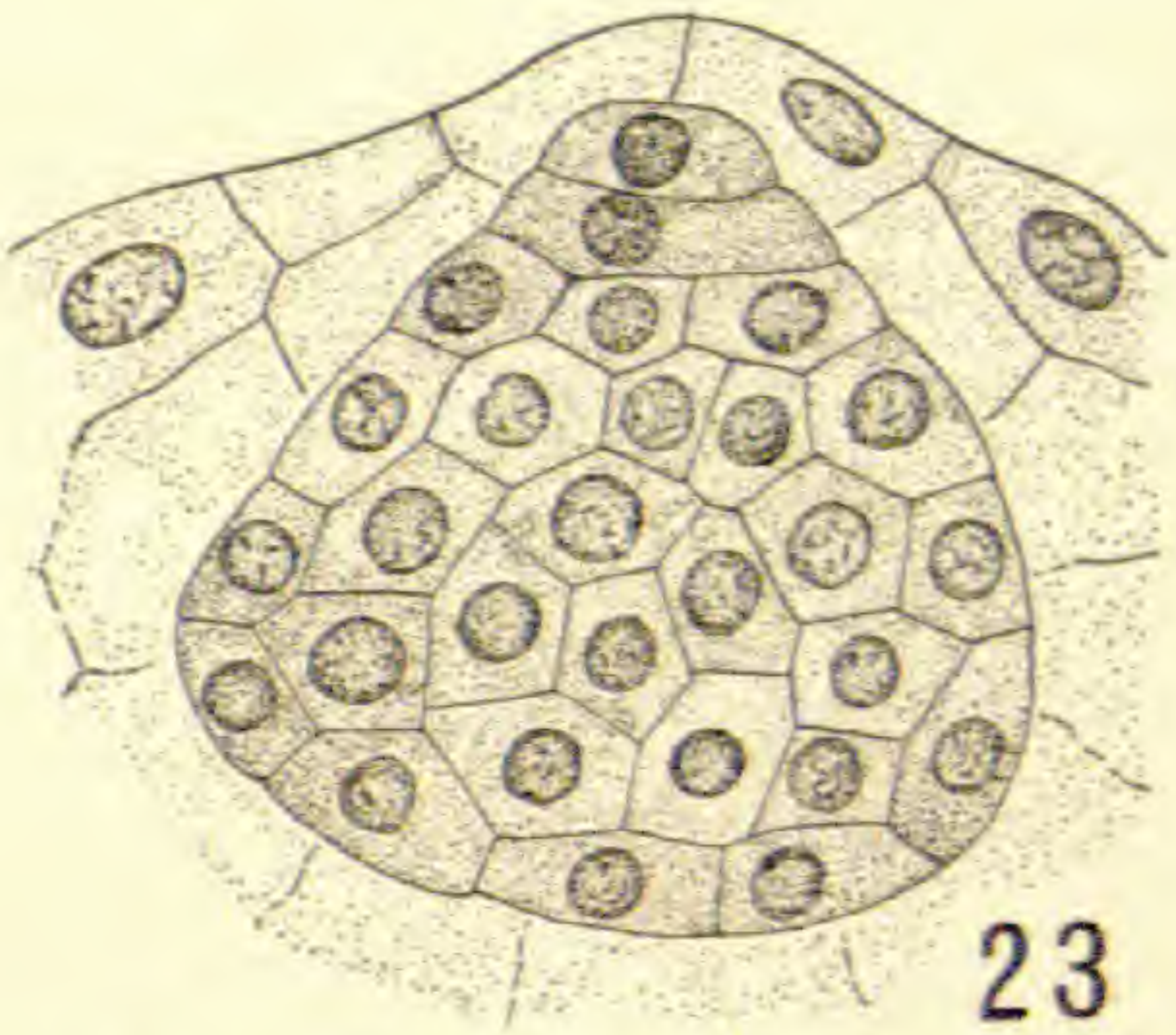
The stigma of Arisaema consists of a mass of loosely compacted, somewhat mucilaginous papillae (*fig. 11*). The pollen tube finds access to the interior of the ovary through an open style which is more or less filled with a loose mass of conducting cells, the lower ones extending well down into the cavity of the ovary (*fig. 14.*) The pollen tube enters by the micropyle, and a number of sections were obtained showing it penetrating the nucellus. One section showed the male nucleus and oosphere (*fig. 15*), but careful search failed to bring to light any case of the fusion of the second male nucleus with the endosperm nucleus. As it is known, however, that this "second" male nucleus enters the embryo sac and approaches the endosperm nucleus (*fig. 16*), it seems probable that the fusion actually takes place.

ENDOSPERM

The first divisions from the endosperm nucleus result in the formation of a number of free nuclei (*fig. 17*). These increase in number by successive divisions until they line the interior of the embryo sac (*fig. 18*). They are well defined and usually multinucleolate, showing an extreme diversity in size. When division has proceeded so far as to produce nuclei sufficient to line completely the inner surface of the embryo sac, walls begin to appear, and soon each nucleus is separated from its neighbors by a well-defined wall. Active growth continues until the embryo sac is filled with endosperm, which now begins to crowd the surrounding cells. The lateral vestiges of the nucellus disappear, as a result of this pressure, and the inner integument is stretched until it becomes finally nothing more than a thin limiting membrane surrounding the endosperm. The outer integument remains intact, its growth keeping pace with the growth of the endosperm. The persistent tip of the nucellus likewise remains intact, and even increases in size. The cells in the base of the nucellus are greatly crowded, and many of them break down. When the endosperm has reached its full size, it is about 3^{mm} in length, and consists of two well-defined parts (*fig. 19*). The upper part (*b*) consists of cells with thin but well-defined walls and at first a distinct nucleus; later they are filled with starch grains. In the lower part of the endosperm there is developed a large vacuole (*d*).

EMBRYO

In the specimens examined, the first division of the oospore does not occur until the formation of endosperm is well under way. As it appears that endosperm formation does not begin until after the pollen tube has reached the embryo sac (and probably not until the endosperm nucleus and the second male nucleus have fused), it would seem that the fertilization of the oosphere is followed by a period of rest before the formation of the embryo begins. The first two divisions are transverse, followed by a longitudinal division (*figs. 20–22*). Beyond this point no definite history of the embryo can be given until the stage illustrated in *fig. 23* is reached. The embryo here shown is six weeks or two months old and has not yet reached its maximum development; *fig. 24* shows a somewhat older embryo. Beyond this stage no good sections of the embryo were obtained.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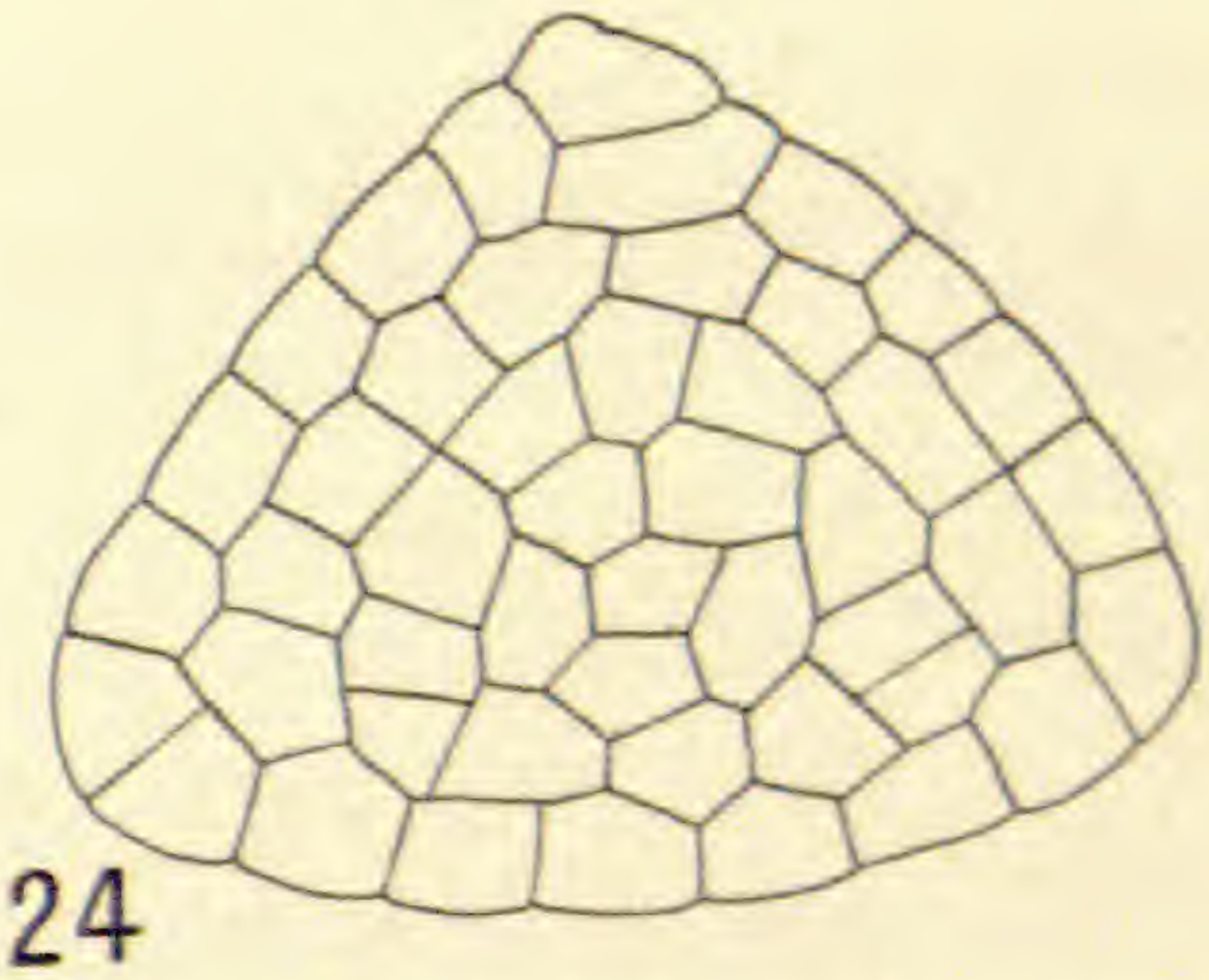
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FIG. 23. Longitudinal section of embryo imbedded in endosperm. ×770.—FIG. 24. Same, at somewhat later stage of development. ×770.

SUMMARY

Although the dioecious character of Arisaema would indicate for it a very low place among the aroid genera, other characteristics do not entirely bear out this view. The erect and orthotropous ovule, it is true, seems to indicate it as a primitive form, but the fact that there are four ovules (typically) in each ovary, and that their basal origin is apparent only, since they are in reality lateral outgrowths of the suppressed axis, seems to prove it a more highly specialized type than Aglaonema, Nephthytis, and Spathicarpa, in all of which each carpel contains a single basal ovule. This view is confirmed if we consider the variations in the number and arrangement of the nuclei in Nephthytis and Aglaonema (CAMPBELL, *l. c.*) as indicating their primitive character. The development of the embryo sac in Arisaema corresponds to the usual type found in other angiosperms, and gives no hint of its possible affinities with lower forms. On the other hand, the early presence of a number of sporogenous cells in the nucellus appears to ally it with the two genera mentioned, both of which exhibit a similar peculiarity.

The development of endosperm in the aroids is usually characterized from the beginning by the formation of prominent cell walls. This characteristic has been noted by CAMPBELL (*l. c.*) in Anthurium, *Nephthytis liberica*, and Lysichiton, and by myself in *Nephthytis Gravenreuthii*. Arisaema is an exception in that the first formation of endosperm is by free nuclear division, although cell walls are soon developed. In this respect it resembles *Dieffenbachia daraquiniana*, whose embryo sac becomes quite filled with endosperm nuclei before the cell walls begin to make their appearance. The resemblance of the suppressed axis to that of Dieffenbachia has already been mentioned. The anatropous character of the ovule in the latter genus, the fact that each of the two ovules is inclosed in a separate carpel, and the fact that there is only one archesporial cell, and that the primary parietal cell originates at its inner instead of its outer end, serve to draw a broad distinction between it and Arisaema, in spite of the resemblance in endosperm formation. The peculiarities mentioned would probably indicate that Dieffenbachia is a somewhat less primitive form than Arisaema, and one whose development has proceeded along different lines. The presence of staminodia among the pistillate flowers also appears to bear out this view.

Arisaema, therefore, appears to be a more highly developed form than Spathicarpa, Aglaonema, and Nephthytis, but somewhat more primitive than Dieffenbachia, and much more primitive than Anthurium, Spathyema, Orontium, and Acorus, since all the latter have perfect flowers.

When the foregoing was written, the writer was not aware of the existence of a paper by MOTTIER[2] on the development of the embryo sac in Arisaema. A comparison of the two papers, however, shows that they do not cover the same ground, since MOTTIER limited his investigation to the earlier phases, and did not discuss the development of the endosperm, the growth of the embryo, or the possible affinities of the species.

GREENFIELD, IOWA

[2] MOTTIER, D. M., On the development of the embryo sac of *Arisaema triphyllum*. BOT. GAZETTE 17:258–260. *pl. 18.* 1892.

THE TOXIC EFFECT OF CERTAIN COMMON SALTS OF THE SOIL ON PLANTS

FLORENCE N. MAGOWAN

(WITH ONE FIGURE)

A number of investigations have been made upon the action of single salts on plants, but in the majority of cases the object has been to determine the limit of endurance or the highest concentration at which the plants could live in the pure salt solutions. These concentrations vary with the salts used, and from the tabulated results it is not possible to tell the difference in growth of plants in the various salts in equimolecular solutions, nor would it be safe to assume this difference to vary uniformly at all concentrations. On a-priori grounds the reverse might be thought to be true, i. e., comparative degree of toxicity of certain solutions at one concentration might be entirely different at another. Again I have found time to be a very important factor in changing this relation, so that experiments lasting only a short period cannot be considered conclusive. The work done by previous investigators[1] differs so greatly in method, and the results are so scattered that no useful comparison with my results is possible.

Wheat was chosen as a well-known agricultural crop, and one with which much experimental work has been done. One variety only was used, the "Early Genesee," which contains a large proportion of starch, and which in preliminary experiments was found to germinate evenly and with a high percentage of germination. Radish and clover seeds were also used for comparison and found to give similar results to wheat.

In practically all experiments that have been made along this line, the seeds to be tested were first germinated in distilled water, then

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[1] References given at the close of the following papers include most of the literature on this subject: "Toxic limits and stimulating effects of some salts and poisons on wheat," by G. H. JENSEN in BOT. GAZETTE 43:42. 1907; also "The variability of wheat varieties in resistance to toxic salts," by L. L. HARTER, U. S. Dept. Agr., Bur. Pl. Industry, Bull. 79. 1905.

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placed in the solutions. As the object of this investigation was to find the growth made by the seeds in the different solutions, this method was not practicable, and the seeds were supported on filter paper[2] so that they might germinate in the solution from the beginning.

Twenty or more seeds were placed in each solution. In general the germination was quite uniform. Usually seven duplicate series of experiments were made. While some series made a better growth than others, the comparative order of toxicity was not changed. In all concentrations final examinations were made when the roots had reached the limit of growth. The solutions were all titrated against standard silver nitrate solution in the beginning and great care was taken in making up the various concentrations.

To keep the solutions at the same strength was a problem which received careful consideration. As little salt was taken by the plants the first few days, it was found perfectly safe to replenish the loss due to evaporation by adding distilled water. When the solutions were found by titration to be too weak, however, they were replaced by fresh solutions.

Several concentrations of chlorids of Ca, K, Na, and Mg were used, these salts being taken because they represent the bases most common in the soil. In order that the effects of the cathions might be clearly evident, only one anion, chlorion, was used. Control experiments with tap and distilled water were always carried on with each series, the water having been distilled with especial care to avoid all traces of metals in solution.

The plants made a better growth in distilled water than in any of the salt solutions, which were thus shown to exert a toxic rather than a stimulating effect at the concentrations used. While a marked difference in the degree of toxicity of Ca, Mg, K, and Na was clearly noticeable in the roots, the tops were more nearly alike in equimolecular solutions of the salts, except in the stronger concentrations of $MgCl_2$, in which little growth of either roots or tops occurred.

The order of germination was noteworthy. The seeds in KCl and NaCl were the first to send out roots, and for several days remained in advance of those in $CaCl_2$ and $MgCl_2$. For the first five days the

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[2] The method is described by OSTERHOUT, BOT. GAZETTE **44**: 259. 1907.

wheat in the $MgCl_2$ solutions made a growth almost equal to those in $CaCl_2$, but after fifteen days the roots in the $MgCl_2$ were dead, while those in the $CaCl_2$ series were still living, even in such high concentrations as 0.12 *m*. After twenty or twenty-five days the plants in $CaCl_2$ solutions were in advance of those in KCl and NaCl. In all the solutions the limit of growth was reached in about thirty days.

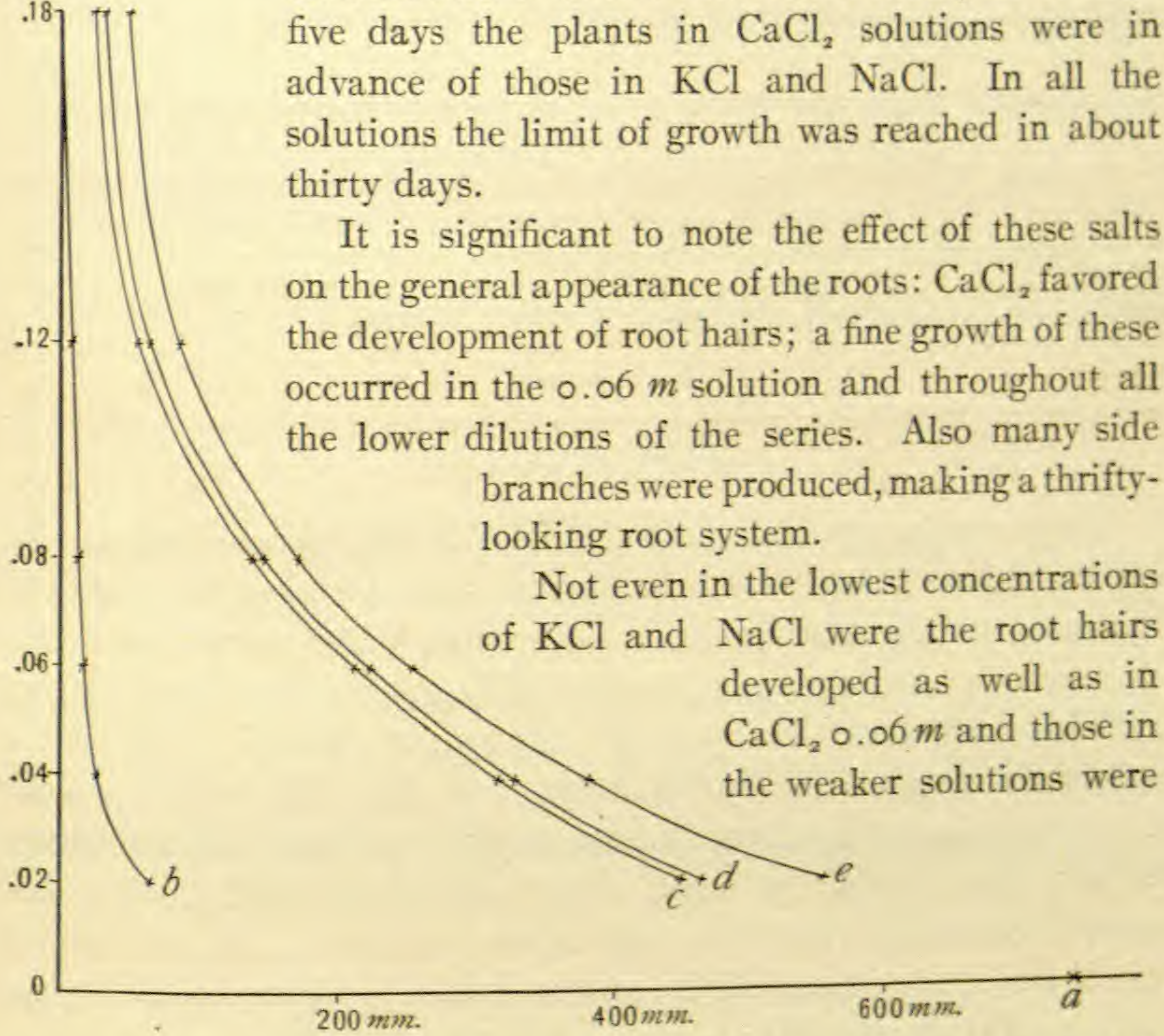

FIG. 1.—Curves showing the growth of roots of wheat in salt solutions; *a*, distilled water; *b*, $MgCl_2$; *c*, NaCl; *d*, KCl; *e*, $CaCl_2$. The ordinates represent concentrations (parts molecular); the abscissae, the aggregate length of roots per plant (in millimeters).

It is significant to note the effect of these salts on the general appearance of the roots: $CaCl_2$ favored the development of root hairs; a fine growth of these occurred in the 0.06 *m* solution and throughout all the lower dilutions of the series. Also many side branches were produced, making a thrifty-looking root system.

Not even in the lowest concentrations of KCl and NaCl were the root hairs developed as well as in $CaCl_2$ 0.06 *m* and those in the weaker solutions were decidedly unthrifty in appearance. In KCl the roots had a dull white color, and as to branching were next to $CaCl_2$, although the branches were thicker and shorter than in $CaCl_2$; the branches were most abundant at 0.02, 0.04, and 0.06 *m*. In $CaCl_2$ branching was practically the same throughout. In NaCl a few stunted branches developed and after twenty days the roots were of a yellow color in all the higher concentrations, down to 0.02 *m*. In $MgCl_2$ neither branches nor root hairs were produced in concentrations 0.08 *m* to

0.005 *m*, but in 0.001 *m* root hairs were developed, and in 0.0001 *m* branches were sent out similar to those in $CaCl_2$ solutions.

The following table gives the average length of roots per plant produced in the various solutions, the figures being based on seven duplicate series of experiments.

SALTS	CONCENTRATIONS					
	0.02 *m*	0.04 *m*	0.06 *m*	0.08 *m*	0.12 *m*	0.18 *m*
$MgCl_2$..............	64$^{\text{mm}}$	26$^{\text{mm}}$	15$^{\text{mm}}$	10$^{\text{mm}}$	6$^{\text{mm}}$	0$^{\text{mm}}$
NaCl...............	446	321	210	134	54	24
KCl................	462	333	220	144	63	31
$CaCl_2$..............	552	386	249	168	84	48

Distilled water, 740$^{\text{mm}}$

These results are also shown in *fig. 1* in the form of curves, the concentrations being used as ordinates and the average length of the roots per plant being used as abscissae. The curves show that little growth took place at 0.18 *m* in sodium, potassium, and calcium chlorids, and none whatever in magnesium chlorid. The small growth in magnesium chlorid throughout the whole series of concentrations is due mostly to its toxic action; but also in some degree to its higher osmotic pressure. The molecule of $MgCl_2$ when dissolved dissociates into three ions instead of two, as in the case of NaCl and KCl; hence $MgCl_2$ solutions have a higher osmotic pressure than equimolecular solutions of NaCl and KCl.

All the more striking is the behavior of $CaCl_2$ which in spite of high osmotic pressure (approximately as high as $MgCl_2$) nevertheless permits greater growth than NaCl or KCl; hence it must be much less toxic than any of the other salts.

RESULTS

1. Each of the principal soil bases (Na, K, Mg, Ca) is toxic. The following is the order of toxicity of their chlorids: (1) $MgCl_2$, (2) NaCl, (3) KCl, and (4) $CaCl_2$.

2. Experiments lasting but a short time (such as have been made by most previous investigators of the toxic action of salts) cannot be considered as conclusive as longer experiments. After six days,

plants in NaCl and KCl made more growth than in $CaCl_2$, but after twenty-five or thirty days the plants in $CaCl_2$ were in advance of all the others.

These investigations were carried on under the direction of Professor W. J. V. Osterhout, to whom the writer wishes to express sincere thanks for his kindly assistance and valuable suggestions.

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Berkeley, Cal.

BRIEFER ARTICLES

THE PHYSIOLOGICAL CONSTANTS OF PLANTS COMMONLY USED IN AMERICAN BOTANICAL LABORATORIES

The object of this paper is identical with that of its predecessor,[1] namely, to ascertain which of the plants commonly grown or readily raised in greenhouses is best adapted for the demonstration of a particular physiological process, and how much may be expected of it and of the others. The work is being done in the Laboratory of Plant Physiology at Smith College, with the advice of Professor W. F. GANONG. Others of the series are in advanced preparation.

II. ROOT PRESSURE AND EXUDATION

Osmotic processes, of course, are of the greatest importance in the life of the plant, and of these osmotic root absorption is perhaps best known. The most direct way of demonstrating the osmotic absorption and pressure of the root is by measurement of the quantity and pressure of exudation from the stem cut just above the ground. I have undertaken to find for those greenhouse plants which are practicable for the purpose the pressure of root exudation and the quantity exuded. The only work of importance done upon potted plants, so far as I can find, is that of WIELER[2] in 1892, who recorded the pressures of some fifteen greenhouse plants, and this is the source of the figures in current books. Single measurements have also been made by DETMAR and a few others. The pressures given by trees lie outside the present work.

The first requisite for the study of exudation pressure is a gauge which will register the pressure accurately, however small the quantity of liquid exuded. Most of those used for demonstration are faulty in principle to an extreme degree, as has been recently pointed out.[3] After some preliminary trials, I have fixed upon one which is practically that described by MORSE and FRASER[4] for the measurement of osmotic pressures. It is a mercury manometer of the usual form, of 0.5$^{\mathrm{mm}}$ bore barometer tubing, with a bulb blown in the middle arm. In order to test the variations in

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[1] BOT. GAZETTE 40:302–305. 1905.

[2] Cohn's Beiträge zur Biologie der Pflanzen 6:1–210. 1892.

[3] W. F. GANONG in School Science and Mathematics 6:300. 1906.

[4] Am. Chem. Jour. 34:3–5. 1905.

the diameter of the bore, two gages were calibrated. It was found, however, that while for high pressures, involving the measurement of short lengths of tube, it would be necessary to make the correction for the differences in diameter of the bore, this is not needed in dealing with small pressures, of about one atmosphere, because in the length of tube used the inequalities, which are very slight, balance one another.

For use the gage is first filled with clean, dry mercury; then with the lower end dipping into boiled water mercury is forced out into the water by pressure with a rubber bulb upon the upper end until there remains only the required amount, namely, enough to fill the gage half-way up the bulb. When the force is removed, water replaces the mercury in the lower end. The long arm is then filled with air, dried, by being drawn through tubes containing calcium chlorid and phosphorus pentoxid, and is quickly sealed with hot, melted shellac. Proper tests were of course applied to prove that the shellac made a perfectly tight joint with the glass. Sealing wax was used at first, but it is apt to crack from the glass when the range of temperature is great. The gage was then attached by means of stout-walled rubber tubing to the stump of a plant whose top had been cut off $2–3^{cm}$ above the ground. The cutting was done under water to prevent the entrance of air, which would rise in the gage later. The rubber tubing was fastened on the stump with stretched rubber bands, or wire in case of a stout stem, and to the manometer by wire and was wound with tire-tape to prevent stretching under internal pressure.[5]

Where the stem was larger than the gage, a glass sleeve was cemented with sealing-wax outside the latter, to make it nearly the size of the stem. The height of the mercury in the long arm was noted and when it had reached its highest point the pressure was computed by BOYLE's law. A source of error in the use of the gage, not noted until too late for correction, consists in the expansion of the mercury through rise in temperature. Subsequent tests were made to ascertain its extreme possible amount, with a result that under no circumstances could the error exceed 0.0134 atmospheres or 0.2^{lb} per square inch, and in nearly all cases it was very much less than this.

The quantity of exudation was found at the same time for plants of the same age and approximate size as those used in the exudation pressure experiments. For this a piece of glass tubing was attached to the plant and led into a graduated cylinder which contained a film of oil to prevent evaporation.

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[5] A fuller description of this gage with directions for use is given by W. F. GANONG in a catalogue of apparatus now being published by the Bausch & Lomb Optical Company.

All the plants used were young, in good growing condition, and in most cases were taken just before flowering. It will at once occur to the reader that in some cases conclusions have been drawn from too small a number of plants, but since in these experiments the plants must be sacrificed completely, it was impossible to obtain more than three of some species—one for exudation and two for pressure. However, this does not involve so great a possibility of error as it seems at first sight, for, although it is possible that the plant used may be abnormal, the probabilities, according to QUETELET's law, are that it will fall near the mean. The temperature of the laboratory during observation of the experiments was 18°–22° C. The soil temperature at time of highest pressure was 17°–18°C.

The results obtained for quantity and pressure of exudation are given in the following table, in which the first and fourth columns give the number of plants used for exudation and for pressure experiments, respectively.

From the table on page 53 it will be seen that the plants giving the highest mean pressures, in order of amount, are *Salvia involucrata*, *Helianthus annuus*, *Fuchsia speciosa*, with others following according to the table. When considered in order of maximum pressures, the highest are given by *Fuchsia speciosa*, *Salvia involucrata*, and *Lycopersicum esculentum*. The highest minimum pressures are given by *Helianthus annuus*, *Salvia involucrata*, and *Senecio Petasitis*.

For the practical purpose of demonstration, taking into consideration high pressures, ease of manipulation, and abundance of plants, the very best are *Fuchsia speciosa*, *Chrysanthemum frutescens*, and *Pelargonium zonale*. Since they have stout, woody stems, there is no danger of compressing the vessels in making a tight joint with the pressure gauge. With the pressure gauge attached they maintain a high pressure for about a week, usually giving the highest pressure between 10 and 12 o'clock on the second day. The next best are *Heliotropium peruvianum*, *Pelargonium peltatum*, and Abutilon. These give lower pressures than the preceding, but they have a slight advantage in another way. The stems of vigorous young plants have about the same diameter as the manometer tubing and therefore can be more readily attached to the gauge. Other plants which give high pressures are *Lycopersicum esculentum* and *Phaseolus vulgaris*. These have somewhat soft stems, though with care it is not difficult to attach the gauge.

The following plants cannot be used advantageously for this experiment: *Euphorbia pulcherrima*, because of its latex which thickens and closes the ducts in a very short time; *Ricinus communis*, because of its hollow stem; *Senecio mikanioides* and *Tropaeolum majus*, because their

Name	No. of plants	Exudation quantity in cc.	Durat'n of flow in days	No. of plants	Pressure in atmospheres
		Min. Mean Max.			Min. Mean Max.
Abutilon (golden bells)...	3	1 - 1.9-3.3	2-3	4	.757- .795- .840
Begonia coccinea........	1	168	29	2	.818- .858- .898
Chrysanthemum frutescens (marguerite).....	4	24 -40 - 55	9-21	4	.847-1.014-1.230
Coleus.................	2	11.5-14.2- 17	8-9	2	.822- .897- .972
Cucurbita Pepo (squash)	1	27	12		
Euphorbia pulcherrima (poinsettia)...........	1	2.5	1	2	.573- .637- .701
Ficus elastica (rubber plant)................	1	23	31	2	.737- .817- .897
Fuchsia speciosa........	6	16.5-99.2-263	12-34	8	.887-1.246-1.605
Hedera Helix (English ivy).................	1	1.5	2	2	.323- .430- .538
Helianthus annuus (sunflower)...............	1	30	16	2	1.210-1.276-1.343
Heliotropium peruvianum (heliotrope).....	1	7	8	4	.809-1.045-1.294
Impatiens Holstii........	1	3	3	2	.851- .855 .860
Lupinus albus (white lupine)...............	1	4	3	2	.793- .807- .821
Lycopersicum esculentum (tomato-dwarf stone)................	1	13	5	4	1.015-1.164-1.349
Pelargonium domesticum (Lady Washington geranium)........	1	17.4	13	2	.714- .861- .909
Pelargonium peltatum (ivy-leaved geranium)	1	4.5	9	2	.940-1.001-1.063
Pelargonium zonale (horse-shoe geranium)	3	14-15.5-17.5	19-20	10	.620- .881-1.116
Phaseolus vulgaris (string bean)..........	2	4- 4.2- 4.5	5	4	.908-1.002-1.149
Ricinus communis (castor bean).............	1	8.5	17	2	.666- .692- .718
Salvia involucrata........	1	14	12	2	1.151-1.369-1.571
Senecio mikanioides (German ivy).........	1	27	7	2	.518- .633- .648
Senecio Petasitis.........	1	110	14	2	1.137-1.191-1.246
Tropaeolum majus (nasturtium)..............	1	13.5	8	2	.759- .779- .800
Zea Mais...............	1	4	6		

stems are too soft and weak. I could not attach the gauge successfully to the following: *Cucurbita Pepo*, *Zea Mais*, and *Vicia Faba*. Obviously stemless plants cannot be used. Thus comparatively few of the common plants are practicable.

The minimum pressures for any given plant in the table were obtained in the winter months and the maximum pressures in April and May, showing a variation in pressure with time of year which is probably widespread.

In order to determine the individual variations of plants taken at the same time of year and treated in exactly the same way, the following species were studied with appended results:

April, 5 plants Pelargonium zonale, .620, .645, .767, .782, 1.035 atmospheres.
June, 5 plants Fuchsia speciosa, 1.406, 1.428, 1.467, 1.500, 1.605 "

While an average of the pressures given by the above plants may seem of no great value, nevertheless there is a certain advantage in knowing this mean, which may be taken as a general expression of the root pressure of common greenhouse plants. The mean pressure of the 22 plants studied is approximately 0.9 atmosphere, or in round numbers 13^{lb} for the square inch.

Turning from pressure to quantity of exudation, the above table shows that the plants giving the greatest quantity of exudation are *Fuchsia speciosa* and *Begonia coccinea*. Different plants of *Fuchsia speciosa* vary greatly in the quantity given off, and this seems to be correlated with the formation of new shoots—the longer this is delayed the greater the flow. This is true in less degree of most of the plants which form new shoots. In others—*Impatiens Holstii*, *Lycopersicum esculentum*, *Pelargonium peltatum*, and *Tropaeolum majus*—the stems decay and the roots soon die.

Some observations were made on periodicity of exudation and of exudation pressure. These facts will be published in a separate note later.—Sophia Eckerson, *Northampton, Mass.*

THE CONDITION OF CERTAIN WINTER BUDS

During 1905–1906 an effort was made to ascertain the seasonal stages in the microspore development of certain woody plants, with the following results:

	Mother cell stage	Dividing nuclei	Mature spores
Populus deltoides..............	....	....	October 23
Fraxinus americana............	Oct. 23—Feb. 15	....	....
Celtis occidentalis.............	October 24	April 12	April 15
Carpinus caroliniana...........	October 24	April 12	April 15
Cornus florida..................	August 20	August 22	August 24
Cercis canadensis..............	October 31	April 17	April 20

William Moore and Mary E. Behney, *Ursinus College, Pa.*

CURRENT LITERATURE

BOOK REVIEWS

Danish strand vegetation

WARMING[1] has published the first of a series of important monographs on the vegetation of Denmark. The aim is to treat the entire vegetation of the country from the standpoint of ecology. In view of the many years of work of the author in this field, these monographs are certain to take front rank in studies of this kind, and it is to be regretted that publication in the Danish language makes them inaccessible to many who would read them with keen interest. The first monograph deals with the strand, and the fact that more than 300 pages are devoted to the strand of a small area, and at that without including the sand-dune vegetation, gives some conception of the wealth of detail and thoroughness of treatment of the study. In WARMING's many years of work in Denmark, the strand and dune vegetation of his country have especially attracted his interest, and the work here reviewed may be regarded as a summary of all his previous work on the strand. There is no doubt that it takes its place at once as the classic contribution on this subject. The volume is amply illustrated with many halftone reproductions from photographs and a large number of drawings, representing life-habits and structural characteristics of the strand plants.

The first chapter considers the sea-cliffs, a physiographic formation that is but slightly developed in Denmark. The granites of Bornholm are inhabited by halophytic lichens, and the Cretaceous calcareous rocks furnish a habitat for rock-boring algae. Chapters follow on the gravel and shingle shores. There are all gradations from rough stony coasts with no vegetation to older and higher gravel coasts with almost closed vegetation carpets. Between large stones there often appear the characteristic plants of salt marshes. The most widespread shore formation is the sand strand, and here successive belts of vegetation are seen, first a plantless zone, then a zone of sand algae, followed by a zone of halophytic seed-plants, mostly chenopods; last of the truly maritime formations is a zone in which various non-halophytic psammophytes mingle with the halophytes. A chapter is devoted to the vegetation of the seaweed heaps, mostly made up of ruderal chenopods. The shallow lagoons, known as "vader," are poor in plants but rich in animal life (for a fuller account of these interesting habitats, see BOT. GAZETTE **41**: 78, 79. 1906); sometimes various diatoms and *Salicornia herbacea* are the only plants of such places. In similar situations, where only spring tides bring contact with salt water, are some remarkable alga societies, chiefly com-

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[1] WARMING, EUG., Dansk Plantevaekst. 1. Strandvegetation. 8vo. pp. vi+325. *figs. 154.* Copenhagen and Christiania. 1906.

posed of Chlamydomonas, Pleurococcus, various blue-green algae, Myxophyceae, and diatoms. The animals of the "vader" and these algae are of enormous importance in reclaiming land from the sea. A chapter is devoted to the enalids, that is, the plants of submerged meadows, among which Zostera, Ruppia, and similar plants dominate.

An entire chapter is devoted to the ecology of *Salicornia herbacea* (another evidence of the thorough treatment of the book), which is the pioneer emergent plant of the salt marsh; its upper line is determined by the tide, since over three hours of complete emergence seems detrimental. Beyond the Salicornia zone are various halophytic plants, largely grasses, whose distribution varies largely with soil changes. Sand-binding grasses like *Glyceria maritima* dominate in the sandy marshes of this type. In the chapter which compares the salt marshes of the North and East seas, it is noted that the North Sea flora is much more halophytic than is that of the less salty East Sea. It is very curious, however, that some true halophytes of the East Sea region are not known from the North Sea. Reed swamps of Phragmites, Scirpus, etc., are found in brackish waters (up to 3 per cent. salt). Of much interest are the bacterial swamps in which the purple sulfur bacteria and Beggiatoa dominate; these occur of course where there is decaying vegetation. A chapter is devoted to the unevennesses of salt marshes (such as mounds formed by ants, moles, etc.), and their vegetation, which differs much from the ordinary flora of the marsh. The influence of artificial land reclamation is the subject of another chapter; dike-building soon results in the disappearance of salt from the marshes, and the development of the vegetation into an artificial meadow. The final chapters deal with the ecological characteristics of halophytes. Most species are hapaxanthic turf-builders. Woody plants are missing except for two half-shrubs. Succulence and leaf isolaterality are common. The flowers are largely wind-pollinated, and the seeds are scattered more by water currents than by other means. The thoroughness of this work makes us long for the other members of the series.—H. C. COWLES.

A new textbook of biology

That the teaching of biology in secondary schools has not yet reached an accepted method is evident with the appearance of each new textbook. Formerly courses in general biology were recommended, without any separation into botany and zoology. Later it became more common to offer unit courses in zoology, or botany, or physiology. During all this time, however, there have been adherents to the elementary course in general biology, and some excellent arguments for such a course have been recognized by almost every teacher.

In a recent book by HUNTER[2] we have another attempt to solve this problem. It is intended to present botany, zoology, and human physiology in one course to students in the first year of the high school, and has been used in the New

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[2] HUNTER, GEORGE WILLIAM, Elements of biology. pp. 445. New York: American Book Company. 1907.

York city schools. The sequence is botany (146 pp.), zoology (139 pp.), and human physiology (124 pp.). Preceding the presentation of botany, there is an introductory chapter, another on "Experiments in chemistry and physics," and a third on "Protoplasm and the cell." Evidently there is no attempt to follow the older type of courses in general biology, but rather to present in one book short courses in the three sciences. Directions for experiments accompany the discussions of the text and are often not dissociated from them. The preface states that "it would not be wise to attempt all of the work outlined in this book;" and also, "it is thought that each successive chapter, although related to that immediately preceding it, is yet distinctive enough to allow of the omission of a chapter or chapters without in any way interfering with the continuity of the work." This universal plasticity suggests a general lack of organic relationship.

The botany begins with a chapter on flowers. The first two pages are almost entirely filled with fine print, which the author says in his preface is "less important;" and with this luminous beginning we find on the third page the astonishing title "Fertilization of flowers," in connection with which appear diagrams and terms presenting ovules, embryo sac, eggs, pollen grains, and pollen. The attitude of mind which makes possible the attempt to present this difficult topic in this way is shown, not only by the misleading title given above, but by the following sentence: "The first beginning of the growth of the seed takes place at the moment of fertilization." The remainder of the chapter discusses pollination; and then follow chapters on "Fruit" and "Seeds and seedlings." It is not until the seventh chapter that there is any discussion of the structure or functions of the working parts of the plants. Even then the chapter on "Roots and their work" is followed by a chapter on "Buds and stems," instead of the chapter on "Leaves and their functions." In such an arrangement no close working relation is developed between the different regions. Ecology is presented in one chapter (8 pp.), and the botany closes with a somewhat long chapter on "Flowerless plants."

If throughout this part of the book there were evident an attempt to correlate the material with that of zoology or physiology, we might understand the plan, but no such correlation appears; in fact there is not even any correlation of the botanical material itself. Furthermore, the statements are often faulty. In defining matter, the author says: "It may be living, or may have been alive at some previous time, in which case we speak of it as organic matter; or it may never have been alive. The latter state of matter is called inorganic." Under the topic "chloroplasts," the process of photosynthesis is discussed, and in italics the statement is made that "the chloroplasts, by means of the energy received from the sun, manufacture starch out of certain materials." An accompanying diagram, labeled "Diagram to illustrate the formation of starch," attempts to visualize the process; but not a word appears to indicate that starch is not formed directly by chloroplasts, or that anything besides starch is made by photosynthesis.

The treatment of zoology is from the standpoint of increasing complexity, from Protozoa to Mammalia. For this subject the author is equipped, so that probably it is free from such faults as have been indicated in the chapters on botany. The section on human physiology attempts to cover all the important topics, and meets the demands of certain state laws by indicating the effects of alcohol. The absurd extent to which this may be carried is shown in the chapter on "The nervous system," in which ten pages are given to alcoholism and eight to the entire nervous system.—OTIS W. CALDWELL.

Wettstein's Handbuch [3]

A third instalment of WETTSTEIN'S *Handbuch* contains a general discussion of the angiosperms and a special taxonomic treatment of the choripetalous dicotyls. The next and final instalment, which is promised in the spring of 1908, will deal with the rest of the dicotyls and with the monocotyls. After a presentation of the general morphology of angiosperms, the writer discusses their phylogeny. He believes that the monocotyls have been derived from the dicotyls, and that among the dicotyls the Monochlamydeae contain the most primitive forms. Consequently, they would be most likely to show characters which might indicate relationship with groups below. The pollen-tube structures and the flowers of angiosperms are thought to indicate a gymnosperm origin. It is not claimed that the flowers of living gymnosperms and angiosperms furnish an easy transition, but merely that the types can be reconciled both morphologically and ecologically. The Choripetalae are arranged in thirteen series, beginning with the Verticillatae, Casuarinaceae being the lowest family, and ending with the Centrospermae, in which the Caryophyllaceae are the highest family. The Dialypetalae contain twelve series, beginning with the Polycarpicae, Magnoliaceae being the lowest family, and ending with the Umbelliflorae. The description of families is full and clear and profusely illustrated with excellent figures, many of which are new.—CHARLES J. CHAMBERLAIN.

MINOR NOTICES

Das Pflanzenreich.[4]—Parrt 30 contains the Styracaceae, prepared by JANET PERKINS. After the usual introductory account of the characters and geographical distribution of the family, the six genera are presented as follows: Pamphilia (3 spp., 1 new), Styrax (97 spp., 11 new), Bruinsmia (2 spp.), Alniphyllum (3 spp.), Halesia (3 spp.), and Pterostyrax (3 spp., 1 new).

Part 31 contains the Potamogetonaceae by ASCHERSON and GRAEBNER, prefaced by an unusually full account of the structure and habits of this interesting family. The systematic presentation is as follows: Zostereae containing Zostera

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[3] WETTSTEIN, DR. RICHARD R. v., Handbuch der systematischen Botanik. II Band. 2 Theil (erste Hälfte). pp. 161–394. *figs. 165* (995). Leipzig und Wien: Franz Deuticke. 1907. *M* 9.

[4] ENGLER, A., Das Pflanzenreich. Heft 30. Styracaceae von J. PERKINS. pp. 111. *figs. 18* (191). *M* 5.60. Heft 31. Potamogetonaceae von P. ASCHERSON und P. GRAEBNER. pp. 184. *figs. 36* (221). *M* 9.20. Leipzig: Wilhelm Engelmann. 1907.

(6 spp.) and Phyllospadix (3 spp.); Posidonieae containing Posidonia (2 spp.); Potamogetoneae containing Potamogeton (89 spp., 1 new, and numerous hybrids) and Ruppia (1 sp.); Cymodoceae containing Cymodocea (7 spp.) and Diplanthera (2 spp.); and Zannichellieae containing Zannichellia (2 spp., 1 new) and Althenia (4 spp.).—J. M. C.

Giesenhagen's Lehrbuch.—The fourth edition of GIESENHAGEN'S *Textbook of botany*[5] is not essentially different from the third edition, which was reviewed in this journal.[6] A few details have been modified, a few figures added, and the text in general brought up to date. The most noticeable change is the presentation of current theories, which many botanists believe to be out of place in a textbook. The author, however, believes that important theories and problems, even though still lacking definite proof, are valuable if the presentation makes it clear that they are only theories.—CHARLES J. CHAMBERLAIN.

Plants of Bolivia.—The fourth part of the enumeration of Bolivian plants collected by MIGUEL BANG has been issued as a Bulletin of the New York Botanical Garden.[7] The previous parts appeared in the *Memoirs of the Torrey Botanical Club* in 1893, 1895, and 1896. The present part completes the enumeration with the exception of Gramineae, and contains descriptions of 227 new species. Three new genera are also characterized: *Tournefortiopsis* and *Poederiopsis* (both Rubiaceae), and *Vassobia* (Solanaceae).—J. M. C.

Genera Siphonogamarum.—The eleventh fascicle of DALLA TORRE and HARMS'S[8] list of the genera of seed-plants completes the work, the last entry being Zyzygium. There are included also title-page, preface, and list of families (290 in number).—J. M. C.

NOTES FOR STUDENTS

Morphology of bryophytes.—Several studies of bryophytes come to hand almost simultaneously. DACHNOWSKI[9] has been working again upon the much-studied *Marchantia polymorpha*. The growth of the rhizoids, the origin of dorsiventrality, the plagiotropic position, the formation of reproductive organs, and fertilization have been reexamined. His conclusions are in part like those of previous investigators and in part a slight modification of them. He finds moisture the most

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[5] GIESENHAGEN, DR. K., Lehrbuch der Botanik. Fourth edition with 561 illustrations. 8vo. pp. xiv+463. *figs. 561*. Stuttgart: Fr. Grub. 1907.

[6] BOT. GAZETTE **37**:225. 1904.

[7] RUSBY, HENRY H., An enumeration of the plants collected in Bolivia by MIGUEL BANG. Part 4. Bull. N. Y. Bot. Garden **4**:309–479. 1907.

[8] DALLA TORRE, C. G. DE, and HARMS, H., Genera Siphonogamarum ad systema Englerianum conscripta. Fasc. 11. pp. 801–921. Leipzig: Wilhelm Engelmann. 1907. *M* 6.50.

[9] DACHNOWSKI, A., Zur Kenntnis der Entwicklungs-Physiologie von *Marchantia polymorpha*. Jahrb. Wiss. Bot. **44**:254–286. *figs. 4. pl. 4.* 1907.

important factor in calling forth rhizoids. Dorsiventrality is fixed in 10–20 hours after sowing and "rests upon the reciprocal relation between determinative cooperating external factors and internal conditions of maturity"—the latter dependent in large measure upon the age of the gemmae. The plagiotropic position is a functional relation, influenced by illumination, and resulting from the cooperation of diaheliotropism and negative geotropism. Moisture and other variable external factors exercise a far greater influence than diageotropism, autogenous hyponasty, and photepinasty, to which it has been previously referred. As to reproductive structures, they seem to arise only when the plants are brightly illuminated, whereas weak illumination and much moisture permit only vegetative growth. Natural parthenogenesis was not observed.

Györffy has had opportunity to study that curious hybrid, *Physcomitrella Hampei*, which has three forms, differing according to the male parent, *Physcomitrella patens* being in all cases the female parent. One of these is *P. patens* × *Physcomitrium sphaericum;* the second, *P. patens* × *Physcomitrium eurystomum;* and the third *P. patens* × *Physcomitrium pyriforme*, which Györffy himself discovered. He has made a comparative anatomical study of the first and third hybrid forms and of their parents. This is a paper that will be useful for the writer of a comparative anatomy of mosses, but for readers it is wordy and tiresomely detailed, and might easily have been compressed, to its advantage, into half the space. It seems hardly necessary in such a paper to describe the form of an archegonium and the mode by which the canal is opened for the sperm, when these do not have any peculiarities; the paper is full of such repetitious and irrelevant matter. This begins in the title, which is an excellent example of what a title ought *not* to be.[10] Györffy makes one particularly interesting observation, which is important if true; he figures the spore mother cells as capping the columella in *P. patens*. This is contrary to Limpricht's observations, and as Györffy lays no special stress on the point, it seems doubtful if he appreciated its significance. In general he finds, as others have done, that the vegetative characters are those of *P. patens*, while the characters of the sporophyte are intermediate in certain respects. Thus the foot and the external form of the sporophyte of *P. Hampei* I are those of *P. sphaericum*, while the internal structure is more like that of *P. patens*. In *P. Hampei* III, strangely enough, the form of the capsule is like that of *P. Hampei* I, though the foot and long seat are those of *P. pyriforme*.

Servit discusses the mode of branching of the bryophytes,[11] supporting the novel views of Velenovský,[12] which take no account of the source of branch

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[10] Györffy, I., Ueber die vergleichenden anatomischen Verhältnisse von *Physcomitrella patens* (Hedw.) Br. et Sch., *Physcomitrium pyriforme* (L.) Brid., *Physcomitrium sphaericum* (Ludw.) Brid., und *Physcomitrella Hampei* Limpr. [*Physcomitrella patens* (Hedw.) × *Physcomitrium sphaericum* (Ludw.), resp.: *Physcomitrella patens* (Hedw.) × *Physcomitrium pyriforme* (L.)]. Hedwigia **47**:1–59. 1907.

[11] Servit, M., Ueber die Verzweigungsart der Muscineen. Beihefte Bot. Cent. **22**^{1}:287–293. *figs. 3.* 1907.

[12] Velenovský, J., Vergleichende Morphologie **1**:130. Prag. 1905.

initials, and determine morphological relationships merely by "how things look in the mature condition."

SERVIT extends VELENOVSKÝ'S "angular leaf" idea from pteridophytes to bryophytes, and describes such a leaf in Mastigobryum, where it is easily distinguished by its transverse insertion as well as by its form. It is situated at the base of two branches and he considers it as formed by the concrescence of the first leaf of both. This angular leaf, he says, is present in all liverworts whose branches arise from the half-segment; but it is elsewhere not so typical (this sounds Goethean) as in Mastigobryum. It is lacking in those whose branches arise only from the basiscopic part of the half-segment.

In intercalary branching both of liverworts and mosses the branch is endogenous and breaks through a sheath, which may be split into segments that SERVIT calls *Blättchen*. He has confirmed in all cases VELENOVSKÝ'S "law" (that the branches in mosses arise without exception in the axil of the leaf below them!) by examining "extraordinarily abundant material;" but he does not say that in any case he has determined the relation of the branch initial to the leaf initial. Until that is done the current view, which rests upon careful studies of LEITGEB, will prevail.

BUCH has made a thorough study of the two modes of vegetative propagation of *Blasia pusilla*,[13] especially of the development and germination of the gemmae produced in the flasklike receptacles. These arise both on male and female plants, but are rare on the latter if they form embryos. While the brood-buds (modified tips) are the chief means of propagation in summer, the gemmae probably do not germinate in the summer and certainly not in the autumn, but hibernate and start new plants in the spring. Shoots arise from either side (seldom both), where there is a zone of four small cells. One of the upper two produces a thallus initial by three successive divisions of the protruded tube, while the two lower ones produce rhizoids.—C. R. B.

Mutations of Oenothera.—MACDOUGAL[14] and his collaborators have published a further account of evolutionary studies upon the Oenotheras. Taxonomic descriptions of several of the mutants from *O. Lamarckiana*, as they grew in the New York Botanical Garden, are given, including *O. albida*, *O. oblonga*, *O. scintillans*, *O. brevistylis*, and *O. lata*. These will be valuable for comparison with cultures elsewhere. The English "*O. biennis*," as growing in the vicinity of Liverpool, has been found to be *O. Lamarckiana*, and growing wild with it are two of the mutants, *O. rubrinervis* and *O. lata*, the latter maturing pollen, contrary to its habit elsewhere. The cultures of *O. Lamarckiana* from DEVRIES' seeds gave six of the mutants described by that author and also nine other types which could

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[13] BUCH, H., Ueber die ungeschlechtliche Vermehrung von *Blasia pusilla* (Mich). L. Öfvers. Finska Vet. Soc. Förhdl. **49**: no. 16. pp. 42. *pls. 2. figs. 7.* 1907.

[14] MACDOUGAL, D. T., VAIL, A.M. and SHULL, G. H., Mutations, variations, and relationships of the Oenotheras Carnegie Institution of Washington, Publ. no. 81. pp. 92. *pls. 22. figs. 73.* 1907.

not be identified. These aberrant forms are of special interest from the standpoint of variation, and it is hoped that further careful records will be obtained of them and their offspring.

SHULL presents a continuation of his earlier studies[15] on variation in *O. Lamarckiana* and some of its mutants. On account of the unsatisfactory character of height, branching, and leaf form for statistical comparisons, the studies are extended to include bud characters. The fact that all his collections of buds were made late in the season, and that there is a marked seasonal variation in the size of the buds, would render somewhat doubtful his statement that the periodicity is probably too small to need consideration. The bud characters chosen are length and thickness of ovary, hypanthium, and cone, the forms studied being *O. Lamarckiana, O. rubrinervis, O. gigas,* and *O. lata*. A formidable series of variation and correlation tables is presented, the general conclusion being that the mutants tend to show greater variability than the parent form, though there is no decrease in correlation of parts. MACDOUGAL concludes on these premises that instead of mutations being the cumulative results of ever-increasing fluctuation, they are the initial process, of which fluctuations in the mutants, gradually decreasing to a minimum, are an after-effect. The hypanthium of Oenothera, which is the most variable part of the bud, is considered an example of a phylogenetically new character showing increased variability.

Miss VAIL contributes taxonomic accounts of *O. grandiflora, O. Simsiana, O. Oakesiana, O. parviflora,* and *O. muricata*. *O. parviflora*, known in Europe since 1759, has recently been found native in Maine.

Cultures of *O. grandiflora* and *O. biennis* showed the presence in the former of at least two mutants, and that under the latter there is a swarm of elementary species distributed throughout North America. Two interesting cases of bud-sports were observed, one in *O. Lamarckiana*×(*O. Lamarckiana*×*O. cruciata*), and the other in *O. ammophila*, which is suspected of being a hybrid derivative of *O. biennis*. In both cases self-fertilized seeds of the mutant branch came true to the type of the sport.

A careful account is given of MACDOUGAL'S injection experiments. After some negative results with other forms, mutants were obtained from injected ovaries of *O. biennis* and *Raimannia odorata*. The solutions used were $ZnSO_4$ (1:500), $Ca(NO_3)_2$ (1:1000), and 10 per cent. sugar solution. Other inflorescences were treated with a pencil of radium emanations. In the *O. biennis* experiments, in addition to the usual forms, one rosette appeared which differed widely from any known type, and its progeny was like it. In the case of *Raimannia odorata*, a group of mutants appeared in the progeny of injected ovaries. It was also found that "plants of the progeny of the first treatment which were apparently normal yielded seeds which gave a few atypic forms."

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[15] MACDOUGAL, D. T., assisted by VAIL, A.M., SHULL, G. H., and SMALL, J. K., Mutants and hybrids of the Oenotheras. Carnegie Institution of Washington, Publ. no. 24. pp. 57. *pls. 22. figs. 13.* 1905.

BOULENGER,[16] from observations of the evening primroses growing wild at South Kensington, England, and in La Garde St. Cast, Brittany, concludes that *O. Lamarckiana* and *O. biennis* cannot be distinguished as separate "species," and that *O. Lamarckiana* originated from *O. biennis* through some hypothetical process of hybridization among the different forms of the latter. His conclusions rest on an examination of the variability of the plants taken *en masse* in the wild condition, and the unproven assumption that all the plants observed at St. Cast, Brittany, came from a single stock. Many different forms, both in Europe and America, go under the name of *O. biennis*, as MACDOUGAL has shown, and if *O. Lamarckiana* were capable of giving rise to any of these we might have expected to find them in DEVRIES' cultures. The study of the Oenotheras has long since reached the stage where cultures are necessary to determine the relationships of the various strains, and without such studies speculations drawn from casual observations of variability among forms growing wild are not likely to affect seriously the results obtained from culture work such as that of DEVRIES.—R. R. GATES.

A biometric study of Ceratophyllum.—A recent memoir by PEARL[17] on variation and differentiation in Ceratophyllum presents an admirable illustration of the successful application of mathematics to the working-out of important biological problems. The work is too compact to allow a brief review to present all the important relations which are clearly demonstrated by biometric analysis of the variation in the number of leaves in a whorl as related to the position of the whorl on the plant. It is shown that the whorls on each grade of branches, e. g., main stem, primary, secondary, tertiary, etc., are differentiated as a class from each other grade by several distinctive features of their variability. In all these grades the correlation between number of leaves in a whorl and its position on the stem is considerable. It is least in the main stem and increases as we pass to the more peripheral divisions. The regression line is not linear, but logarithmic, being of the type $y=A+C\log(x-a)$, in which A, C, and a are constants, y is the number of leaves per whorl, and x the position of the whorl. The curves so derived fit beautifully the observed data, and allow the derivation of what is believed to be a very fundamental biological law which is called the "first law of growth" in Ceratophyllum, and is stated thus: "On any axial division of the plant the mean number of leaves per whorl increases with each successive whorl in such a way that both the absolute increment and the rate of increase diminish as the distance (measured by the number of nodes) of the whorl from a fixed point increases."

By the fact that the equation fits data from plants collected from different habitats, by merely changing the factor A which represents the actual size and

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[16] BOULENGER, G. A., On the variations of the evening primrose (*Oenothera biennis* L.). Jour. Botany **45**:353–363. 1907.

[17] PEARL, RAYMOND, assisted by OLIVE M. PEPPER and FLORENCE J. HAGLE. Variation and differentiation in Ceratophyllum. Carnegie Institution of Washington, Publ. no. 58. pp. 136. *pls. 2. figs. 26.* 1907.

not at all the size of parts relative to position, it is shown: (1) that A is the environmental factor, and (2) that environment affects size but not at all the progress of differentiation represented by the logarithmic curve. It is found that the curve for the main stem is most nearly horizontal and that for each successive grade of branches is steeper and steeper, a change in the curve dependent upon the increasing value of the constant C, which is therefore regarded as the constant which expresses the action of internal formative factors.

On any axial division the first whorls are the most variable in number of leaves, and this variability decreases at first rapidly, then less and less rapidly as more and more distal positions are reached, the theoretical limit attained being perfect constancy. This constitutes the "second law of growth" in Ceratophyllum. It is shown that these two laws are true in branch production as well as leaf production in this species.

Although few other studies have been made which can be definitely cited to demonstrate the general validity of these two laws of growth, the essence of the second law was recognized by the reviewer[18] in the case of *Sium cicutaefolium*, and other published observations are mentioned which appear to indicate that these are general laws. It is believed that this second law is the same which in the physiological field has been stated by JENNINGS[19] thus: "An action performed or a physiological state reached is performed or reached more rapidly after one or more repetitions, so that in time it becomes 'habitual.'" It is shown that in psychology it is probably the same law that lies at the basis of memory, namely, the ease, as measured by rapidity and accuracy, with which a state once reached is reached again. It seems probable, at any rate, that suitable analysis of all these cases will show that they are governed by logarithmic laws, and though no suggestion has been made as to the nature of the common causes upon which such general validity of the logarithmic law must rest, their nature will doubtless be brought to light very soon when once it is recognized that they exist.

The necessity of more studies of a similar nature is apparent. It is important to know how these laws are affected by more complex environmental conditions. The ease and conclusiveness with which they were demonstrated in Ceratophyllum probably depended upon the fact that in this species environmental modifications are reduced to their lowest terms. It is to be hoped that the aims and methods of this paper may serve as guides for many biometric studies, for the successful analysis of the laws of differentiation within the individual must precede studies which involve repeated structures in different individuals. Studies of the latter type are insecure until the laws of intra-individual differentiation and variation are known for the particular species of plant or animal under investigation.—GEO. H. SHULL.

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[18] SHULL, G. H., Stages in the development of *Sium cicutaefolium*. Carnegie Institution of Washington, Publ. no. 30. pp. 28. *pls*. 7. 1905.

[19] JENNINGS, H. S., The methods of regulation in behavior and other fields. Jour. Exp. Zool. 2:473–494. 1905.

Taxonomic notes.—C. WARNSTORF (Hedwigia **47**:76–112. 1907), in describing new species of Sphagnum, includes 5 from the United States and 9 from S. America.—ED. FISCHER (Ber. Deutsch. Bot. Gesells. **25**:372–376. 1907) describes in a preliminary way some hypogeous fungi from California, collected by SETCHELL and GARDNER, including a new monotypic genus, *Pseudobalsamia Setchellii*, and a new species of Pseudogenea and of Hysterangium. He also refers Pseudhydnotrya to Geopora (Pezizaceae).—R. C. BENEDICT (Bull. Torr. Bot. Club **34**:445–458. 1907), in a first paper on the genus Antrophytum, has presented a synopsis of the subgenera and the American species, the latter 9 in number, 2 of which are new.—F. D. KERN (*idem* 459–463) has published new western species of Gymnosporangium (3) and Roestelia (3).—W. A. MURRILL (*idem* 465–481), in a list of Philippine Polyporaceae, has published 24 new species and transferred 21.—J. N. ROSE (Smithson. Miscell. Coll. **50**:330. 1907) has published a new Nopalea from Guatemala; and in collaboration with N. L. BRITTON (*idem* 331–333. *pls. 43, 44*) has established a new genus (*Pereskiopsis*) of Cactaceae, by raising to generic rank § Pereskiopuntia of Opuntia, including 11 species, 2 of which are described as new.—L. M. UNDERWOOD and W. R. MAXON (*idem* 335, 336) have described 2 new species of Lindsaea, one from Columbia, the other from Cuba.—W. F. WIGHT (*idem* 174. *pl. 17*) has described a new Alaskan larch (*L. alaskensis*).—C. V. PIPER (*idem* 195–202), in publishing some new plants of the Pacific slope, describes 3 new subspecies of *Cassiope Mertensiana;* presents a revision of *Orthocarpus tenuifolius* and its relatives, including a new species; and describes new species under Lupinus, Castilleia, and Valeriana.—F. S. COLLINS (Rhodora **9**:197–202. *pl. 76.* 1907) has described new species of green algae under Pleurococcus, Chaetomorpha, Cladophora, and Vaucheria (2).—A. DAVIDSON (Muhlenbergia **3**:105–108. 1907), in a discussion of several species of Sphaerostigma, describes a new species.—In the first paper (Bull. Soc. Bot. France **54**:1–30. 1907) of a series to contain descriptions of new plants collected in tropical Africa by AUG. CHEVALIER, CASIMIR DECANDOLLE describes a new genus (*Charia*) of Meliaceae.—In the 19th number of "Diagnoses Africanae" (Kew Bull. 1907:360–365), W. B. HEMSLEY has described a new genus (*Cordeauxia*) of Leguminosae (Caesalpinieae), and H. BOLUS a new genus (*Peglera*) of Rhizophoraceae.—N. L. BRITTON (Bull. N. Y. Bot. Gard. **5**:311–318. 1907), in a fourth contribution to the flora of the Bahama Islands, describes new species under Zamia, Ibidium, Badiera, Passiflora, Rochefortia, and Tetranthus.—G. W. WILSON (Bull. Torr. Bot. Club **34**:387–416. 1907), in a second paper on N. Am. Peronosporales, presents the Phytophthoreae and Rhysotheceae, and describes *Rhysotheca* as a new genus of 12 species, to include Peronospora § Zoosporatoparae DeBary.—P. A. RYDBERG (*idem* 417–437), in his 18th paper on the Rocky Mountain flora, breaks up Thelypodium, as heretofore recognized, into 6 genera, the 5 new genera being *Thelypodiopsis, Pleurophragma, Hesperidanthus, Stanleyella,* and *Heterothrix;* establishes a new genus (*Chlorocrambe*) on *Caulanthus hastatus* S. Wats.; and describes new species under Homalobus (5), Diholcos, Kentrophyta, Aragallus (3), Hedysarum, Lathyrus, Capnoides (2), Lepidium (3), Thelypodium (2), Pleurophragma, Sophia (2), and Arabis.—J. M. C.

Ammonifying soil nitrogen.—LIPMAN has made another valuable contribution to soil bacteriology.[20] His experiments were exceedingly well planned, were fundamental, and in most cases gave consistent results. So much detail is included in the report that a satisfactory summary of the article is impossible.

He first tested the ability of various soils to ammonify nutrient solutions of peptone, and solutions containing peptone and inorganic salts. The addition of the salts favors the growth of certain species of bacteria and also ammonification under certain conditions. These species were unequally distributed in soils. Inoculation with soil infusion was decidedly inferior to inoculation with soil itself. But soil infusions in the same sterile soil exhibit relations similar to those of the corresponding direct soil inoculations. The inoculation of sterile soils in various ways shows that in ammonification the bacteriological soil factor is much more important than the chemical one. Manured soils showed a superior ammonifying power. Peptone salt solutions which are not provided with too large a quantity of mineral are most suitable for study of the general condition of the bacterial flora of the soil. The differences in the power of ammonification exhibited by several soils seem to bear no direct relation to their mechanical composition. The amount of the sterile soil which is inoculated affects the yield of ammonia, the amount of ammonia being larger, in nearly every case, in the larger samples of soil. This may be due to increased production of ammonia by ammonification of the soil nitrogen; or, more probably, to the larger amount of other mineral substances present which may be useful to the organism; or possibly to the absorption of deleterious products by the soils. Old samples of soil kept in a laboratory for some months had decidedly lower ammonifying power than the younger samples. Original differences in the ammonifying power of the different soils, however, were not entirely obliterated, even after keeping the samples in the laboratory for several months.

Lime added to the soil often stimulated the activity of decay bacteria, the stimulus increasing with the amount of lime up to two tons per acre. The author suggests that it may be possible to develop a bacteriological method of determining the lime requirements of soils in general. More consistent results were obtained by soluble nitrogenous material, such as peptone and gelatin, than by insoluble substances, like albumen, casein, and cotton-seed meal. As a rule, ammonia is produced very rapidly; the amounts given off in three days were nearly as large as in thirty-two days, and in some cases they were larger.

Working with Azotobacter, LIPMAN found that small quantities of soil did not furnish enough mineral salts, whereas in larger quantities a normal growth was obtained. He therefore suggests that it may be possible to measure the mineral

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[20] LIPMAN, G. B., Chemical and bacteriological factors in the ammonification of soil nitrogen. Report N. J. Agric. Exp. Sta. **1906**: 119–187. 1907.

deficiencies of soils by determining how much soil is required to give normal Azotobacter formation.—F. L. STEVENS.

Fossil Osmundaceae.—KIDSTON and GWYNNE-VAUGHAN[21] describe the structure of two osmundaceous stems from the Jurassic of New Zealand. On account of the difficulty of distinguishing anatomically between Osmunda and Todea, they put these stems under the provisional genus *Osmundites*. One of the stems, *O. Dunlopi*, is characterized by an extremely small central cylinder, comparable with that found in the filmy Todeas of the present day. In this species the foliar gaps are much reduced and are said by the authors even to be absent sometimes. The other new species, *O. gibbiana*, has a better developed stele and its foliar gaps are very similar to those of living species of Osmunda. The authors add something to our knowledge of the *Osmundites skidegatensis* of PENHALLOW, from the Lower Cretaceous of Western Canada. In this species they describe well-developed internal phloem. The medullary tissues clearly become continuous with those of the cortex through the very broad foliar gaps. Concerning this species the authors say: "At first sight (it) appears to provide something very like the dictyostelic ancestor postulated by JEFFREY's theory." They conclude, however, with BOODLE and the majority of other British anatomists, since there is an absence of internal phloem in the young plant of living species of Osmunda, that on the hypothesis of recapitulation internal phloem and the accompanying structures must have been absent in the ancestors of the Osmundaceae. This argument, however, is quite fallacious. It would apparently be just as reasonable to assume that the mesarch foliar and peduncular bundles of the Cycadales are not an ancestral feature, as is generally admitted, especially in Great Britain, because forsooth they have not been shown to occur in the stem of cycadean seedlings. Moreover, those who hold with GWYNNE-VAUGHAN, WORSDELL, and others, that the condition without internal phloem is ancestral in the case of the tubular central cylinder of the stem in the Filicales, find likewise no ontogenetic support whatever for their views among the very numerous ferns in which the tubular central cylinder of the adult is siphonostelic with internal phloem. Ontogeny at present throws no light whatever on the subject of the presence or absence of internal phloem in the primitive tubular central cylinder of the fern series. Argument accordingly must be based on comparative anatomy alone, and that being the case there is apparently as good reason to consider the concentric type of bundle to be ancestral for the leaf and stem of the Osmundaceae as there is to regard the mesarch xylem bundle as ancestral for leaf and shoot in the cycadean series. The authors follow SCOTT in regarding the Botryopterideae as among the most primitive ferns. They consider that their *Osmundites Dunlopi* furnishes evidence that the Osmundaceae have come from this group. There is apparently no better reason for regarding *Osmundites Dunlopi* and *O. gibbiana* as primitive Osmundaceae than the similarly austral types, Agathis and Araucaria, as primitive araucarian conifers.—E. C. JEFFREY.

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[21] KIDSTON, R., and GWYNNE-VAUGHAN, D. T., On the fossil Osmundaceae. Trans. Roy. Soc. Edinburgh **45**:759–780. *pls. 1–6.* 1907.

Ophioglossaceae.—CAMPBELL[22] has published an account of his studies of East Indian species of Ophioglossum. He collected *O. moluccanum*, *O. intermedium*, and *O. pendulum*, securing both gametophytes and sporophytes in considerable number. The spores of *O. moluccanum* germinated freely, but owing to a failure to become associated with the mycorhizal fungus the prothallia died after reaching the four-celled stage. Prothallia of *O. pendulum* made the mycorhizal connection and reached the thirteen-celled stage. They did not develop any chlorophyll on exposure to light, although a few chloroplasts developed in the prothallia of *O. moluccanum*. The adult gametophyte in all the species studied is subterranean, without chlorophyll, and radiate in structure. CAMPBELL'S account agrees closely in this regard with the accounts of METTENIUS, LANG, and BRUCHMANN. The account of the development of the antheridium is also essentially the same as that given by these authors. The statement is made that in the mitosis immediately preceding the formation of sperms the number of chromosomes is much larger than in the earlier divisions. Most cytologists will regret that this interesting situation was not investigated somewhat further. The development of the sperm agrees closely with that of Equisetum. The archegonia resemble those of the Marattiaceae. There is either a binucleate neck canal cell or two neck canal cells. The ventral canal cell is difficult to demonstrate, but was shown to be present in *O. pendulum* and probably in the others. The primary wall in the embryo is transverse in most cases, but varies considerably, particularly in *O. pendulum*. The entire hypobasal half goes to the formation of the foot. There are three types of embryo: (1) that of *O. moluccanum*, in which only root and leaf are developed, the plant being annual and no stem being produced; (2) that of *O. vulgatum*, in which root and stem are first produced, and much later a leaf; and (3) that of *O. pendulum*, in which root only is produced, stem and leaf arising later by means of an endogenously developed bud from the primary root at some distance from the gametophyte; the vascular bundles of leaf and stem arise separately from the bundle of the root and remain separate.

In *O. moluccanum* the primary root is monarch and the bundle of the leaf is collateral, and CAMPBELL says that it is merely a question of terms whether one calls the root monarch or collateral. The primary root of *O. pendulum* is diarch, as are also the later roots. The *O. moluccanum* type of embryo is probably the most primitive and is most nearly related to the embryos of Marattiaceae and Equisetineae, especially to the former. The author's well-known claim that Ophioglossum is a primitive type of pteridophytes is emphasized by his somewhat detailed explanation as to how the *O. moluccanum* type might have arisen from an Anthoceros-like ancestry.—L. LANCE BURLINGAME.

Sterility in hybrids.—TISCHLER[23] has published a preliminary account of further investigation on the causes of sterility in hybrids. He has studied *Potentilla*

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[22] CAMPBELL, D. H., Studies of the Ophioglossaceae. Ann. Jard. Bot. Buitenzorg II. 6:138–194. *pls. 9–19.* 1907.

[23] TISCHLER, G., Weitere Untersuchungen über Sterilitätsursachen bei Bastardpflanzen. Ber. Deutsch. Bot. Gesells. 25:376–383. 1907.

Tabernaemontani×*P. rubens*, *Syringa vulgaris*×*S. persica*, and Mirabilis forms including *M. Jalapa*×*M. tubiflora* and *M. Jalapa*×*M. longiflora*. In some of these the usual irregularities in the reduction mitoses of sterile hybrids are reported. In *Potentilla Tabernaemontani*×*P. rubens* the x number of chromosomes is 16. The hybrid shows no difference from the parent *P. Tabernaemontani* in pollen formation, except that the former produces a larger number of imperfect pollen grains. In *P. rubens* nearly all the pollen grains are good. The latter is a constant species, while the former is very variable or "mutable." By subjecting this hybrid to etiolation in hothouse temperature, complete sterility was produced. The reduction divisions were apparently normal, but there was a lack of cytoplasm and of chromatin, the scarcity of the former beginning as early as the archesporium stage. Similar but less marked results were obtained by subjecting *P. rubens* to the same conditions. Some of TISCHLER's interesting conclusions, which will be further discussed in the forthcoming paper, may be briefly stated as follows: (1) Sterility of hybrids does not depend upon any form of chromatin repulsion. (2) Sterility results from the fact that two sexual cells are thrown together whose developmental tendencies are not identical. When the individual reaches the critical reproductive stage the disturbance of harmony in its development makes itself apparent. (3) By modification of the external conditions in plants which are not hybrids, the sexual cells may be so influenced as to produce conditions similar to those which result from hybridization. (4) The sterility of hybrids is a purely relative matter. (5) A true splitting or segregation of characters does not occur in the reduction divisions. One of the arguments in support of this conclusion is that certain characters Mendelize which concern not single rudiments but the constitution of the whole of the idioplasm. (6) The assumption that the individual characters are connected with distinct and separated pangens is abandoned. (7) The chromatin is not of exclusive significance as a hereditary substance. (8) There is a specific idioplasm having a definite constitution. (9) A distinction is to be maintained between chromatin and linin. (10) Apogamy is merely an aid in reproduction in cases of pollen sterility, and is not the primary condition to which pollen obliteration is secondary. (11) Similarly, as shown by CORRENS, in species which are passing from the monoecious to the dioecious condition, disturbances similar to those of mutation occur, resulting in contabescence of the anthers or ovaries. (12) There is, as DARWIN believed, a close relation between the sterility of hybrids and that of plants under cultivation. The full account of this work will be awaited with interest.—R. R. GATES.

Anatomy of Araliaceae.—A very comprehensive treatment of the Araliaceae has been published by VIGUIER,[24] dealing with the history of the family, the external morphological characters, and the internal structure of stem and leaf of about sixty genera. Besides being a valuable contribution to the knowledge of plant anatomy in general, the paper offers an excellent proof of the importance of ana-

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[24] VIGUIER, RENÉ, Recherches anatomiques sur la classification des Araliacées. Ann. Sci. Nat. Bot. IX. 4:1–208. 1906.

tomical characters to systematic research. As a matter of fact, the anatomical characters as described by VIGUIER are not only very useful, but to some extent really necessary for gaining a thorough knowledge of this family and its affinities. From an external morphological point of view the Araliaceae exhibit a number of very conspicuous types, represented by herbaceous, frutescent, and arborescent species; among these the herbaceous are of a special interest, and it is to be regretted that the author could not examine the root structure of the very peculiar Panacineae, for example, to which the ginseng belongs. The stem structure seems to be very constant. The cuticle is often thick; the epidermis consists only of a single layer; the cortex is differentiated as an external continuous zone of collenchyma and an inner parenchymatic zone frequently traversed by secretory ducts; the pericycle is stereomatic and forms arches, always accompanied by secretory ducts; the cork develops constantly in the peripheral hypodermal stratum of cortex, the only exception being Echinopanax. A very considerable variation exists in the occurrence of ducts in the pith, of mestome strands in the pith, in the presence or absence of secretory cells (*poches sécrétrices*) in the secondary hadrome, etc., which is readily noticeable from the anatomical table. The petiole shows also a very characteristic structure, and among the generic characters may be enumerated the arrangement of the mestome in one or several concentric bands; the various disposition of the ducts; the collenchyma continuous or interrupted by cortical, chlorophyll-bearing parenchyma, etc. The structure of the petiole becomes very much complicated when secondary formations set in, as observed in several species. In the blade of the leaf the structure is quite variable, as follows: secretory cells occur only in Gilibertia; the midrib may be composed of several mestome strands arranged in a circle, or may consist only of an arch of leptome and hadrome; hypodermal collenchyma is developed in some genera, but not in others. The anatomical keys are drawn so well that the genera may be easily identified in a number of cases. Ten tribes are recognized by the author, and some new genera have been proposed. Aralidium has been excluded; it lacks secretory ducts and might be placed more naturally as a member of Cornaceae, near Torricellia, Melanophylla, and Kaliphora.—THEO. HOLM.

Embryology of Sechium.—LONGO[25] has found that in the maturing seed of Sechium (Cucurbitaceae) the outer integument enlarges, consisting of homogeneous parenchyma traversed by vascular strands and rich in starch. No "testa" development occurs, but the ovule is closely invested by the pericarp. The outer integument is thus a food-storage region for the embryo, which uses up the endosperm, all of the nucellar tissue, and the inner integument. In germination the cotyledons are differentiated into two regions, intraseminal and extraseminal. The stomata of the two regions differ markedly, in the intraseminal region being more circular in outline and with more circular slits than shown by the decidedly elongated outline of guard cells and slits of the extraseminal region. The differentiation is possibly associated with the differentiation into absorbing and photosynthetic regions.—J. M. C.

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[25] LONGO, B., Sul *Sechium edule* Sw. R. Accad. Lincei **16:** 470–472. *figs. 2.* 1907.

Seed dispersal in Polygonum.—REED and SMOOT[26] find that *Polygonum virginianum* has a somewhat unique means of seed dispersal. When an object hits the rigid persistent style, the akene is violently ejected. It is found that a definite separation layer is developed, and that a considerable tension arises in the pedicel through the growth of the pith below the separation layer. This growth is sufficient to cause the compression of the pith above the layer, since the bounding vascular cells are rigid. The release of these cells from this pressure, when the akene is detached by a stroke, causes the shooting of the akenes to a distance of three or four meters.—H. C. COWLES.

Solution tension and lipolysis.—POND[27] has investigated the effect of various toxic salts upon the saponification of ethyl butyrate by a commercial product, holadin. Whereas MATHEWS, MCGUIGAN, and CALDWELL concluded from their results with eggs of Fundulus, diastatic digestion, and proteolysis, respectively, that toxicity was an inverse function of solution tension, POND concludes that in lipolysis this is not true of the salts tested. He points out also various discrepancies in the results of these investigators, emphasizing the want of agreement and the extent of the divergence of observed from calculated values.—C. R. B.

Swiss vegetation.—Few countries are better known phytogeographically than Switzerland. GRISCH[28] has given a detailed account of one of the less known districts. The entire area studied contains only subalpine, alpine, or nival vegetation. Much is made of the influence of snow, and it is pointed out that snow is as detrimental to some plants as it is favorable to others. This was brought out by some interesting experiments that supplemented the field study. A detailed treatment of the various plant formations, and an annotated list of the species make up the body of the paper.—H. C. COWLES.

Heliotropism in a lichen.—NĚMEC has tested experimentally an inference of SACHS from observations in nature that the thallus of *Peltigera canina* reacted to light like the thallus of Marchantia.[29] He finds *Peltigera aphthosa* distinctly heliotropic, without having the rate of this reaction influenced by geotropism, and with a non-reversible inherent dorsiventrality. The young rhizines are negatively heliotropic. The perceptive and active regions have not been determined.—C. R. B.

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[26] REED, H. S., and SMOOT, I., The mechanism of seed-dispersal in *Polygonum virginianum*. Bull. Torr. Bot. Club 33:377–386. 1906.

[27] POND, R. H., Solution tension and toxicity in lipolysis. Am. Jour. Phys. 19: 258–283. 1907.

[28] GRISCH, ANDR., Beiträge zur Kenntniss der pflanzengeographischen Verhältnisse der Bergünerstöcke. Beih. Bot. Centralbl. 22²:255–316. 1907.

[29] NĚMEC, B., Die heliotropische Orientation des Thallus von *Peltigera aphthosa* (L.) Hoffm. Bull. Int. Acad. Sci. Bohême 11:1–5. 1906.

NEWS

DR. PERCY GROOM has been appointed lecturer in botany at the North London Polytechnic.

DR. C. DETTO of Leipzig, known for his treatise on the theory of adaptation and for other ecological studies, is dead.

MR. OAKES AMES, North Easton, Mass., has presented his collection of living orchids to the New York Botanical Garden.

DR. N. L. BRITTON, N. Y. Botanical Garden, has been elected an honorary member of the Philadelphia College of Pharmacy.

DR. A. A. LAWSON (Chicago), recently of Stanford University, has been appointed assistant in botany at Glasgow University, to succeed Mr. GWYNNE-VAUGHN.

HARVARD UNIVERSITY received in June last a bequest of $50,000 to be used in connection with the Gray Herbarium. It is known as the Sarah E. Potter endowment fund.

MR. R. P. GREGORY has been appointed lecturer in botany at Cambridge University, to succeed Mr. A. W. HILL, who has become assistant director of the Royal Garden at Kew.

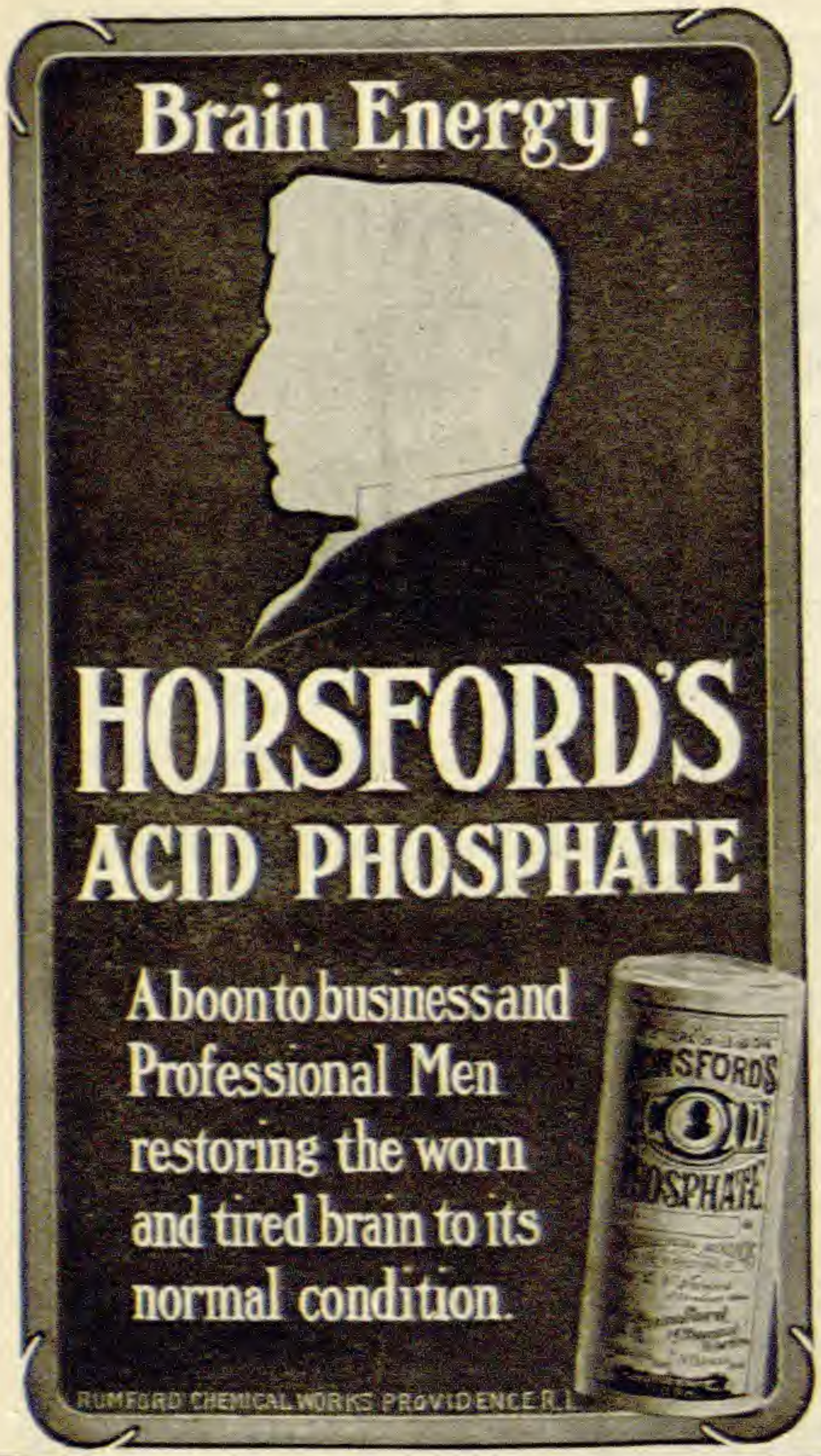
PROFESSOR WILLIAM BATESON, Cambridge University, during his recent visit to the United States, lectured at the Brooklyn Institute of Arts and Sciences, and at The University of Chicago.

DR. HANS FITTING, *privat-docent* at the University of Tübingen, has received for 1907 the stipend of the German government which provides for a visit to the Buitenzorg (Java) botanic garden.

DR. J. REYNOLDS GREEN, lately professor of botany to the Pharmaceutical Society of Great Britain, has been appointed to the Hartley lectureship on plant physiology at The University of Liverpool.

THE DEATH IS ANNOUNCED of Dr. FRANZ KRAŠAN of Graz, Austria. Dr. KRAŠAN is perhaps best known by his writings concerning evolution, which contain records of interesting experiments, as well as somewhat speculative conclusions. In the field of plant geography, he was interested especially in the influence of the earth's heat as a phytogeographic factor, and also in the vegetation of Styria.

A NEW BOTANICAL JOURNAL has been established at Stockhlom, with the title "Svensk Botanisk Tidskrift." It is published by the Swedish Botanical Society, the editorial committee being O. ROSENBERG, K. BOHLIN, G. LAGERHEIM, N. SVEDELIUS, and R. SERNANDER, with ROSENBERG as editor. Four parts will be issued each year, the subscription price being 15 kroner. Two parts for 1907 have appeared, containing about 300 pages.



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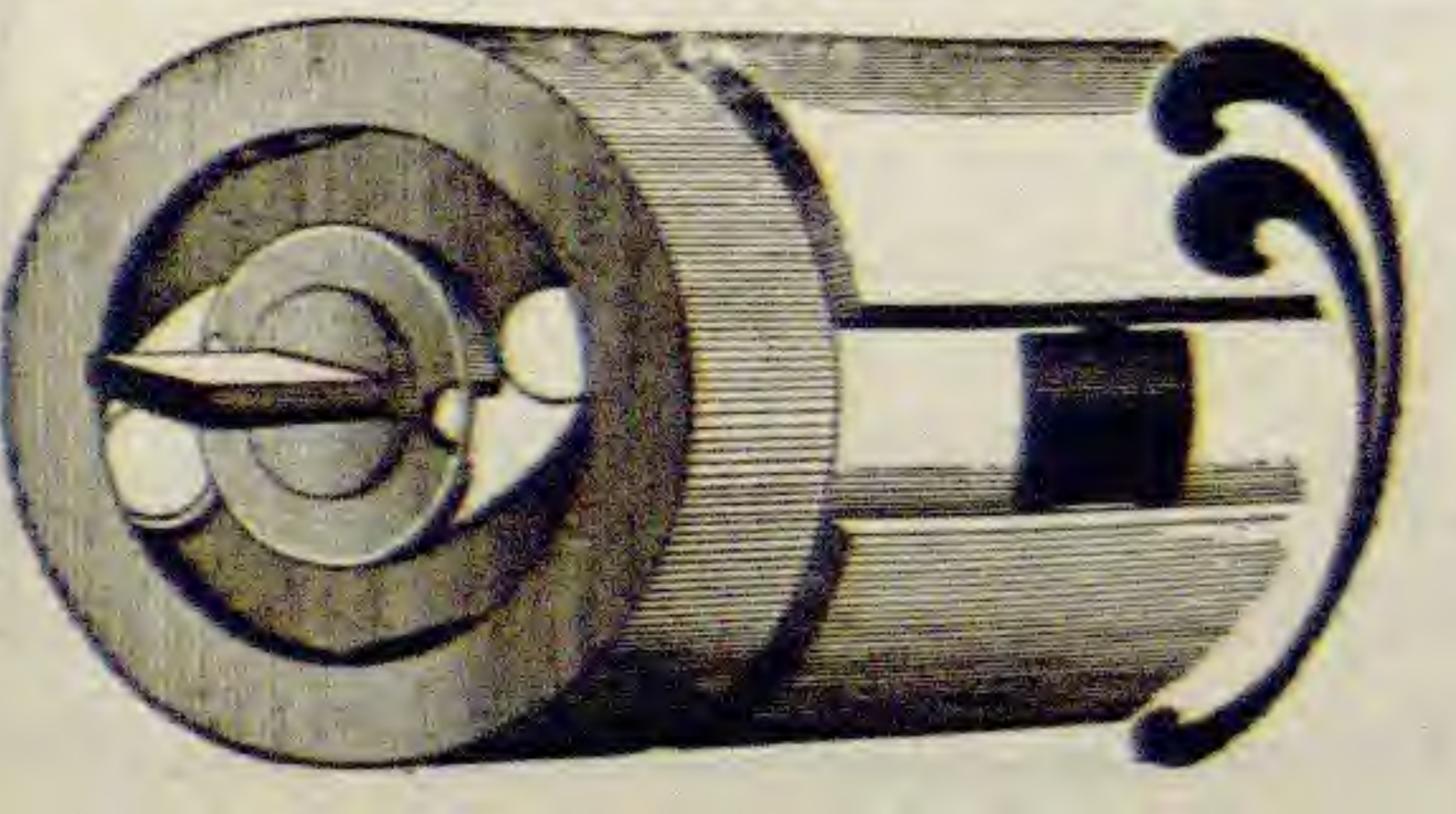

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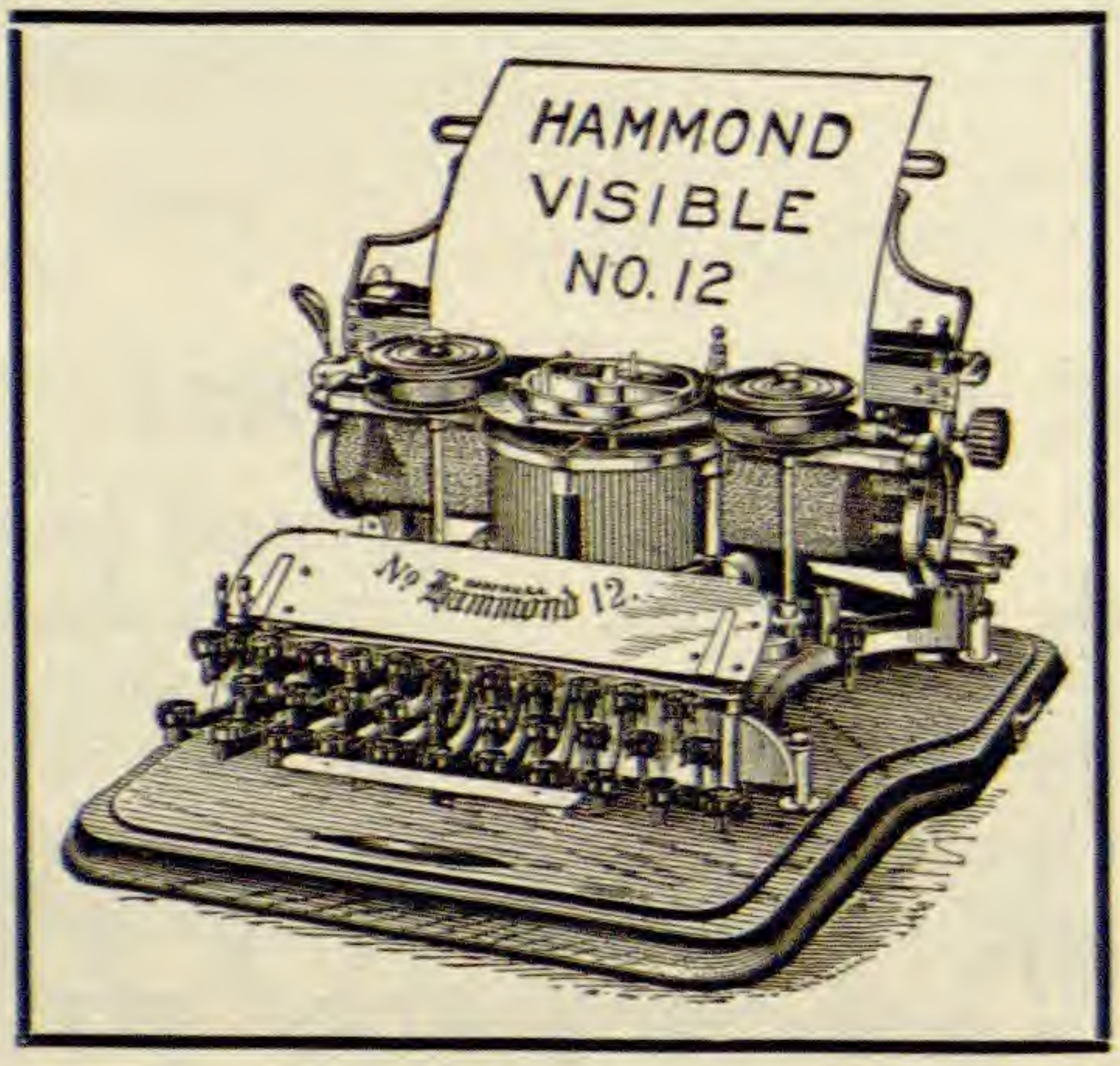

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Vol. XLV No. 2

THE
BOTANICAL GAZETTE

February 1908

Editors: JOHN M. COULTER and CHARLES R. BARNES

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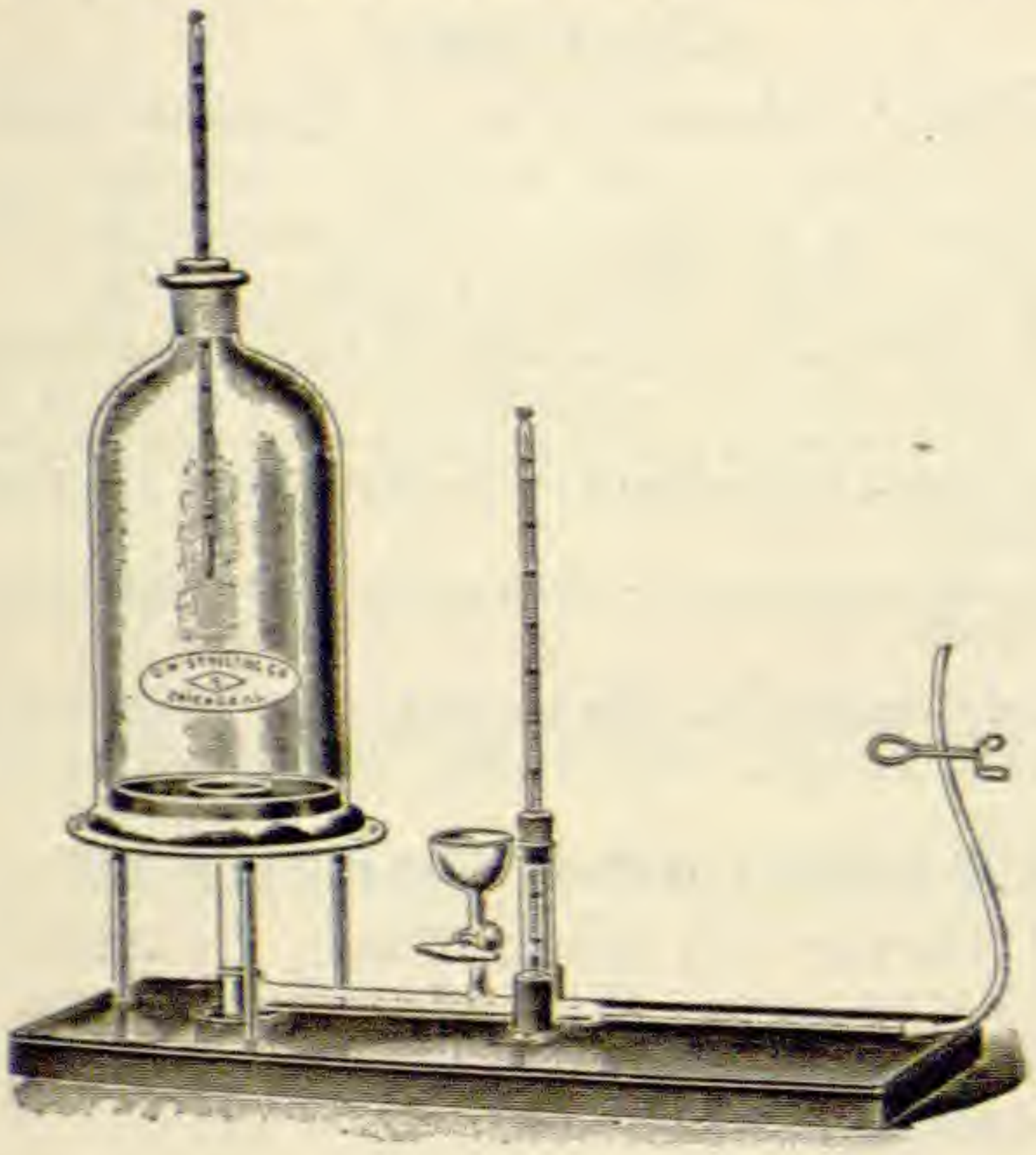

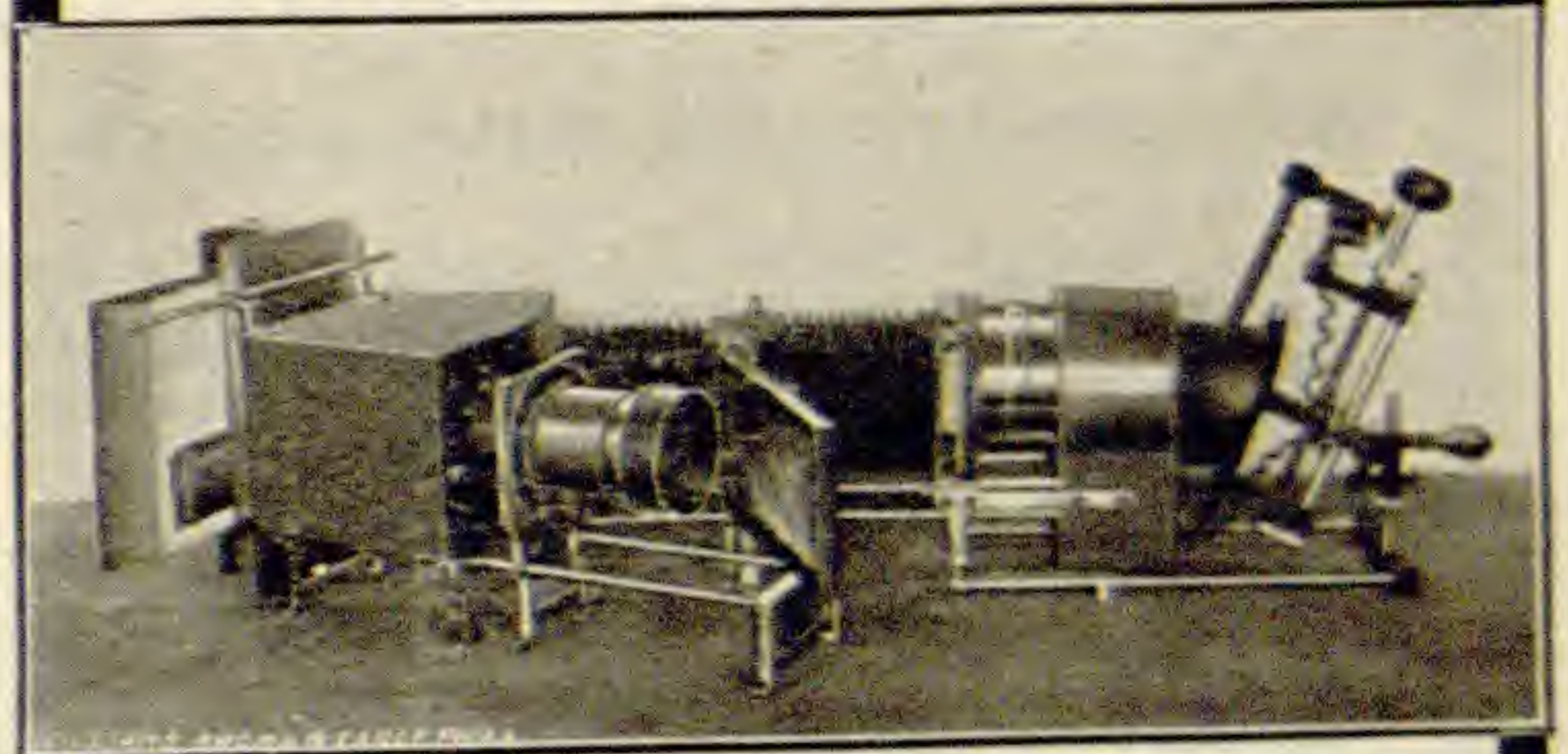

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THE TOXIC ACTION OF CERTAIN ORGANIC PLANT CONSTITUENTS

OSWALD SCHREINER AND HOWARD S. REED[1]

(WITH SEVEN FIGURES)

The present paper is the result of a study of the toxic action of certain compounds which, with few exceptions, occur naturally in vegetable tissues. The laboratories of the Bureau of Soils have been engaged for several years in studying the toxic properties of aqueous extracts of unproductive soils. As a result of these studies it has been found, as was shown in *Bulletins 28* and *36*, that many soils are unproductive because of the presence in them of toxic organic compounds. It has also been found that toxic excretions are produced by growing roots (cf. *Bulletin 40*) and that these excretions, if allowed to remain in the soils, may exert a harmful effect upon a second crop of the same or closely related species. Since these excretions are to be regarded, in the light of our present knowledge, as products of plant metabolism which escape from the tissues of the plant, it becomes of some interest to study the action of compounds which are known to exist as such in the tissues of plants and might persist in the soil or arise from related bodies.

Many instructive studies of toxic action of various inorganic and organic compounds upon plants have been made. Those investigators who studied the action of organic compounds have employed, quite naturally, those which are more or less toxic to animal life and to bacteria. As a result, we have much information upon the action of organic acids, phenols, aldehydes, terpenes, cyanogen, and volatile oils. The present study is intended to add some data upon the action

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of compounds which occur naturally in plants. A few compounds which have not been found to occur as such in vegetable tissues are included in the study, for reasons which will be evident to the reader.

Material and methods

The organic compounds were dissolved in water and the solutions employed as water cultures for wheat seedlings. Most of the compounds used were obtained from Merck & Co. and were used without further purification. The water used in making up solutions was shaken with washed carbon-black, allowed to stand thirty minutes, and then filtered clear. This treatment, as described by LIVINGSTON (:06), gives physiologically pure water. Continued use of the method proves that it is as efficient in removing the toxic properties of ordinary distilled water as careful redistillation in the presence of strong oxidizing or reducing agents.

The solutions when made were placed in salt mouth glass bottles having a capacity of 250^{cc}, each of which accommodated ten seedling wheat plants. The solutions were made up in the ratio of a certain number of parts per million by weight. Since electrolytic dissociation plays a very subordinate rôle in determining the toxic properties of most organic compounds, the concentration of the solutions was expressed in terms of the mass of the respective substance present. Whenever possible the highest concentration employed was 1000 parts per million, for the sake of brevity designated as "1000 ppm." A number of substances used were soluble only in amounts less than 1000 ppm.; in these cases the range of concentration employed was much smaller, and is noted in the last column of Table II.

The wheat was germinated and water cultures conducted according to the method described by LIVINGSTON (:06) and in *Bulletins 23* and *40* of the Bureau of Soils. The seedlings were put into the water cultures at the stage when the first true leaf was beginning to emerge from its sheath. Twenty plants in two cultures of ten each were used in testing each concentration, and comparison was made with an equal number of plants growing in pure distilled water under like conditions. During the seasons of the year in which the conditions for growth were the best, each experiment was continued for eight to ten days, but during the cloudy winter weather some of the experiments were continued for twelve to fourteen days; the exact length of time for each experiment is given in the third column of Table II.

Two and sometimes three criteria of growth were employed, viz., transpiration, green weight, and turgidity, no one of which can be regarded as absolute; but they usually agree in indicating the order of results, which was the point sought in the experiments. The jars containing the water cultures were closed with cork stoppers notched and carefully fitted to receive the seedlings, so that there was practically no chance for loss of water except that transpired by the seedlings. Therefore, the loss in weight of the cultures represents very closely the amount of water transpired by the plants, and in the case of wheat the transpiration has been shown by LIVINGSTON (:05) and JENSEN (:07) to be a very accurate measure of growth when comparisons are made with control cultures. Transpiration is more nearly proportional to the growth of both roots and tops, while the green weight is not at all an indication of root growth and is liable, moreover, to some error in indicating the actual growth of tops. In computing the effects of the toxic agents employed, the transpiration figures are believed to be a more reliable indication of the growth of wheat, so far as the entire plant is concerned, than the weight of the green tops. Many of the substances employed produced the greatest effect upon the roots of the seedlings, and it was found that the transpiration record was a better indicator of the growth in such cases than the green weight of the tops.

The weight of the tops or of the entire plants in the fresh condition was taken as a second means of measuring their growth and as a means of comparison with the control plants.

The condition of the root tips was also noted as an aid in determining the lethal concentration. When killed, the root tips lose their turgidity and soon become slimy. Before this ensues they generally show indications of injury by discoloration in the region occupied by the most actively growing cells or meristem. In some solutions the tips stop elongating and become swollen, with more or less simultaneous discoloration.

The photographs which are appended show in a general way the growth of the plants in comparison with the controls.

The investigations here reported were intended to reveal the character of the toxic action of the several compounds rather than to establish the exact limits for their toxicity. Strict mathematical accuracy is not claimed for the data, but it is believed that they indicate correctly the order of the results, and that, within the experimental error, they approach the toxic limits for each compound. A general summary of the results is presented in Table II and the details are described in the following pages. This table shows the lowest concentration of the compounds which caused the death of the wheat plants, provided they were killed by a concentration of 1000 ppm. or less; the lowest concentration causing injury; and the concentration causing stimulation, where such a phenomenon was observed.

It would be manifestly improper to make strict comparisons between these results and those obtained by other investigators who determined the concentration necessary to kill or prevent growth in twenty-four hours or less. Obviously, a concentration which is sufficient to check growth in a short interval may cause death in a period of several days.

Experimental results

THE ACTION OF SOME SIMPLE AND SUBSTITUTED AMINO-ACIDS

Recent researches upon the chemistry of proteids have brought the amino-acids into prominence physiologically. These bodies are found in relatively large amounts in plants, especially in those of the legume and mustard families, not only in the seedling stage of the plants but also in the roots, stems, and storage organs of mature plants. According to DOJARENKO (**:02**) the amino-acid nitrogen forms a very considerable portion of the nitrogen of humus bodies (22 to 70 per cent.).

The action of amino-acids upon plants has been studied by a number of investigators. Asparagin (amino-succinic acid) has received the most attention because of its importance as a nutrient for heterotrophic plants. MAYER (**'69**) demonstrated the value of asparagin as a nutrient for fungi. LAURENT (**'90**) studied the effect of glycocoll, leucin, aspartic acid, and asparagin upon yeast. He found that a

1 per cent. solution of glycocoll was harmful to yeast and that 1 per cent. solutions of leucin and aspartic acid were assimilated when carbohydrates were present, but asparagin was assimilated even in the absence of carbohydrate. As classed by LOEW ('99), alanin, leucin, asparagin, and glycocoll form good sources of carbon for bacteria—a classification which is sustained by the result of numerous investigations upon the nutrition of bacteria. NAKAMURA ('96) also showed that asparagin was a favorable nutrient for barley seedlings. KLEBS ('96) found that 1 per cent. solutions of asparagin and glycocoll inhibited zoospore formation in *Conferva minor*.

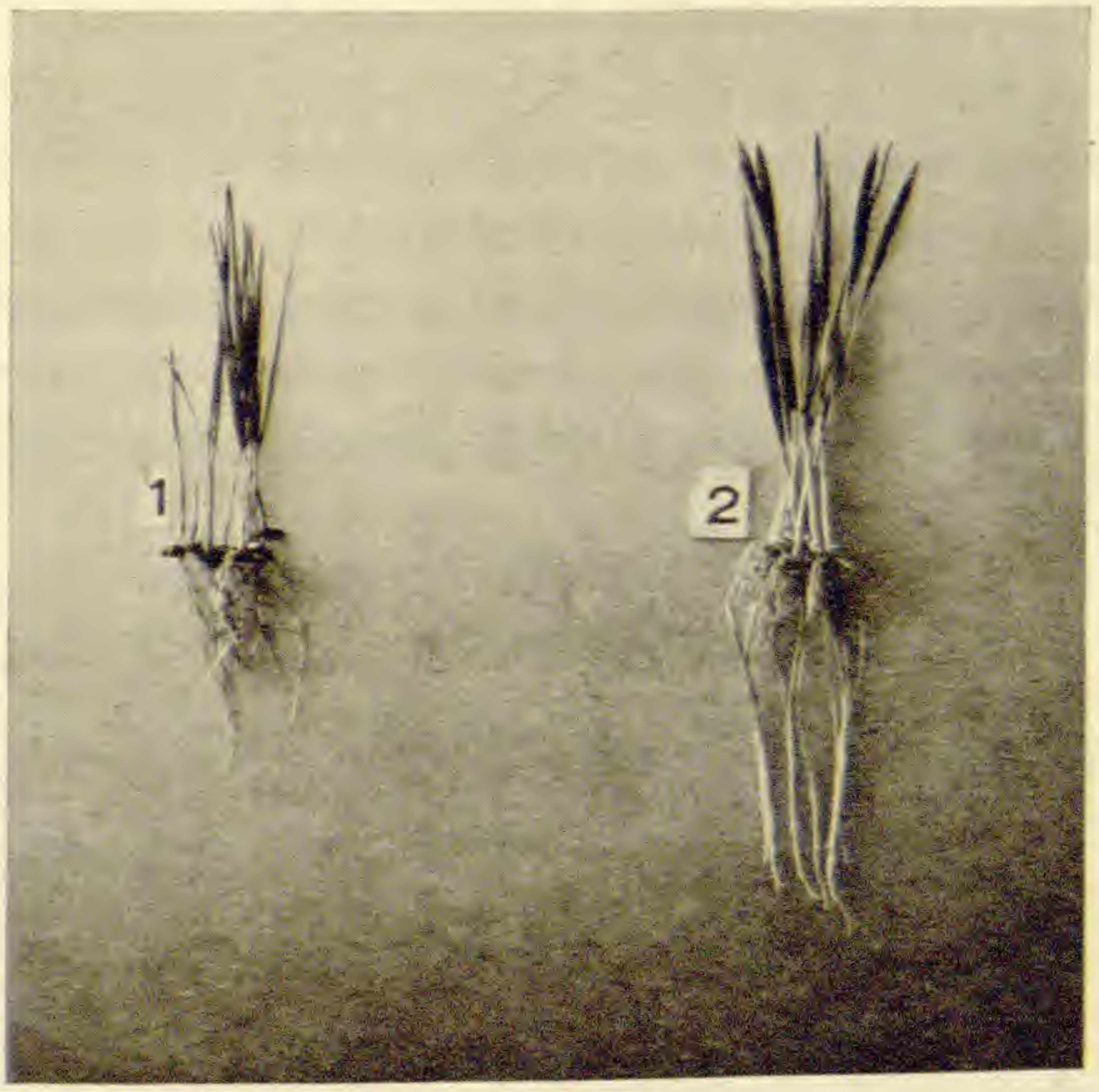


FIG. 1

Our experiments showed that leucin and asparagin are not at all toxic to wheat seedlings. Alanin and glycocoll were slightly injurious at the higher concentrations. Aspartic acid was rather toxic, probably due to its acidic qualities. Tyrosin was more toxic than any of the other amino-acid compounds tried (*fig. 1*). At the expiration of eleven days plants in a solution of tyrosin containing 16 ppm. showed marked injury. At a concentration of 100 ppm. it killed the roots and injured the tops of the plants. At a concentration of. 1 ppm. there was, in some cases, a slight stimulation causing an increase in growth over the controls. The physiological action of different samples of tyrosin was found to vary somewhat.

Upon long standing with exposure to air the tyrosin solutions appeared to have undergone oxidation, all of them having become quite dark in color. The changes wrought were probably the same as those produced by the enzyme tyrosinase, which oxidizes tyrosin to homogentisinic acid, which in turn gives rise to the dark-colored compounds.

An instructive result was obtained by studying the action of such a tyrosin solution which had suffered discoloration incident to age. The tyrosin solution had become so dark in color that it resembled manure extract. It was diluted so that it was equivalent to a 16 ppm. solution of fresh tyrosin. This solution was used for growing wheat seedlings in comparison with a freshly prepared solution of tyrosin of equal strength.

In a few days the plants in the two solutions showed pronounced differences, which became greater as the experiment was continued. *Fig. 1* shows the appearance of the plants when the experiment was discontinued at the end of the twelfth day. The leaves were narrow and beginning to die at the tips; the roots were dead and had become slimy. The plants in the discolored tyrosin had made excellent growth; they resembled plants grown in manure extracts. The leaves were rich green, broad, and making vigorous growth, and the roots showed a similar good development. During the course of the experiment the ten plants in the fresh tyrosin had transpired 19 grams of water, and attained a green weight of 580 milligrams. The plants in the discolored tyrosin had in the same time transpired 38 grams of water and attained a green weight of 980 milligrams.

These results are believed to have importance in explaining the value and action of the so-called green manures used in agricultural practice. The extensive work of E. SCHULZE and others has shown that tyrosin and related compounds are quite widely distributed in the vegetable kingdom. These substances may even increase in number and amount when vegetable matter is allowed to decompose in the soil. Our experiments indicate that bodies like tyrosin and cholin may have a toxic action upon plants, but that these substances in a more highly oxidized stage are actually beneficial to plant growth.

It is of great interest to note in this connection that green manures may have the same action. When first applied to the soil they may exert a depressing effect upon the growth of the crop. After enough time has elapsed for the decomposition of the vegetable matter, with change to other compounds, the crop shows a marked benefit from the action of the green manure.

THE ACTION OF COMPOUNDS RELATED TO LECITHINS

The lecithins are readily decomposed and split off cholin and complex glycerophosphoric acids, which upon further decomposition yield fatty acids, glycerin, and phosphoric acid. It is such a decomposition that takes place in germinating seeds and also in seedlings in the absence of light. ZALEWSKI (**:06**) believes that this decomposition of the lecithins is due to enzyme action. He has shown that the organic phosphorus compounds are quickly and almost completely decomposed during germination, only 2 per cent. remaining unattacked. SCHULZE (**'91**) and other investigators have reported the occurrence of cholin in various plant tissues associated with lecithin, and it does not follow that it must always be regarded as a product of the decomposition of lecithin.

Neurin may arise from lecithin by the life-processes of certain microorganisms, as SCHMIDT and WEISS (**'91**) have shown. It may also be formed from cholin by the action of bacteria or by boiling with baryta water. Betain or oxyneurin may be prepared artificially by the oxidation of cholin. LOEW (**'99**) reports that betain is a favorable and cholin is a moderately good source of carbon for bacteria.

In our experiments with wheat seedlings, neurin was found to be very toxic, cholin moderately toxic, while betain was entirely harmless. Wheat plants were killed by solutions containing 250 ppm. of neurin and injured by solutions containing 25 ppm. It was thought that at least a part of the injury might be due to the strong basic properties of neurin. The experiment was accordingly repeated, using neurin which had been neutralized with acetic acid. The result, however, was not different from the experiment in which the neurin was not neutralized. It may be possible that a part of the difference in toxicity may be due to the fact that neurin contains the vinyl group. LOEW ('93) has pointed out that vinylamin ($H_2C=CH{\cdot}NH_2$) is more toxic to animals than ethylamin ($H_3C{\cdot}CH_2NH_2$). Substances containing the vinyl group possess the power of forming addition compounds.

Solutions of cholin containing 1000 ppm. did not cause the death of wheat seedlings. Injury was caused, however, by solutions containing 1000 and 500 ppm. As shown by *fig. 2*, the injurious effect was most marked upon the roots of the wheat plants. The weakest concentration (1 ppm.) caused a slight increase in growth, which was probably due to stimulation.

FIG. 2

In contrast to the toxic action of neurin and cholin, the more highly oxygenated betain was not at all toxic to wheat seedlings, but was even slightly beneficial. The concentration of the different solutions used ranged from 1000 to 5 ppm. and in all of them the plants grew as well or better than the controls in distilled water.

THE ACTION OF SOME UREA DERIVATIVES

Four compounds which are structurally related to urea were studied with regard to their action upon plants. Alloxan and guanidin are sufficiently soluble in water to permit of being used in a concentration of 100 ppm. Guanidin was used as guanidin carbonate. Guanin and xanthin are so slightly soluble in water that the highest concentrations used were only 40 and 25 ppm. respectively.

Guanidin has been shown previously to be toxic to diatoms and filamentous algae by LOEW (**'93**); toxic to barley seedlings by KAWAKITA (**:04**), who also found that some fungi were unable to utilize guanidin as a source of carbon, although they could utilize it as a source of nitrogen. In our experiments with wheat seedlings a concentration of 100 ppm. of guanidin carbonate was sufficient to kill, and all the lower concentrations employed, including 1 ppm., caused serious injury. Alloxan, the ureid of mesoxalic acid, was stated by LOEW (**'99**) to be an unfavorable source of carbon for bacteria. It is quite toxic to wheat, but less toxic than guanidin. Seedlings were killed by solutions containing 1000 ppm., and injured by lower concentrations down to 100 ppm. Guanin was tested only in concentrations of 40, 20, 10, 5, and 1 ppm. on account of its slight solubility. In all these concentrations there was no harmful effect to be noticed. Xanthin is also so slightly soluble in water that the range of concentrations employed was small. In concentrations of 25, 10, 5, and 1 ppm. it was slightly beneficial to the growth of wheat plants.

THE ACTION OF COMPOUNDS CONTAINING THE PYRIDIN NUCLEUS

Pyridin forms the nucleus upon which are built many of the alkaloids. It is obtained chiefly from coal tar, but may also be obtained from a number of alkaloids (trigonellin, spartein, cinchonin) when these are highly heated, treated with alkalies, or distilled with zinc dust.

It has been observed by FALKENBURG (**'91**) that the vapor of pyridin and some of its homologues is poisonous to bacteria. The action of pyridin and its homologues has received additional importance as a result of the investigations of SHOREY (**:06**), who obtained pyridin by the dry distillation of soil. The author cited says (p. 37): "As the fat in this soil was found to be only 0.005 per cent. it does not seem likely that the pyridin formed was due to the formation of the pyridin ring by condensation, but rather that it exists already in some form in the soil." Pyridin was also obtained from the same soil after extraction with ether, hence it seems that the view taken is the correct one.

In the experiments with wheat seedlings it was found that pyridin, although not sufficiently toxic at a concentration of 1000 ppm. to kill wheat plants in nine days, was nevertheless very injurious, especially to the growth of the green parts of the plants. In a concentration as low as 50 ppm. the growth of the tops was inhibited and the leaf-tips turned brown. In the lower concentrations there was no stimulation of growth.

Picolin ($C_5H_4N{\cdot}CH_3$), or methyl pyridin, was toxic to wheat plants but acted fatally only in the concentration of 1000 ppm., and caused injury at 500 ppm., but not in lower concentrations. The injury seemed to be manifested by the tops more than by the roots, thus resembling the action of pyridin.

Piperidin ($C_5H_{11}N$), or hexahydropyridin, differs in chemical constitution from pyridin by having two atoms of hydrogen attached to each carbon and one atom of hydrogen attached to the nitrogen atom. Solutions of piperidin killed and injured at a lower concentration than either pyridin or picolin. A sample of piperidin, which was neutralized with acetic acid, proved to be more toxic than the strongly alkaline piperidin itself. Piperidin seemed to injure the roots more severely than the tops. By reference to Table I, it will be seen that piperidin is more toxic than pyridin, a fact which may be due to the additional hydrogen it contains.

Chinolin (C_9H_7N) forms the nucleus of many alkaloids found in plants belonging to the families Rubiaceae and Loganiaceae. FALKENBURG ('91) found that chinolin was toxic to bacteria in a concentration of 2000 ppm. Reference to Table II shows that chinolin was sufficiently toxic to kill wheat plants at a concentration of 500 ppm. So low a concentration as 5 ppm. affected them injuriously in six days, but none of the lower concentrations caused stimulation of growth.

PHENOLS

Only a part of the phenols used occur naturally in plants, but in order to make the study more complete several phenols were studied and the results herewith reported.

Monatomic phenols.—Phenol (carbolic acid) is well known on account of its toxic properties, and an enumeration of the literature dealing with its toxicity would be superfluous at this place. Suffice it to say that TRUE and HUNKEL (**'98**) found that a concentration of *m*/400 phenol prevented the growth of lupine roots. This concentration would be about 235 ppm. and stands quite near the concentration (250 ppm.) which was required to bring about the death of wheat plants subjected to it for eight days.

The wheat plants made a small amount of growth before death in solutions containing 500 and 250 ppm. In solutions containing 100 and 50 ppm. the tops were alive at the end of eight days, but the roots were dead. The plants in solutions of 5 ppm. were slightly poorer than the control plants, but those in 1 ppm. were fully as good as the control plants.

Diatomic phenols.—Pyrocatechin (ortho-dioxybenzene) has been reported in a number of different plants. According to WEEVERS (**:03**) it occurs in several species of willows and the characteristic black color which the leaves of certain species of willows assume upon drying is probably the result of the oxidation of the pyrocatechin through the activity of an oxidase. YABE (**'94**) found that pyrocatechin was fatal to yeast cells and bacteria when used in concentrations of 0.4 to 0.58 per cent. TRUE and HUNKEL (**'98**) found that lupin roots were prevented from growing by a *m*/800 solution of pyrocatechin, i. e., about 137 ppm.

Wheat plants were killed by pyrocatechin in solutions of 500 ppm. and injured by solutions containing as low as 25 ppm. The stronger solutions became dark colored after standing a few days and were undoubtedly somewhat more toxic on that account, as was pointed out by TRUE and HUNKEL (**'98**). At the conclusion of the experiment the roots of the plants were colored purplish brown by the stronger concentrations. The effects of different concentrations of pyrocatechin are represented in *fig. 3*.

Resorcin (meta-dioxybenzene), which appears to be more stable in solution than pyrocatechin, is less toxic than that compound. TRUE and HUNKEL (**'98**) found that the growth of lupin roots was prevented by *m*/200 solution (about 550 ppm.), thus exhibiting less toxicity than pyrocatechin. By our method of experimentation we found that the lowest concentration causing death was 1000 ppm. In concentrations of 500 and 250 ppm. the roots of the plants were entirely dead, although the tops were still alive at the end of the experiment. Concentrations of 100 ppm. stopped the growth of most of the plant roots and adventitious roots from the lower part of the stem ceased to grow as soon as they dipped into the solution. Fifty ppm. was the lowest concentration which appeared to cause injury to the plants.

Hydrochinone (para-dioxybenzene) undergoes oxidation when its solution is exposed for some time to the air. TRUE and HUNKEL ('98) found that for lupine roots hydrochinone was more toxic than pyrocatechin or resorcin. With wheat plants the order of results differed somewhat, hydrochinone and pyrocatechin appearing to possess toxicity of about equal intensity. In our experiments a concentration of 500 ppm. of hydrochinone was fatal, 25 ppm. caused injury, and 5 ppm. caused stimulation.

FIG. 3

Triatomic phenols.—Phloroglucin (1:3:5 trioxybenzene) is not found as such in plants, but may be derived from several aromatic plant constituents. It was found to suffer some oxidation when plant roots were allowed to grow in its solutions, the stronger solutions showing a distinct yellow color after a few days. The cultures of wheat seedlings indicated that phloroglucin possesses about the same relative toxicity as pyrocatechin and hydrochinone. TRUE and HUNKEL (**'98**) reported that for lupines phloroglucin was somewhat less toxic than either of the two dioxybenzenes mentioned.

In order to study the relative toxicity of phenols containing 1, 2, and 3 hydroxyl groups, a series of cultures was set up in which solutions of phenol, resorcin, and phloroglucin were employed. It seemed best to use resorcin and phloroglucin for two reasons. First, they appear to be more stable in solution; and second, because of the similarity in the arrangement of the hydroxyl groups; resorcin having the 1:3, and phloroglucin the 1:3:5 arrangement. The cultures were grown under the same conditions for eight days and records of transpiration and green weight taken, although the transpiration is regarded as the more reliable in this experiment. Table I shows the records for the growth of the plants in the different solutions, and *fig. 4* shows the plants grown in two series of parallel concentration.

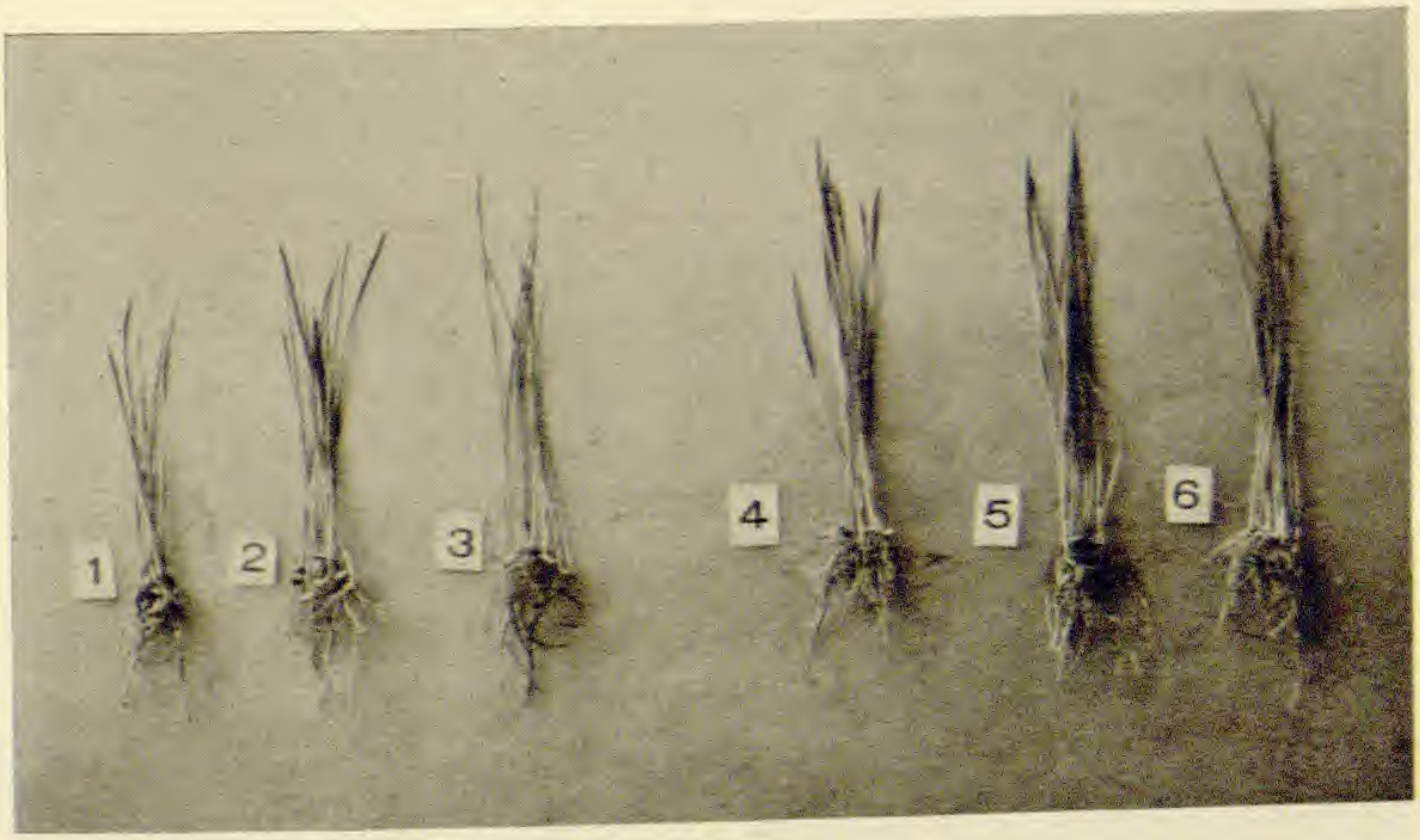


FIG. 4

It would appear from these figures that the toxicity of phenols is not directly dependent upon the number of hydroxyl groups they contain. Resorcin appears to be the least toxic of the three compounds and phenol the most toxic. TRUE and HUNKEL found that

TABLE I

Growth of wheat plants in solutions of phenol, resorcin, and phloroglucin

Solution	Transpiration grams	Green weight grams
Phenol 500 ppm.	dead	....
Phenol 250 ppm.	dead	....
Phenol 100 ppm.	20.6	0.93
Phenol 50 ppm.	27.8	0.64
Resorcin 500 ppm.	19.9	0.53
Resorcin 250 ppm.	23.8	0.76
Resorcin 100 ppm.	26.4	0.84
Resorcin 50 ppm.	41.0	0.98
Phloroglucin 500 ppm.	15.0	0.54
Phloroglucin 250 ppm.	16.7	0.69
Phloroglucin 100 ppm.	22.9	0.98
Phloroglucin 50 ppm.	31.0	0.80

the toxic limit of phenol was not altered by the addition of sodium hydroxid, hence the greater toxicity of this compound is probably not due to its acid properties, but is to be attributed to the properties of the phenyl group. The introduction of additional hydroxyl groups into phenol appears to alter its toxicity variously, so far as it is possible to judge of the toxicity of the different unstable compounds. Pyrogallol was not studied in this connection, because it becomes converted upon standing to purpurogallein, which is more toxic, according to TRUE and HUNKEL, than pyrogallol.

There seems to be no evidence, therefore, from our experiments that the toxicity of phenols to wheat is diminished as the number of hydroxyl groups is increased.

DERIVATIVES OF SOME OXYBENZENES

Arbutin, which may be regarded as a glucoside of hydrochinone, is quite widely distributed among plants, especially among members of the Ericaceae.

LAURENT ('90) found that arbutin seemed to be assimilated in small quantities by yeast. KLEBS ('96) found that a 0.5 per cent. solution inhibited zoospore formation in *Conferva minor*, although it did not appear directly to cause the death of alga cells in the first day.

In studying the action of arbutin upon wheat seedlings, concentrations of 1000, 500, 100, 25, and 1 ppm. were employed. At the end of three days it was noticed that the wheat roots growing in the stronger solutions had oxidized part of the arbutin to an insoluble, light-yellow dye which was deposited upon the roots. When the experiment was discontinued at the end of twelve days, the plants in arbutin solutions containing 1000 and 500 ppm. were dead. The roots of these plants were discolored at the tips and had become slimy. The plants in solutions containing 100 and 25 ppm. were alive, but had made much less growth than the controls in distilled water. The plants in the solutions containing 1 ppm. of arbutin had been stimulated to make slightly better growth than the control plants.

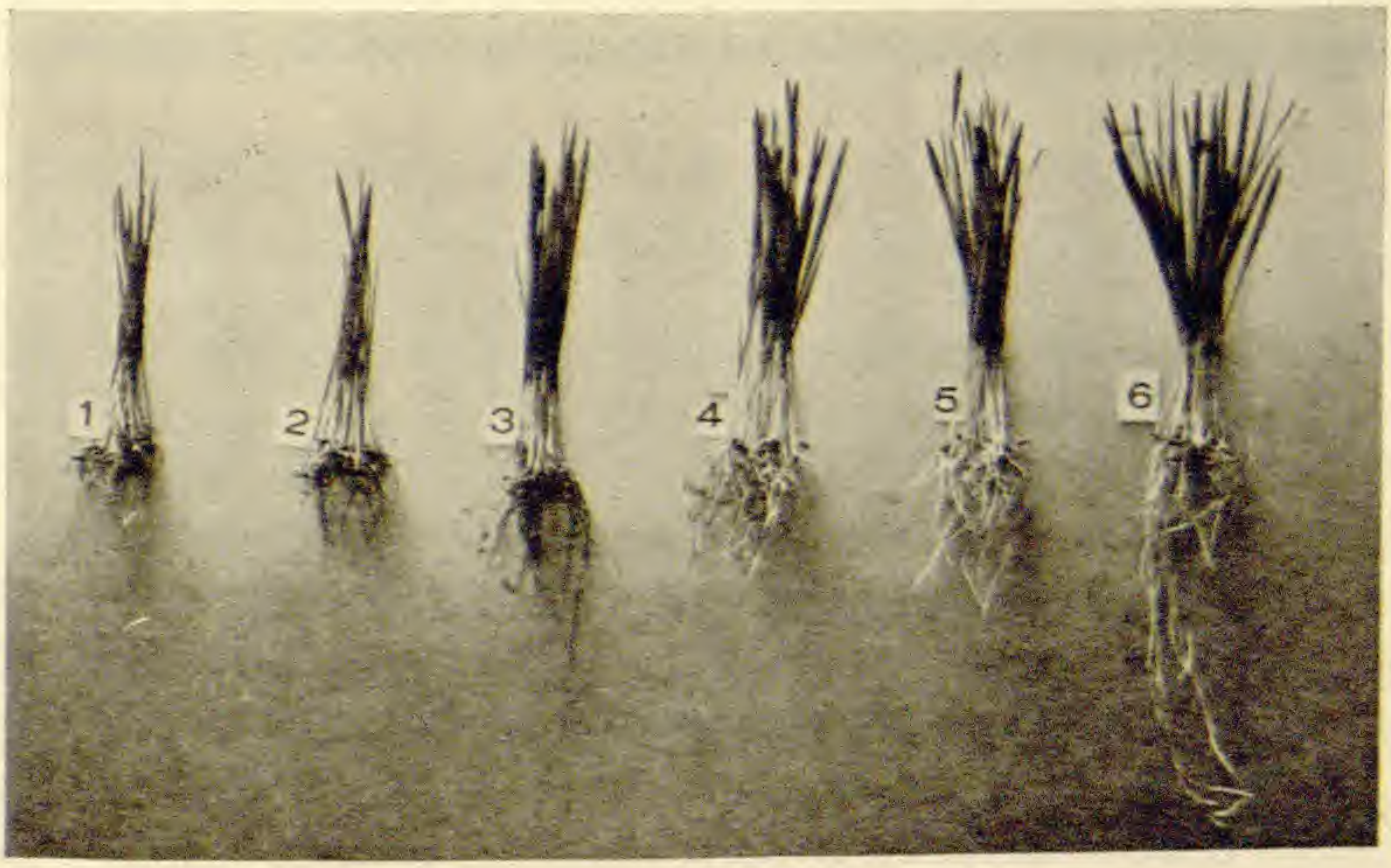


FIG. 5

Vanillin, the aromatic principle of the vanilla bean, is a methoxy-ether of protocatechuic aldehyde. It probably does not occur as such in plants, but exists in the form of a glucoside which breaks into vanillin and a sugar when the plant organs are dried.

KLEBS (**'96**) found that saturated and half-saturated solutions of vanillin were fatal to *Conferva minor.* Our experiments showed that wheat seedlings were killed in nine days by solutions containing 1000 and 500 ppm. of vanillin. In solutions containing 100 ppm. of vanillin the tops made fairly good growth, but the roots were killed. In the most dilute solutions, i. e., 10 ppm. and less, there was sometimes a slight stimulation of growth (*fig. 5*).

The roots of the plants grown in the stronger solutions oxidized some of the vanillin to a purplish-brown, insoluble dye, which colored the roots. The general character of the oxidation has been described in a preliminary communication by SCHREINER and REED (:07*c*).

Vanillic acid, a monomethylprotocatechuic acid, showed greater toxicity to wheat plants than vanillin. The plants were killed in seven days in solutions of 100 ppm. or stronger. In solutions containing 25 ppm. of vanillic acid, the only inferiority noticed in the plants was their root development; but the plants grown in concentrations of 5 and 1 ppm. were better than the controls in distilled water. Although vanillic acid is more toxic to wheat plants than vanillin, it appears to be less inhibitory of root development.

Chinone (benzochinone) may be prepared artificially from quinic acid or from hydrochinone. It is a compound of interest in the study of soil problems since BEIJERINCK (:00) has found that a soil fungus, *Streptothrix chromogena*, has the ability to form chinone from proteids.

Chinone has been shown to be toxic to the seedlings of various plants, algae, and fungi by FURUTA (:02). In concentrations of 1000 and 500 ppm. the roots of soy bean and wheat seedlings were injured within a few hours and killed in three or four days. Filaments of algae were killed in a concentration of 1000 ppm. in three or four hours, and fungi in a somewhat longer period.

Chinone is one of the most toxic compounds whose effect upon wheat seedlings was studied. A concentration of 100 ppm. was fatal in nine days, and even 1 ppm. produced an injurious effect. In the intermediate concentrations the chinone was correspondingly injurious. The plants scarcely survived in a concentration of 50 and 25 ppm. and only in concentrations of 10 and 1 ppm. was there any growth comparable to that of the controls in distilled water.

The great toxicity of chinone is probably due to two of its chemical properties. The first of these is its strong oxidizing power, by virtue of which it is probably able to oxidize labile compounds which exist in cells of the plants and render them unsuitable for use in metabolism. The second property which gives chinone a toxic action is its ketone nature. Chinone readily forms bromin addition products. It unites with one molecule of hydroxylamin to form chinonoxime; with two molecules of hydroxylamin it forms chinondioxime.

The ketones, as is well known, are distinctly toxic to plants, and taken together these two properties undoubtedly account for the action of chinone upon plants.

Cinnamic acid occurs in small amounts in the secretions of certain plants and its esters are found in the leaves of various tropical plants. By proper treatment it yields all the different phenol acids.

TRUE (**:00**) found that a concentration of cinnamic acid equivalent to *m*/12,800 (12 ppm.) prevented the growth of the root tips of *Lupinus albus* in 24 hours. He also found that sodium cinnamate was toxic to root tips when employed in a concentration of *m*/800 (184 ppm.).

Using a slightly different method, the results obtained with wheat seedlings varied, naturally, from those obtained with lupine. The same relations, however, hold true in both cases. Cinnamic acid is strongly toxic for seedlings, and its sodium salt is moderately toxic.

A concentration of 100 ppm. of cinnamic acid was fatal to wheat plants and 25 ppm. was injurious. Cinnamic acid is particularly toxic to root growth, and it was only in the concentrations below 25 ppm. that anything like normal root growth was exhibited. When a wheat root is killed by cinnamic acid it is not swollen nor discolored in the usual fashion, but on the contrary appears perfectly white.

Sodium cinnamate is much less toxic than cinnamic acid. Wheat plants were not killed by a concentration of 1000 ppm. and were not injured at concentrations weaker than 100 ppm.

Cumarin is found in a number of different plants and gives the odor which is characteristic of Hierochloa and Melilotus. It is a lactone of cumaric acid which may be regarded as orthoxycinnamic acid. It is also of interest to note that GOSIO (**:06**) has found that cumarin may be formed from carbohydrates by the action of certain mold fungi, e. g., *Aspergillus glaucus*, *A. novus*, *A. flavescens*, and *A. varians*.

Orthocumaric acid and *o*-hydrocumaric acid (melilotic acid) are found in Melilotus (sweet clover) in addition to cumarin. Parahydrocumaric acid, although it has not been reported in plants, is of interest on account of its relation to tyrosin. Through the action of bacteria, the amid group in tyrosin is broken up and ammonia liberated, thus giving rise to *p*-hydrocumaric acid.

KLEBS (**'96**) found that *Conferva minor* was quickly killed in saturated and half-saturated solutions of cumarin. Our results show that cumarin is extremely poisonous to wheat plants. At the end of five days the plants in 250 ppm. and stronger were dying, the roots and tops having made practically no growth. The root tips were swollen and slightly discolored, although the roots themselves were quite turgid. When the experiment was discontinued at the end of eight days the plants in the solutions of 100 ppm. were dead, although they had made a slight growth at the beginning of the experiment. The roots were discolored for a distance of 3 to 6^{mm} from the tip and their surface was very slimy, due to the death of the outer layers of cells which were then beginning to peel off. The leaves of the affected plants were short and broad, a feature not brought out by the illustrations, but which was very characteristic in all experiments with cumarin. The meristematic tissue of the stem appeared to be injured in such a way that only the first leaves were unfolded, and in most cases the sheathing leaf base was more or less swollen by the abnormal growth of the inhibited leaves within it.

FIG. 6

The plants in the solutions of 50 ppm. were alive at the end of eight days, but all growth had ceased. The root tips were discolored and badly swollen. The leaves were dead for a considerable distance back from the tips. In the solution of 1 ppm. the development of tops, as shown by the illustration (*fig. 6*), was practically equal to that of the control plants in distilled water. The root development was not as good, however, the tips being slightly discolored but not swollen.

Daphnetin, which occurs in various species of Daphne, is a lactone, which is to be regarded as (3, 4) dioxycumarin. On account of its slight solubility in water it was impossible to test daphnetin in concentrations greater than 50 ppm. Daphnetin was relatively less toxic than cumarin. At a concentration of 50 ppm. the green weight of tops produced in twelve days was 76 per cent. of the controls. In concentrations of 25 ppm. and less the growth of tops was practically as good as in distilled water, and the development of roots was only slightly inferior.

Esculin is of particular interest on account of its relation to cumarin. It is the glucoside of esculetin, which is a lactone isomeric with daphnetin, and has been shown by TIEMANN and WILL ('82) to be (4, 5) dioxycumarin.

Both esculin and esculetin have been found in plants, but little is known concerning their distribution. The esculin solutions had a blue fluorescence when prepared, which was lost after plants had grown for a time in them. The roots which grew in the stronger solutions were colored dark yellow as a result of their oxidative activities, the dye formed being insoluble and remaining upon the surfaces of the roots where oxidation had occurred. This effect is shown in *fig. 7*. Esculin, as shown by the figures, is decidedly less toxic to plants than cumarin, but more toxic than daphnetin. KLEBS ('96) found that a saturated solution of esculin inhibited the formation of zoospores in *Conferva minor*, but did not prevent the formation of chlamydospores in *Mucor racemosus*. The toxic effects of esculin were more marked upon the roots of wheat plants than upon the tops. In concentrations of 1000 and 500 ppm. wheat plants were killed, and were injured even in a solution containing 1 ppm. (*fig. 7*). In the solutions containing 100 ppm. there was a distinct stimulation of the growth of tops.

Heliotropin, or piperonal, is the aldehyde of piperonylic acid. It possesses the pleasant odor of heliotrope. The growth of wheat plants in solutions was markedly affected by the presence of heliotropin. Although the plants were not killed by a concentration of 1000 ppm. or less, they were injured by such slight quantities as 1 ppm. It is worthy of remark that the tops of the wheat plants were more affected by the heliotropin than the roots. The roots were healthy and of nearly equal development in all solutions of heliotropin used.

FIG. 7

MISCELLANEOUS COMPOUNDS

A few of the compounds whose effects were studied do not permit of classification with the majority of compounds used. These bodies are skatol, ricin, mucin, and quinic acid.

In solutions skatol proved to be somewhat toxic to wheat plants. A concentration of 200 ppm. was sufficient to kill seedling plants in nine days, and in the same time a concentration of 50 ppm. was injurious. In those cases where skatol had a harmful effect, the roots of the wheat plants were more injured than the tops.

Ricin is the very poisonous (for animal life) principle of Ricinus;

its chemical composition is unknown, but it is probably a toxalbumen. Bokorny (**'96**) found that ricin is slightly toxic to algae and infusoria. On account of its slight solubility, we were not able to make tests of solutions having a concentration greater than 50 ppm. The wheat plants were somewhat injured by concentrations of 50 and 40 ppm.

Mucin is also comparatively insoluble in water and hence a wide range of concentrations could not be employed. In a saturated solution there was practically no growth of the roots of the wheat plants. In a solution of 100 ppm., which is slightly below the point of saturation, the plants were seriously injured in the growth of both roots and tops. A solution containing 5 ppm. was slightly stimulating to the growth of wheat plants.

Quinic acid, which occurs in cinchona bark associated with the alkaloid quinin, proved to be quite toxic to seedlings. In all except the more dilute solutions, quinic acid was very toxic to wheat plants. At the end of ten days the entire plants in concentrations of 1000 and 500 ppm. were dead; in 100 ppm. only the roots were dead. In concentrations below 50 ppm. the plants were as good as the controls grown in distilled water; and in the solutions containing 5 ppm. there was a slight stimulation of growth.

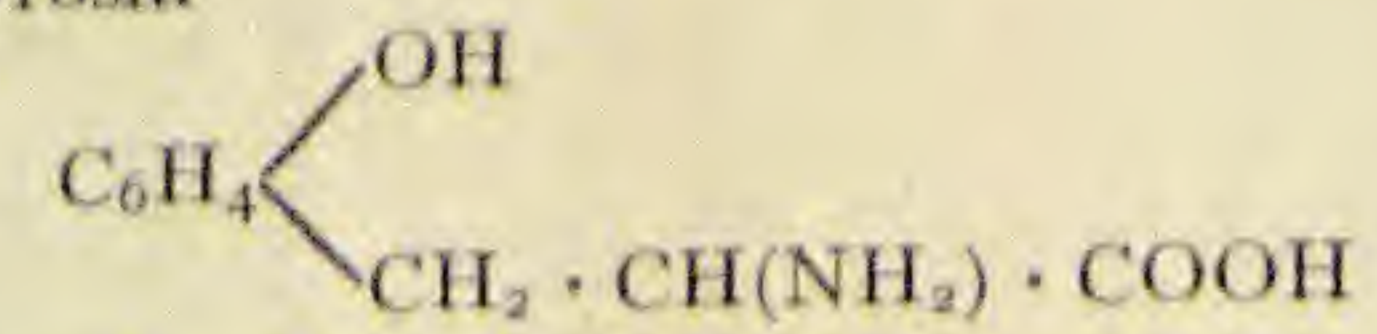
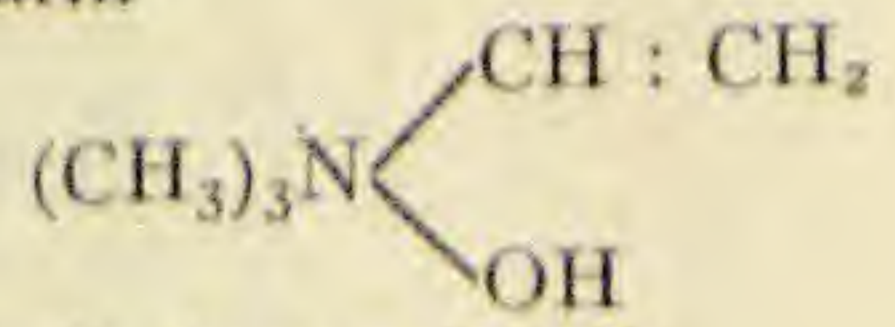
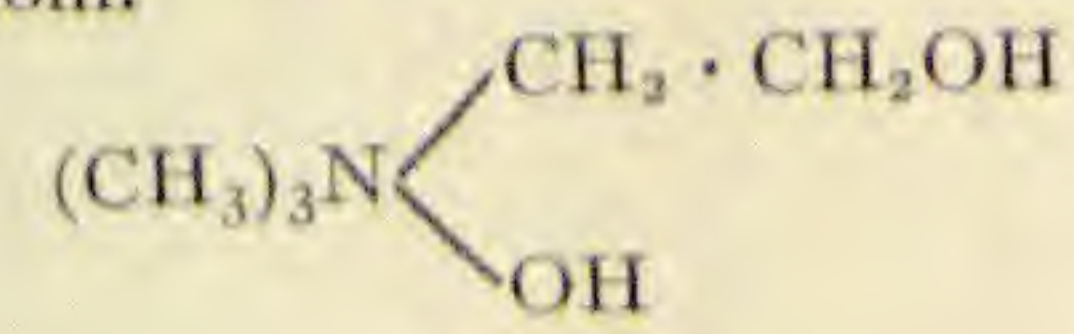
Discussion and summary

The data presented show that a number of the compounds occurring naturally in plants have toxic effects upon wheat seedlings. The toxicity of certain compounds appears to be greater for plants than for animals; in the case of other compounds the reverse relations seem to exist.

The amino-acid compounds display a relatively low toxicity, and some of them appear to serve directly as nutrients to the plants. Two exceptions deserve mention. Tyrosin, which was found to cause injury in concentrations of 25 to 10 ppm., may be regarded as oxyphenylalanin. Reference to the table shows that alanin itself was slightly injurious in the highest concentrations, and it is easy to understand that increased toxicity should follow the introduction of the oxyphenyl group. Aspartic acid is the other amino-compound which displays relatively great toxicity to the plants. Aspartic acid differs chemically from the other amino-compounds employed in

TABLE II

Effect of various organic compounds upon the growth of wheat plants (ppm = parts per million).

No.	Compound	Duration of experiment in days	Lowest concentration causing death	Lowest concentration causing injury	Concentration causing greatest stimulation	Remarks
1	Glycocoll $CH_2(NH_2)COOH$	9	......	......	......	Tops of all plants good. Roots sl'tly inj'd at higher conc'tions.
2	Alanin $CH_3 \cdot CH(NH_2) \cdot COOH$	10	......	500 ppm	25 ppm	Only the roots were injured by concentration of 500 ppm.
3	Leucin $CH_3 \cdot (CH_2)_3 \cdot CH(NH_2) \cdot COOH$	9	......	......	......	No injurious action.
4	Tyrosin  $C_6H_4\left\langle\begin{array}{l}OH\\ CH_2 \cdot CH(NH_2) \cdot COOH\end{array}\right.$	11	......	10 ppm	......	
5	Aspartic acid $HOOC \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	10	500 ppm	100 ppm	......	Normal growth in concentrations below 100 ppm.
6	Asparagin $NH_2OC \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	9	......	......	......	No injury in concentrations below 1000 ppm.
7	Neurin  $(CH_3)_3N\left\langle\begin{array}{l}CH : CH_2\\ OH\end{array}\right.$	9	250 ppm	25 ppm	......	
8	Neurin (neutralized)	8	250 ppm	25 ppm	......	
9	Cholin  $(CH_3)_3N\left\langle\begin{array}{l}CH_2 \cdot CH_2OH\\ OH\end{array}\right.$	10	......	500 ppm	1 ppm	Roots were injured more than the tops.

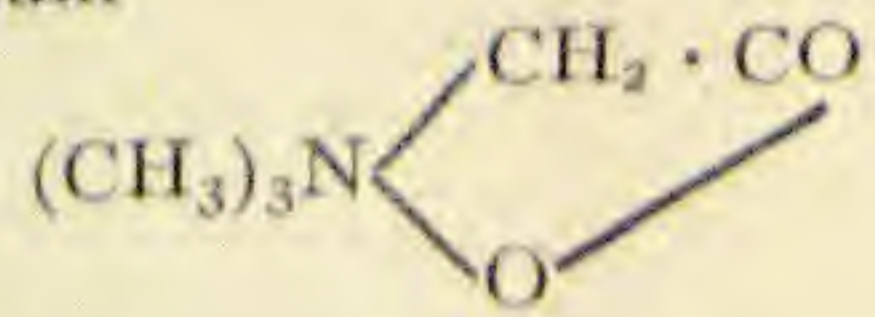
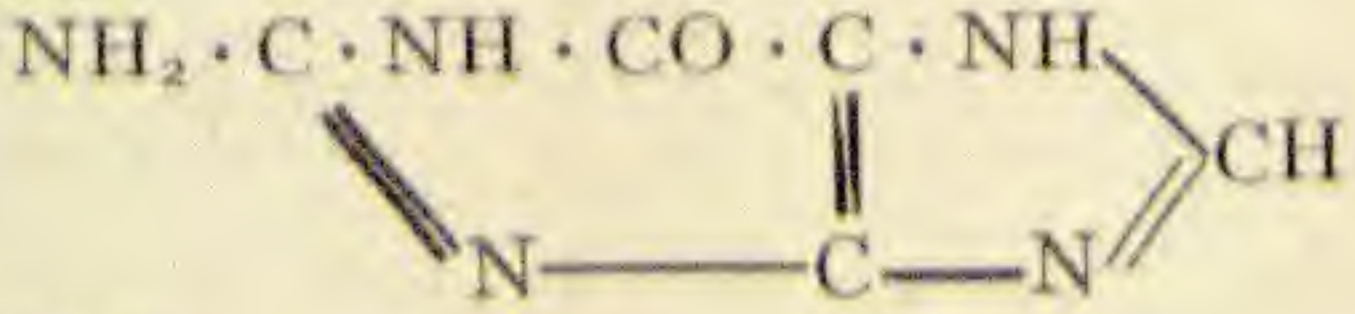
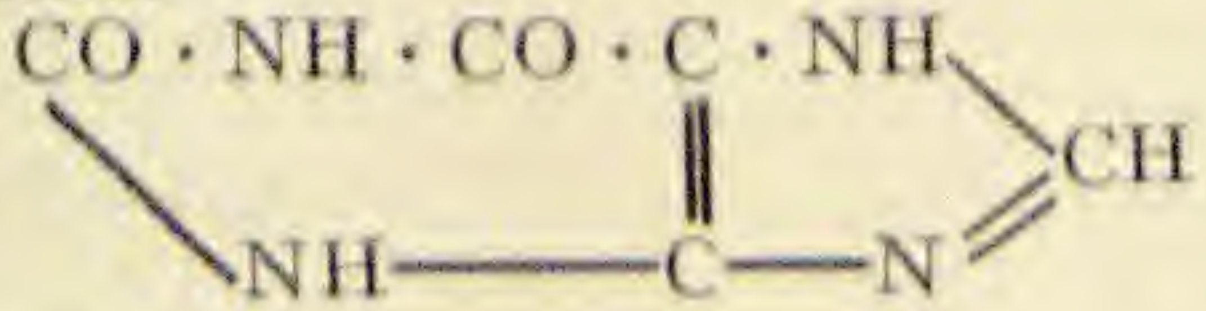
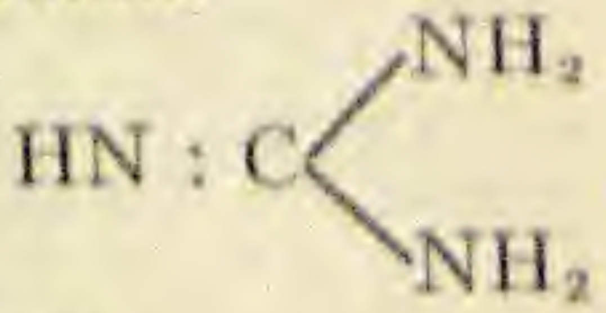
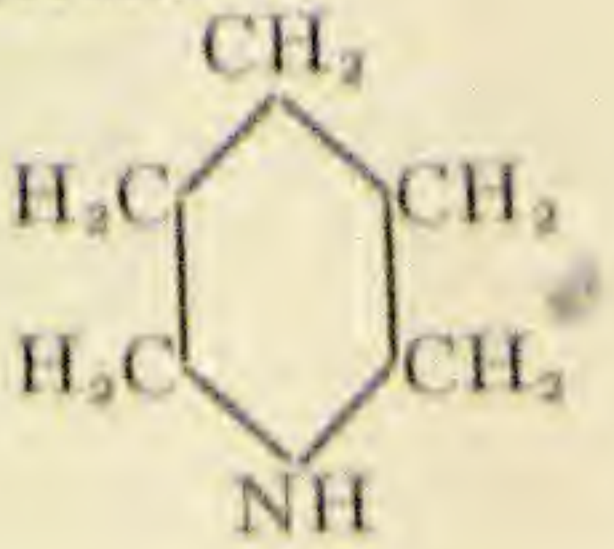
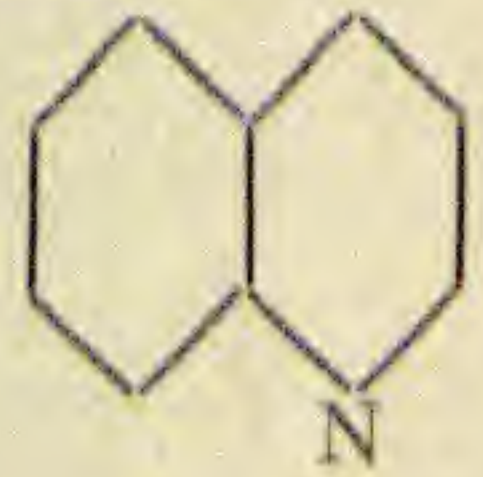
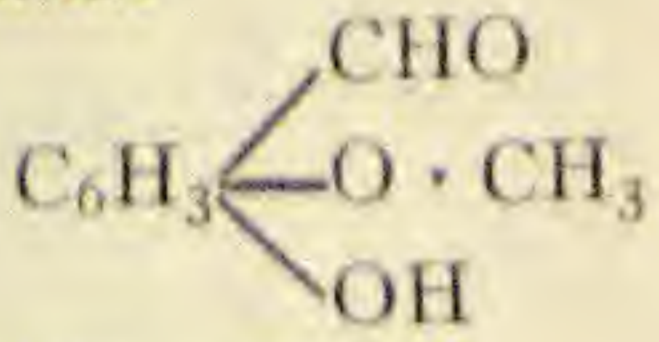
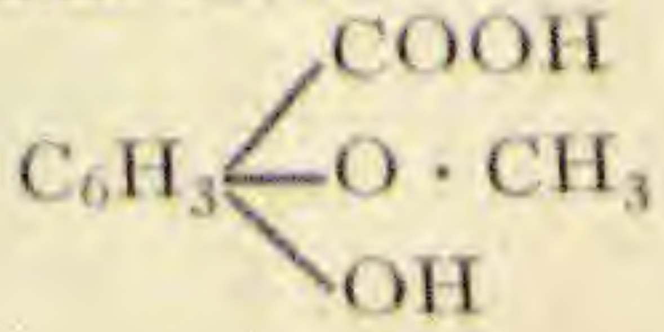
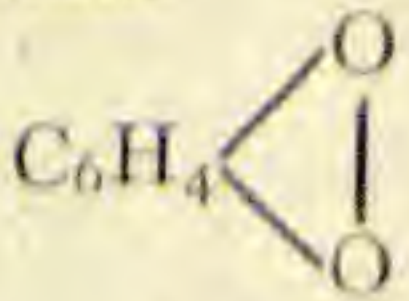
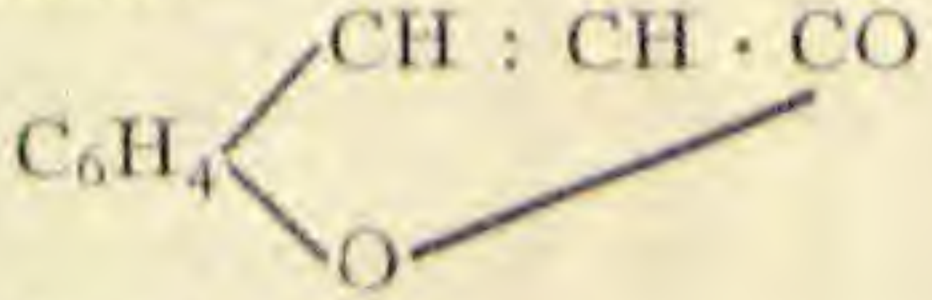
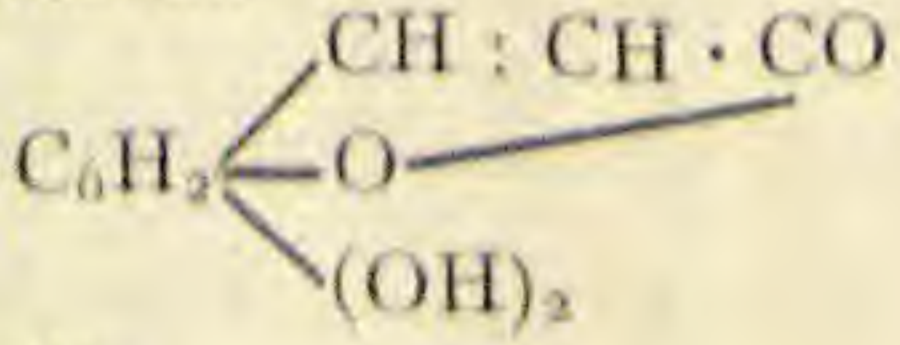
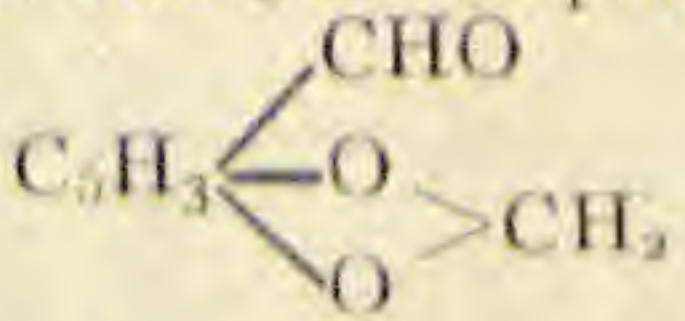
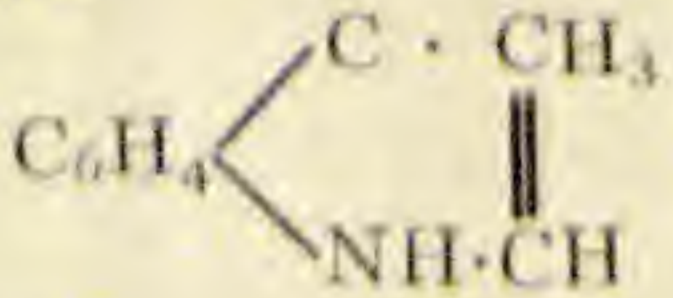
10	Betain 	9	......	......	......	No injury.
11	Alloxan 	10	1000 ppm	100 ppm	......	
12	Guanin 	12	......	......	......	Insoluble above 40 ppm. No harmful effects at that concentration.
13	Xanthin 	10	......	......	......	Insoluble above 25 ppm. No injurious effects at that concentration.
14	Guanidin 	9	100 ppm	1 ppm	......	
15	Pyridin C_5H_5N	9	......	50 ppm	......	In solutions of 50 ppm and less, the root growth was normal.
16	Picolin $C_5H_4N \cdot CH_3$	7	1000 ppm	500 ppm	100 ppm	
17	Piperidin 	7	250 ppm	25 ppm	......	

TABLE II—*Continued*

No.	Compound	Duration of experiment in days	Lowest concentration causing death	Lowest concentration causing injury	Concentration causing greatest stimulation	Remarks
18	Piperidin (neutralized)	7	100 ppm	25 ppm	1 ppm	
19	Chinolin 	6	500 ppm	5 ppm	......	
20	Phenol $C_6H_5 \cdot OH$	11	250 ppm	5 ppm	......	
21	Pyrocatechin $C_6H_4(OH)_2$, 1 : 2	12	500 ppm	25 ppm	1 ppm	
22	Resorcin $C_6H_4(OH)_2$, 1 : 3	10	1000 ppm	50 ppm	1 ppm	
23	Hydrochinone $C_6H_4(OH)_2$, 1 : 4	9	500 ppm	25 ppm	5 ppm	
24	Phloroglucin $C_6H_3(OH)_3$, 1 : 3 : 5	13	500 ppm	25 ppm	1 ppm	
25	Arbutin $C_{12}H_{16}O_7$	12	500 ppm	25 ppm	1 ppm	
26	Vanillin  $C_6H_3\langle CHO, O \cdot CH_3, OH$	9	500 ppm	1 ppm	......	
27	Vanillic acid  $C_6H_3\langle COOH, O \cdot CH_3, OH$	7	100 ppm	25 ppm	5 ppm	

28	Chinone 	9	100 ppm	1 ppm	......	
29	Cinnamic acid $C_6H_5 \cdot CH : CH \cdot COOH$	8	100 ppm	25 ppm	......	
30	Sodium cinnamate $C_6H_5 \cdot CH : CH \cdot COONa$	12	......	100 ppm	......	Roots were stimulated in lower concentrations.
31	Cumarin 	8	100 ppm	1 ppm	......	
32	Daphnetin 	12	......	50 ppm	......	Insoluble above 50 ppm. Roots injured more than tops by 50 ppm.
33	Esculin $C_{15}H_{16}O_9$	13	500 ppm	1 ppm	......	
34	Piperonal (heliotropin) 	7	100 ppm	1 ppm	......	
35	Skatol 	9	200 ppm	50 ppm	......	Roots injured more than tops.
36	Ricin	10	......	40 ppm	......	Insoluble above 50 ppm.
37	Mucin	10	......	100 ppm	......	Not tested in solutions stronger than 100 ppm on account of insolubility.
38	Quinic acid $C_6H_7(OH)_4 \cdot COOH$	10	500 ppm	100 ppm	......	

containing two carboxyl groups, only one of which is linked with the amid group. The behavior of the seedlings grown in solutions of aspartic acid is in harmony with this fact. The injurious effect of the aspartic acid suddenly disappears as soon as diluted below 100 ppm. and the gradual disappearance of toxic effects is not shown. The toxicity of aspartic acid, therefore, seems to be due largely to the carboxyl groups which it contains.

The physiological action of neurin, cholin, and betain presents some interesting facts bearing upon the relation of toxicity to chemical structure. It will be noted, by reference to the structural formulae for these compounds, that they differ only in one of the groups. Neurin contains the ethylene group; cholin contains an ethyl in place of the ethylene group, and has one carbon atom oxidized to the primary alcohol stage; finally in betain the alcohol group is oxidized to carboxyl with subsequent splitting-off of water. Corresponding to the increased degree of oxidation in these three compounds, there is a decrease in their toxicity, neurin being the most toxic, and betain being absolutely non-toxic in concentrations less than 1000 ppm.

On account of the slight solubility of two of the four urea derivatives employed, it is not possible to make comparisons of their physiological actions. The compounds which contain the purin group did not appear to be toxic. Guanidin, however, is distinctly toxic, and alloxan moderately toxic.

The pyridin compounds employed are all toxic, some of them being fatal to wheat plants in relatively small amounts. Pyridin itself appears to be relatively less toxic than any of the other related compounds studied. It was not fatal at a concentration of 1000 ppm. and only injured the tops slightly in concentrations of 100 ppm. and less. The introduction of methyl into the pyridin appears to increase slightly its toxicity (picolin). The addition of more hydrogen to pyridin (reduction) produces a marked increase in its toxicity, as is shown by the physiological action of piperidin, which may be regarded as hexahydropyridin. This fact supports what was said on a previous page concerning the decrease of toxicity accompanying the oxidation of neurin, cholin, and betain. Chinolin appears to be somewhat more toxic than pyridin, as might be expected from its resemblance to naphthalene.

The phenol compounds studied displayed varying degrees of toxicity. In general their toxicity does not appear to depend upon the number of hydroxyl groups they contain. Phenol itself is the most toxic of any of the members of that group which have been reported in this paper. Undue credence, however, must not be given to slight differences in the toxicity of the di- and triatomic phenols, because it is known that they are more or less unstable in solution, and as a result may be converted into more toxic compounds.

The toxicity of cinnamic acid is not difficult to understand when one notes that it is phenylacrylic acid. Sodium cinnamate is much less toxic than the corresponding acid, indicating at least that a part, but not all, of the injurious properties of cinnamic acid are due to the amount of carboxyl it contains. TRUE (**:00**) reported that of a series of sodium salts of organic acids, sodium cinnamate was the most harmful to lupine seedlings. This observation indicates still more strongly that the phenylacrylic group is responsible for a considerable part of the toxicity exhibited by cinnamates.

Cumarin is related to cinnamic acid, and it is worthy of note that this lactone, containing no dissociable hydrogen, is as toxic as cinnamic acid containing dissociable hydrogen.

Daphnetin is another compound which shows a diminished toxicity accompanying a higher stage of oxidation. It differs from cumarin in having two atoms of phenyl-hydrogen replaced by hydroxyl, with the result that the toxicity is somewhat diminished.

While it would be presumptuous to assert that decrease in toxic action always accompanies the introduction of oxygen into organic compounds, it is undoubtedly true that in a number of the cases studied, the more highly oxygenated compounds are less toxic than those containing less oxygen. Many of these substances which are ordinarily regarded as by-products in vegetable metabolism are highly toxic to seedlings when present in sufficient quantities.

It will be noted that certain of the compounds studied produced the effect which LE RENARD (**:02**) has termed "chemauxesis" or stimulation, a phenomenon which has been studied carefully by RAULIN (**'69**), RICHARDS (**'97, '99**), ONO (**:00**), and others. RICHARDS found in the case of the fungi he studied that the presence of a small amount of toxic agent enabled the plants to work more economically with a given amount of nutritive material. It is possible, when toxic agents are present in small amounts, that they excite the cells to greater activity, as a result of which the cells are able to utilize more fully the materials at their disposal, or to overcome retarding conditions of their environment. Such an action would be analogous to the "activating" influence of certain inorganic salts upon enzymes.

The stimulating agents may also act beneficially upon growth by virtue of the changes they cause in the excretory matter of the plant or of the cell. The writers have shown (:07*a*, :07*b*) that deleterious waste products are excreted from living plants, and that the accumulation of these substances may be detrimental to vegetable growth unless some agency removes or destroys the excretory products, either by the formation of harmless compounds, or more simply by precipitating them. It is well known that salts of the heavy metals, which in small amounts exert a stimulating action on plants, will also bring about a precipitation of proteid-like bodies. When larger amounts are present, these substances not only combine with the excreted proteid bodies at the surface of the plant, but also exert this same action on the living protoplasm within the plant, causing death.

If the excretory products mentioned be of the nature of proteids, it is easy to see how beneficial conditions might arise by the continual precipitation of the harmful excretions. In other cases it is possible that the compounds which exert a stimulating action may set up chemical processes which, while not precipitating excretions, nevertheless render them harmless to the plants which produce them.

From this point of view, the stimulation of plant growth by small quantities of toxic agents consists in the removal of deleterious waste products, and the maintenance of a sanitary environment for the growing plant. It is not at all improbable that each of the processes mentioned may act as factors in increasing plant growth at different times and under different conditions.

The oxidizing power of the plant roots was shown in several instances by the formation of colored compounds which stained the roots.

Certain of the substances employed appear to have had a toxic action mainly upon the growth of the tops of the plants; other substances were more toxic to the growth of roots than of the tops.

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SOME NEW CASES OF MENDELIAN INHERITANCE

GEORGE HARRISON SHULL

(WITH FOUR FIGURES)

In crosses between nearly related elementary forms, numerous investigations have shown that a very wide range of characteristics in a great many species of both plants and animals behave in the Mendelian way; that is, they give a uniform progeny in the first hybrid generation (F_1), and show perfect segregation of the various characteristics in F_2 and later generations. So consistent have been the results that there can be no question that MENDEL'S law has fundamental and widespread applicability, but it has also been demonstrated that it has its limitations. Where these limitations lie and why it is thus limited may not be known until a much larger mass of data is on record than is now available.

Several of the cultures at the Station for Experimental Evolution, upon which no report has yet been published, present new instances of Mendelian heredity under conditions which make their consideration desirable, even though only a preliminary account can be given at this time.

HELIANTHUS ANNUUS L.

Among 112 plants of the so-called "Russian" sunflower (*Helianthus annuus* var.) grown at the Station during the summer of 1904, all but one had the usually unbranched stem surmounted by a single large head. Sometimes these had several weak branches in the axils of several of the lower leaves, but there were never more than three or four of these lateral branches, and none ever stood higher on the stem than the fifth node above the cotyledons. The one exceptional individual had strong branches in nearly all of the axils and bore a number of somewhat smaller heads, but was not observed to differ in other respects from its unbranched neighbors (*figs. 1, 2*). The seeds had been purchased at a seed store, and nothing is known of the antecedents of these plants.

Not until late in the season was it decided to investigate the hereditary qualities of the branching habit, and consequently the pollination was not guarded. Attempts at getting self-fertilized seeds from other plants proved unsuccessful, however, and all my experience in breeding the sunflowers during the past four years indicates that they are entirely self-sterile. From this it may be safely inferred that the branched individual (pedigree no. 04109) was cross-fertilized; and as it was the only individual of its kind, it must have been fertilized by means of pollen from the unbranched individuals. Assuming that this branching habit is a Mendelian character, several possible assumptions would lead to different expectations regarding the offspring of this cross: (*a*) If the unbranched habit were dominant over the branched, all the offspring should be unbranched; (*b*) If the branching habit were dominant, there would be two cases: (1) when the branched parent is a pure dominant, the offspring should be all branched; and (2) when the branched parent is a heterozygote (DR),

FIG. 1.—"Russian" sunflower (*Helianthus annuus* var. hort.) showing two pure-bred strains; unbranched on the left, branched on the right.

the offspring should consist of both branched and unbranched individuals in the ratio 1:1.

In the summer of 1905, 59 offspring were reared from this cross, 28 branched and 31 unbranched, showing either that branching is dominant, and the original branched plant was a heterozygote, or that branching is not a Mendelian character. About half of the unbranched plants showed a few weak branches about the base, but none had more than four branches, while none of the branched individuals had

FIG. 2.—Individuals representing two elementary strains of the "Russian" sunflower; leaves removed to display the branching habit.

less than nine branches, the average number of branches in the latter being about 15.

During the same season, 81 offspring of an unbranched plant (04108) which had also been left unguarded showed 72 unbranched and 9 branched. This result would find a ready explanation in case branching is a Mendelian unit-character, simply as the usual result of vicinism. On this assumption the proportion of branched offspring in this family indicates that the pistil-parent (04108) received about one-fifth of its fertilizations from the branched specimen (04109) which was growing near it, and the other four-fifths from the 110 unbranched plants, several of which were growing equally near.

In 1906 the Helianthus cultures were started several weeks too early (April 2) and became tall and slender from long crowding and insufficient illumination before they could be set into the garden. Some of these began to bloom shortly after they were transplanted, and the branching habit was very much deranged. Two families, (05149 and 05150) raised from branched parents having had the pollination fully controlled, resulted in 54 branched and 2 nearly unbranched in the one case, and 68 branched and 2 unbranched in the other, but as the whole branching system was considerably modified the classification could not be made with security. The expectation in these cases, since both parents were DR, would have been branched and unbranched approaching the ratio 3:1. The results secured indicate either that we are not dealing with a Mendelian character or that the conditions of the culture caused an excessive development of branches in normally unbranched individuals. The latter suggestion is strengthened by a small family (05145) reared from fully guarded unbranched parents from the same hybrid family to which the branched parents of the last-mentioned two families belonged. Both parents should be in this case extracted recessives (RR), and all of the offspring should belong to the unbranched class. Only six offspring were produced, and all showed some branching, ranging from one strong branch below the middle to twelve branches from the middle to the top of the stem, the latter type of branching being characteristic of the branched form. The results in 1906 seem therefore to oppose distinctly the idea that the branching character is Mendelian, but still leave a doubt on account of the obvious derangement produced by ill-treatment.

In the summer of 1907 the seeds were sown at the proper time (April 30) and the Mendelian character of the branching habit was fully established by the following facts: (*a*) Reciprocal crosses (06356 and 06357), fully controlled, between two strongly branched specimens belonging to the second of the two DR families mentioned in 1906 (namely, no. 05150) gave 65 fully branched individuals in the one family, 13 in the other family, and no unbranched specimens in either, thus showing that at least one of the chosen parents was an extracted dominant (DD). The number of branches in both these reciprocal families ranged from 10 to 25. (*b*) Properly controlled pollinations were made between two specimens of the last family (05145) mentioned for 1906, supposedly a recessive family. The pistil-parent had two strong branches, one of which showed an abnormal bifission. The pollen-parent had one strong lateral branch which exceeded the main stem. The cross was made between two heads borne by lateral branches. Of the 22 offspring produced (06353), 19 were wholly unbranched, two had one small branch, each near the base of the stem, and one had two small branches similarly located. This demonstrated that both parents were pure recessives and showed that the branches produced the preceding year had no hereditary significance. (*c*) Another sowing (06352) was made of unguarded seed from the same pistil-parent as the last. The 91 offspring consisted of 67 individuals having less than 7 branches each and 24 having more than 7 branches each, the latter group having an average of about 16 branches and the former group an average of less than two branches (*fig. 3*). As the branched type does not usually have more than 4 or 5 vacant axils above the highest branch, one individual having 8 branches and 10 vacant axils probably belongs with the unbranched class. The result in this family shows that about one-fourth of the pollen received by the unguarded mother came from branched individuals, and as this fact was made obvious in the first generation it gives further proof of the dominance of the branching habit over the unbranched.

Furthermore, it illustrates well how impossible it would be to discover Mendelian ratios in cross-fertilizing species or varieties without careful control of fertilization. This requirement accounts for two things, namely, the fact that MENDEL'S law was not discovered by economic plant-breeders and gardeners long ago, and that in certain quarters the notion prevails that Mendelian inheritance is a function only of self-fertilizing or inbred strains.

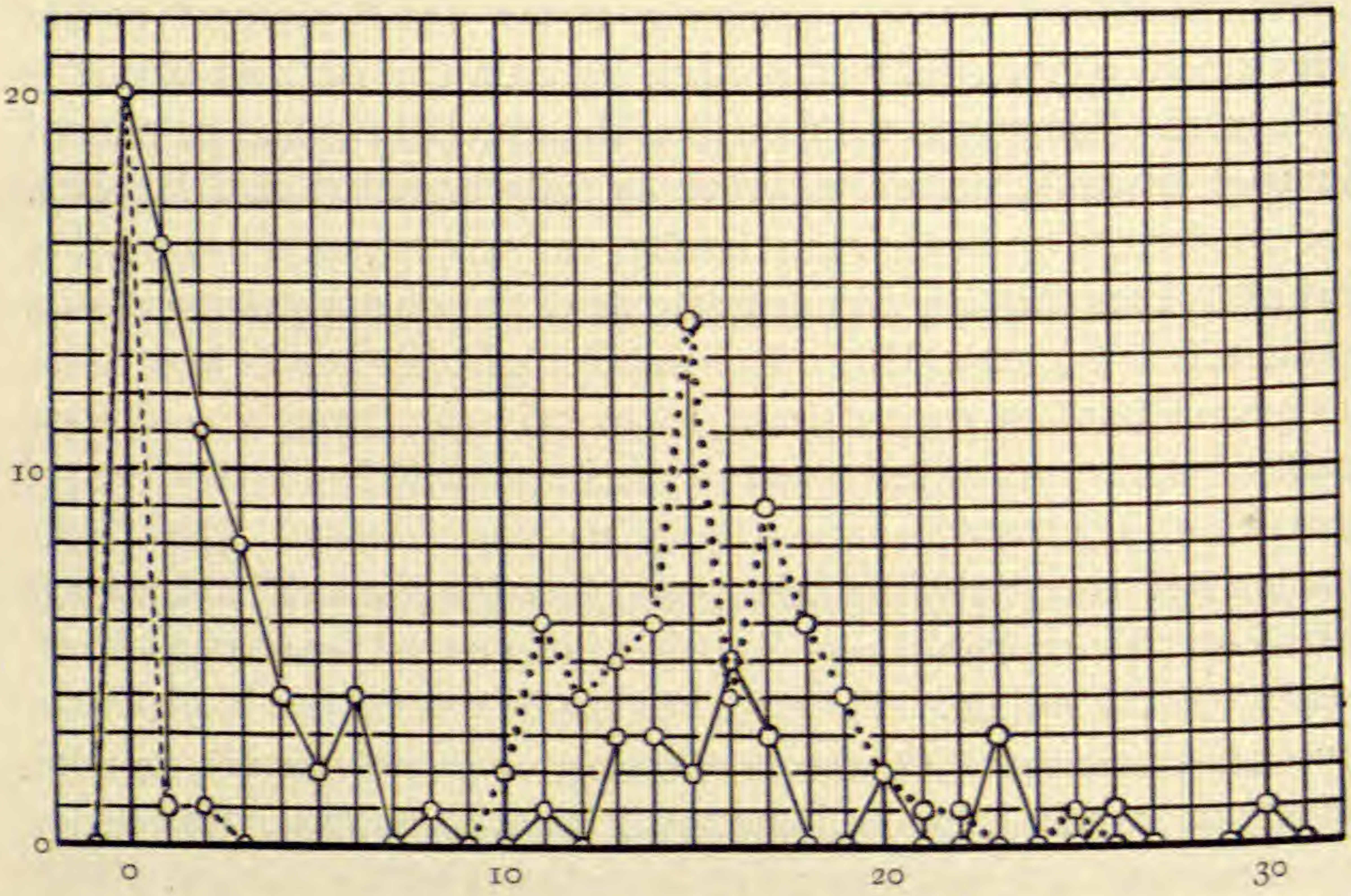
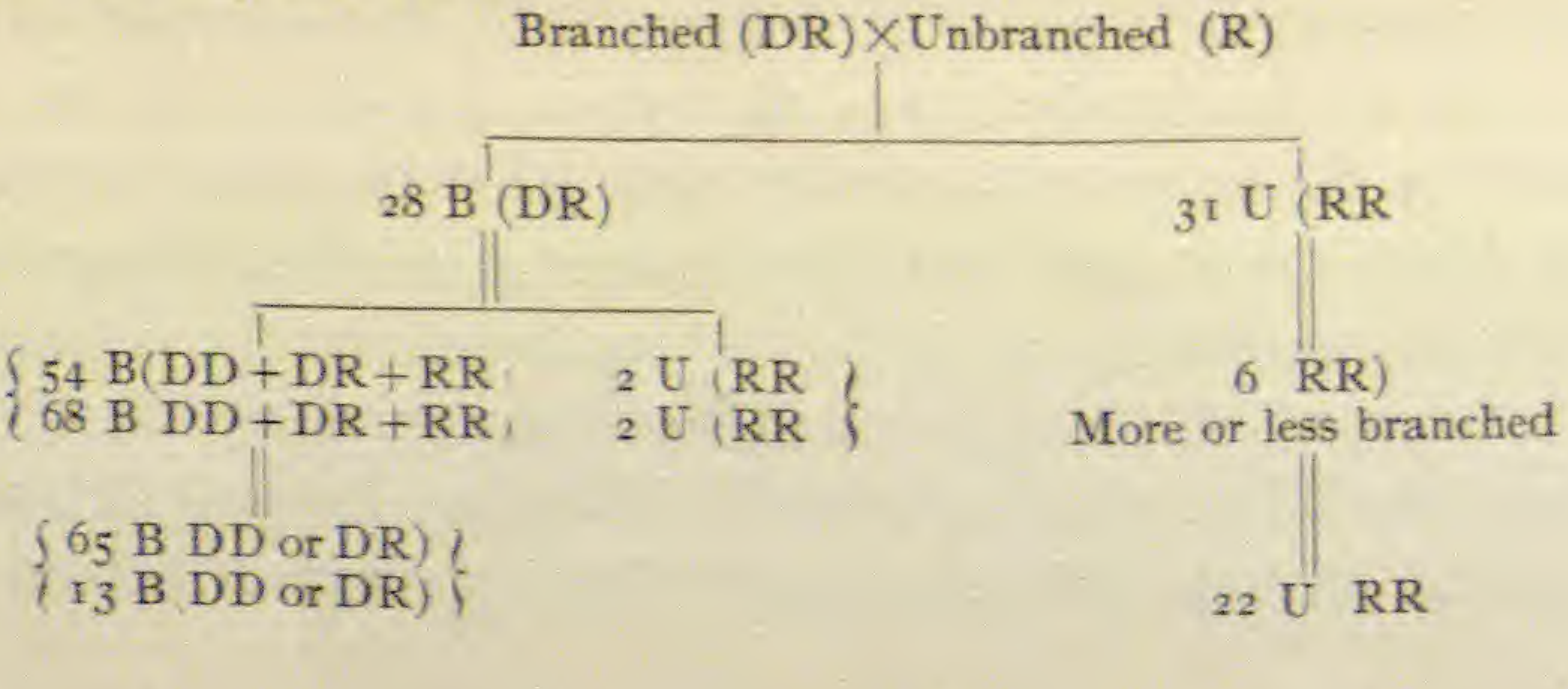


FIG. 3.—Curves showing the extent of branching in three families of "Russian" sunflower; the dotted line at left represents the offspring of two unbranched parents; the dotted line at the center represents the offspring of two branched parents, one of which must have been an extracted dominant; the unbroken line shows the condition of a family from the mother of the pure unbranched family, but with pollination unguarded; the dominance of branching produces a branched offspring in each case that successful fertilization took place by means of pollen from a branched plant.

The foregoing account of experiments on the inheritance of the branching habit in *Helianthus annuus* may be recapitulated in the form of a family tree thus:



During the summer of 1905 experiments were also begun for the purpose of testing the relationship between the Russian sunflower and the wild *Helianthus annuus* of the prairie region. Seed of the latter had been received from Nebraska through the kindness of Dr. C. E. Bessey. Several points of contrast were noted between the plants produced from this seed and the Russian sunflower, the most noteworthy being with regard to branching habit and the color of the disk. The lower branches of the wild sunflower, when given space for full individual expression as they are in my cultures, diverge almost horizontally, having on this account a slightly wider spread than branches arising at higher points on the stem. At the time the first head is ready to spread its rays, the whole plant has a nearly conical form. The development of the primary head checks the growth of the main stem, and some of the upper branches then usually elongate so as to overtop the central axis, thus giving the mature plant a more columnar form. My branched type of the Russian sunflower has all the branches strongly ascending, the lower being enough longer than the upper to reach almost the same level, thus giving the mature plant a corymbose or broadly obconical form.

I have not yet reached a conclusion in regard to the hereditary behavior of these two types of branching, as it is not easy to find decisive criteria for the exact classification of the two forms and their combinations. It has been apparent, however, in my cultures that there is a combination of both types of branching in many if not all of the first-generation hybrids, and at least some segregation in the next generation. The indefiniteness of the character will probably make it difficult to reach satisfactory quantitative results, but the attempt is being made.

A very much simpler character to deal with is the color of the disk. All of the western *Helianthus annuus* that I have thus far observed have a deep purple disk, the color being found in the tips of the paleae, which are of a deep, metallic purple; the margin of the corolla, which is brownish purple; and the style and stigmas, which are reddish purple. In all my Russian sunflowers, the tips of the paleae are yellowish green, the corolla is a clear lemon yellow, and the styles and stigmas usually have the same color as the corolla; but in a small proportion the stigmas are margined with a narrow line of deep crimson, which gives the stigmas the appearance of being orange colored, unless examined closely. No study has been made of the inheritance of this stigmatal margin, but it seems not unlikely that it too may prove to be a Mendelian character. Although the color of the disk affects several organs of very different morphological value, it proves to be a simple character, showing the same kind of correlation that is found in peas and beans which have purple flowers and black seeds, and other plants which have purple flowers associated with more or less purple in the stems.

The cross made during the summer of 1905 between the purple-disked wild sunflower and the yellow-disked, branched, garden sunflower, produced in 1906 a progeny (05153) consisting of 26 with purple disks and 27 with yellow disks; when I would have expected all purple on the assumption that both parents were pure-bred and purple dominant over non-purple, as has been the general experience with the purple color-character in other plants. The conclusion reached was that either the purple disk is not a Mendelian character or the purple parent was a DR instead of a pure-bred purple. As yellow disks have not appeared among my cultures of wild sunflowers during three years, the assumption that the one plant which I used for the cross was itself a hybrid seemed very unlikely, and the probability that this strictly alternative character was not behaving according to Mendelian expectation led me to watch the culture the following season (1907) with great interest. Five F_2 families were raised, two reciprocal families of purple by purple, representing the hypothetical cross DR×DR; two reciprocal families between purple and yellow, representing the cross DR×R; and one family of yellow by yellow, representing the cross R×R. The results were as follows:

DR×DR	No. 06359,	64 p:21 y
	No. 06360,	64 p:16 y
	Total,	128 p:37 y
	Expected,	123 p:41 y
DR×R	No. 06361,	48 p:46 y
	No. 06362,	39 p:41 y
	Total,	87 p:87 y
	Expected,	87 p:87 y
R×R	No. 06358,	96 y
	Expected,	96 y

From all of these results it must be concluded that the purple disk is a strict Mendelian character, and that the first purple-disked parent was a heterozygote. The original cross is being repeated to make the latter point more certain.

LYCHNIS DIOICA L.

This plant is a native of Europe and was doubtless very often cultivated in the gardens of a generation or more ago. It has now commonly escaped from the gardens and is maintaining itself quite generally along roadsides in the eastern United States. The flowers show considerable variation in the intensity of their purple color, and white-flowered ones are quite common. Purple and white flowers have been crossed in so many species and found to follow MENDEL'S law, that there would have been little incentive to study this particular character in *Lychnis dioica*, had it not been for the fact that this species is dioecious, and that in consequence self-fertilization could not be held responsible for the Mendelian behavior if it should be found to exist. Statements in the manuals regarding the dioecious habit of this species indicate that it also occurs as a hermaphrodite, but among about five thousand individuals which I have examined only one was noted which had both stamens and pistils, and that one had but a single flower with both kinds of sporangia.

During the summer of 1905 I made all possible combinations with white and purple flowers, but in one case unfortunately used two different purple-flowered individuals for the pollination of the same white-flowered plant and collected the seeds from both crosses together. The offspring from these crosses reared in 1906 were as follows:

No. 05212, w × w,	90 w
No. 05213, w × 2 p's,	66 p:41 w
No. 05114, p × p,	65 p:23 w
No. 05115, p × p,	96 p

The fact that two white-flowered parents produced nothing but white-flowered offspring, and that in one case two purple-flowered parents produced only purple-flowered young, shows pretty conclusively that we are dealing here with a Mendelian pair of characters, even though the one family (05114), which probably represents the cross DR × DR, shows a considerable departure from the expected ratio of 3p:1w. The ratio of purple to white in family no. 05213 can have no significance, since one of the two purple plants used as the pollen parents was probably a D and the other a DR.

To test the assumption that family no. 05114 represented the cross DR×DR, and to get further evidence on the Mendelian character of the purple color in this species, a series of over eighty crosses was made within this single family. All of the pistillate plants received pollen from a single white staminate plant, and pollen from each of the staminate plants was used to fertilize the different flowers of a single white pistillate plant.

This method was employed because it is the simplest way in which the correct classification of every individual of the first generation of hybrids may be attained. Every single cross in the series had the recessive white as one of its members, so that only three possible combinations could occur among the resultant hybrid families, namely, D×R, DR×R, and R×R, giving respectively, according to expectation, 100 per cent., 50 per cent., and 0 per cent., of purple-flowered offspring, in the ratio 1:2:1. About 50 plants from each of these 83 crosses were reared to maturity in 1907, and, when classified according to the percentage of purple individuals in each family, showed 13 families with more than 95 per cent. purple, 48 having 30–70 per cent. purple, and 22 with less than 5 per cent. purple, the expectation being 21 with 100 per cent. purple, 42 with 50 per cent. purple, and 21 with 0 per cent. purple. The result is close enough to expectation to demonstrate the correctness of the Mendelian law in regard to the flower-color of *Lychnis dioica* L.

The diagram (*fig. 4*) showing the distribution of percentages of purple individuals in the heterozygote families may be used to call attention to what we ought to mean by "Mendelian expectation." Very often when a rather small series of observations shows a considerable departure from the ratios 1:1, 1:2:1, 3:1, 9:3:4, or whatever other ratio represents the theoretical limit appropriate to the conditions of the particular experiment in hand, the statement is made that the results are not in accord with "Mendelian expectation." When the number of observations is small, such a statement is usually due to the fact that too much is expected. This will become clear when attention is called to the fundamental basis of the Mendelian ratios. Equal numbers of both eggs and sperms carry the antagonistic or alternative qualities making up a pair of Mendelian units. These different germ cells unite according to the laws of chance, and we should mean therefore by the expression "Mendelian expectation" that our observations if seriated in the form of a curve will present a normal probability curve within the limits of probable error. Not until the number of observations becomes infinite have we a right to expect absolute agreement with the theoretical ratios.

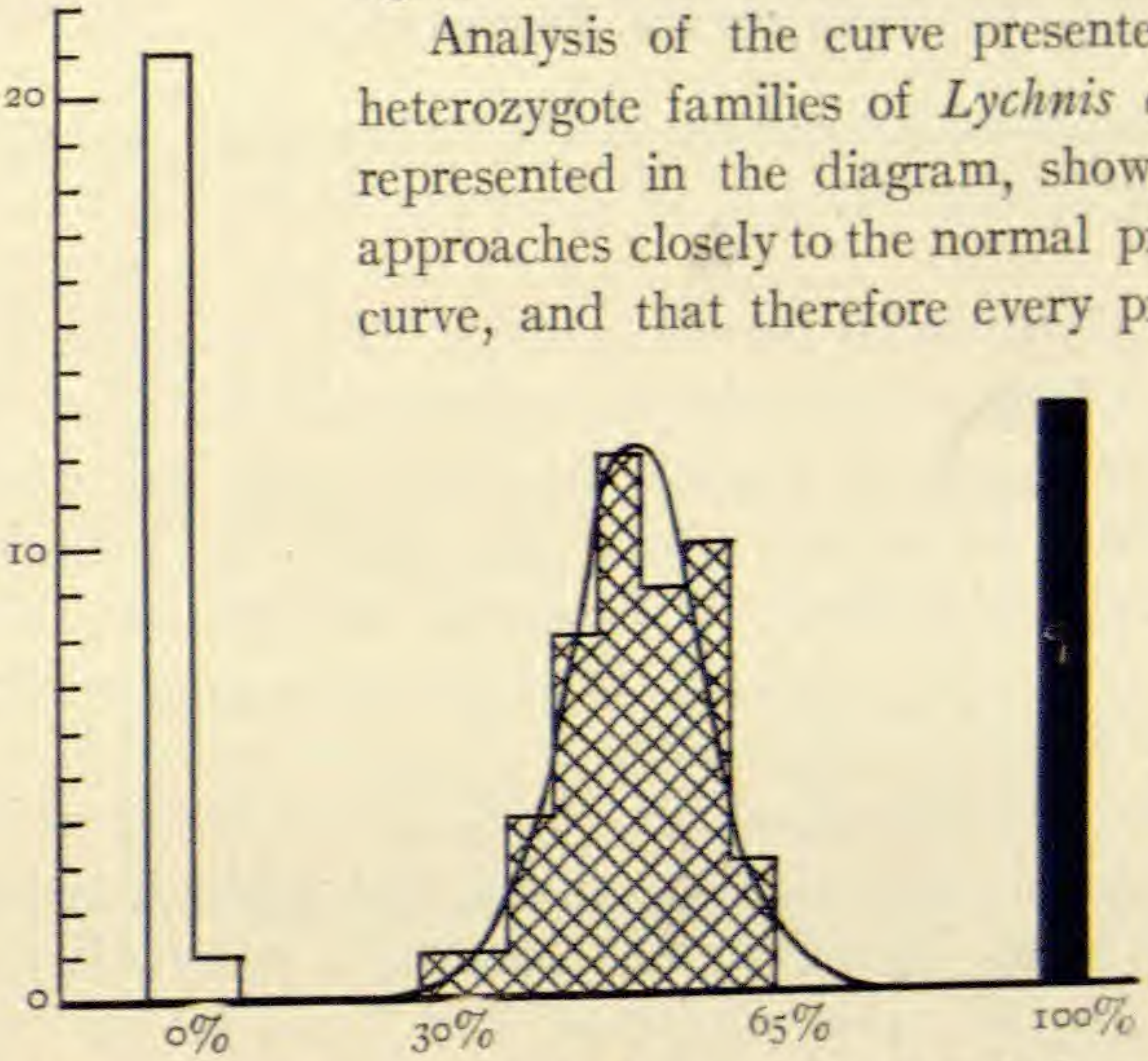


Fig. 4.—*Lychnis dioica* L.: variation in the percentage of purple-flowered offspring in hybrid families of the third generation having the form (D+2DR+R)×R; left-hand group represents the extracted recessives (R×R), the right-hand group the extracted dominants (D×R), and the middle group the heterozygotes (DR×R); superposed upon the heterozygote group is the normal curve having the same standard deviation, showing the close agreement with a perfect chance distribution.

Analysis of the curve presented by the heterozygote families of *Lychnis dioica*, as represented in the diagram, shows that it approaches closely to the normal probability curve, and that therefore every proper expectation is fulfilled. As the departure of variates from the mean is a fundamental part of the law of chance, the fulfilment of expectation is just as complete in the case of the one DR×R family that had only 35 per cent. of purple-flowered individuals, or the three similar families that had about 65 per cent. of purple flowers, as in the twelve families which consisted of about 50 per cent. purple. This is an important fact that needs to be taken into account by both the student and the critic of Mendelian inheritance.

The results here presented both in regard to Lychnis and Helianthus show that Mendelian phenomena stand in no relation to self-fertilization, since neither of these species can self-fertilize. Emphasis upon this point should not be needed, since many of the most typical cases of Mendelian inheritance occur among animals, where the sexes are almost invariably separated.

VERBASCUM BLATTARIA L.

There are two color-varieties of this widely distributed species, one having bright-yellow flowers, and the other having the flowers almost white. If examined carefully, however, the pale flowers are seen to be cream colored rather than pure white. Two considerations made it appear desirable to determine the relation between these two color-varieties by means of crossing. (*a*) Many cases are on record in which albinos are found to be typical Mendelian recessives when paired with certain colors. In *Verbascum Blattaria* the pale flowers approach the albino condition, but both forms possess some color, and it is of interest to know whether this pale-flowered form behaves in the same manner as an albino. (*b*) Several studies in which yellow has been paired with white have shown that yellow is in these cases recessive to white. Thus CORRENS[1] found white *Polemonium coeruleum* dominant over the yellow *P. flavum;* and BATESON[2] has shown that yellow-flowered stocks (Matthiola) are recessive to the white-flowered. CORRENS' results with Polemonium stood alone at the time my experiments with Verbascum were begun, as an instance in which a white-flowered variety dominated one with colored flowers. It seemed important on this account, also, to know what would be the behavior of the yellow-flowered *Verbascum Blattaria* when crossed with its pale-flowered form.

To secure a solution to these problems, several crosses were made during the summer of 1904. The seeds were sown in the spring of

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[1] CORRENS, C., Weitere Beiträge zur Kenntnis der dominierenden Merkmale und der Mosaikbildung der Bastarde. Ber. Deutsch. Bot. Gesells. **21**:195–201. 1903. (See p. 198.)

[2] BATESON, W., SAUNDERS, Miss E. R., and PUNNETT, R. C., Reports to the Evolution Committee of the Royal Society, II. 1905, and III. 1906.

1905, but the plants did not bloom until the summer of 1906. The results then stood as follows:

No. 0446,	y × y,	75(?) y
No. 0447,	w × w,	53 w
No. 0448 × 47,	w × y,	8 y
No. 0448 × self,	y × y,	(?) y (number unrecorded)

Among the members of these families all possible combinations were again made. The offspring of these crosses were mostly induced to bloom the first season by starting early and giving the seedlings the best possible conditions. The results of the second generation were thus secured during the summer of 1907, and showed the following composition:

No. 06241,	D × R,	26 y
	Expected,	26 y
No. 06244,	DR × DR,	5 y: 7 w
	Expected,	9 y: 3 w
No. 06243,	R × DR,	13 y:18 w
No. 06245,	DR × R,	5 y: 8 w
	Total,	18 y:26 w
	Expected,	22 y:22 w
No. 06242,	R × R,	90 w
	Expected,	90 w

Although some of these ratios depart rather strongly from the theoretical limiting ratio, this is due entirely to the small numbers of observations, as pointed out above in the discussion of Lychnis. It is plain therefore that the color-forms of *Verbascum Blattaria* constitute a Mendelian pair, and that the bright yellow is dominant over the pale. The dominance of yellow in this cross is just the reverse of the behavior of yellow in Polemonium and Matthiola, and teaches that there are two kinds of yellows in plants, one of which is dominant to white, the other recessive to white. A microscopic examination of the petals of Verbascum shows that BATESON'S[3] distinction between plastid-colors and sap-colors is probably fundamental, as the yellow color in this species proves to be a sap-color, and its behavior in combinations with white is the same as that of the blue, purple, and red

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[3] Loc. cit.

sap-colors; while the yellow color of Matthiola and of *Polemonium flavum* are plastid-colors and this must account for their different behavior. In Mirabilis CORRENS[4] has noted a yellow-flowered variety, *M. Jalapa gilva*, in which the yellow color seems to be dominant. I do not know that any examination has been made to determine the basis of the yellow color in Mirabilis, but I predict that it will be found to be a sap-color. HURST[5] reports, on the contrary, that the yellow of *Antirrhinum majus*, which is recessive to white, is a sap-color. If this statement is correct, this species seems to stand alone at present in the possession of a sap-color recessive to white. Why a plastid-color should be recessive to the absence of that color calls for an explanation which science is not yet ready to give. This difference between the two kinds of yellow recalls a similar situation in poultry, in which white is usually dominant over colors, but in at least one strain (Silky) the white is recessive.

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[4] CORRENS, C., Zur Kenntnis der scheinbar neuen Merkmale der Bastarde. Ber. Deutsch. Bot. Gesells. **23**:70–85. 1905.

[5] HURST, C. C., Mendelian characters in plants and animals. Rep. 3d Internat. Conference on Genetics, 1906. Roy. Hort. Soc. **1907**:114–128. *figs. 6.*

THE ANTAGONISTIC ACTION OF MAGNESIUM AND POTASSIUM

W. J. V. OSTERHOUT

(WITH THREE FIGURES)

It has been previously pointed out[1] that potassium may inhibit more or less fully the poisonous effects of magnesium and that the abundance of potassium in the soil makes this inhibitory action of importance in soil investigations. LOEW and ASO[2] have criticized this statement. Their objections are that only chlorids were used and that no flowering plants were investigated. In the present paper these objections are fully met. The experiments extend over a wide range of forms and their general agreement furnishes conclusive proof of the above-mentioned action of potassium.

The technique employed has already been described in previous papers in this journal.[3] The material was placed in glass dishes containing 100–300^{cc} of the solution and was covered with glass plates to exclude dust and hinder evaporation. Water twice distilled and salts which were tested for purity were used throughout. The results given in the tables are in all cases averages of several series of experiments.

The first experiments were made upon a marine alga, *Enteromorpha Hopkirkii*, which is able to live in both sea water and distilled water. It was taken from the sea water, rinsed in distilled water, and placed directly in the solutions. The solutions used were of the concentration 0.375m, which has the same osmotic pressure as the sea water in which the plants naturally grow.

In pure $MgCl_2$ .0375m they lived but four days; in pure KCl .0375m seven days; while in distilled water and sea water they were alive and vigorous at the end of twenty days when the experiment was discontinued. It is evident therefore that both KCl and $MgCl_2$ have a poisonous action.

This poisonous effect largely disappears if we mix the two salts

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[1] OSTERHOUT, University of California Publications, Botany 2:235. 1906.

[2] LOEW AND ASO, Bull. Imp. Coll. Agr. Tokyo 7:395. 1907.

[3] OSTERHOUT, BOT. GAZETTE 42:127–134. 1906; 44:259–272. 1907.

($MgCl_2$ and KCl) in proper proportions. In the mixture 100^{cc} $MgCl_2$ $+40^{cc}$ KCl, the plants were alive and in good condition at the end of twenty days, when the experiment was discontinued. It is evident therefore that in the mixture of magnesium and potassium chlorids the plants live five times as long as in pure magnesium chlorid and three times as long as in pure potassium chlorid.

TABLE I

MARINE ALGAE

All quantities given are cubic centimeters of 0.375*m* solutions

Culture solution	Duration of life in days: *Enteromorpha Hopkirkii*
KCl	7
100 KCl 40 $MgCl_2$	20+
$MgCl_2$	4
Distilled water	20+
Sea water (total salts = 2.7 per cent.)	20+
Artificial sea water (total salts = 2.7 per cent.: 100 NaCl 7.8 $MgCl_2$ 3.8 $MgSO_4$ 2.2 KCl 1 $CaCl_2$	20+

The plus sign indicates that the plants were alive at the end of the experiment.

The results obtained from the study of Vaucheria were even more striking. Zoospores were allowed to attach themselves to slides. These were then rinsed in distilled water and placed in the solutions. The results are shown in the following table and also in *fig. 1*.

TABLE II

FRESH-WATER ALGAE

All quantities given are cubic centimeters of .01*m* solutions

CULTURE SOLUTION	DEVELOPMENT DURING 45 DAYS: *Vaucheria terrestris*	
	Length of thallus in mm.	Percentage of increase in length
KCl	0.15	0
100 KCl 40 $MgCl_2$	6.45	4200
$MgCl_2$	0.15	0
Distilled water	10.0	6566.66

A large Spirogyra of the *majuscula* type was used for experiments with the stronger solutions. The results are given in Table III.

TABLE III

FRESH-WATER ALGAE

All quantities given are cubic centimeters of .0937*m* solutions

Culture solution	Duration of life in days: Spirogyra species
KCl	$\frac{3}{4}$
100 KCl 40 $MgCl_2$	12
$MgCl_2$	$\frac{1}{2}$
Distilled water	25+

The plus sign indicates that the plants were alive at the end of the experiment.

A series of experiments was next made with the gemmae of Lunularia. These were allowed to float on the surface of the solutions. A large number was used and the average results given in the following table.

TABLE IV

LIVERWORTS

All quantities given are cubic centimeters of .0937*m* solutions

Culture solution	Duration of life in days: gemmae of Lunularia
KCl	12
100 KCl 50 $MgCl_2$	120+
100 KCl 100 $MgCl_2$	120+
50 KCl 100 $MgCl_2$	100
$MgCl_2$	4
Distilled water	120+

The plus sign indicates that the plants were alive at the end of the experiment.

FIG. 1.—Growth of Vaucheria during 45 days in .01*m* solutions. *1*, KCl, gain 0; *2*, 100cc KCl + 40cc $MgCl_2$, gain 4200%; *3*, $MgCl_2$, gain 0. ×25.

It will be noticed even when magnesium greatly preponderates in the mixed solutions the plants live twenty-five times as long as in pure $MgCl_2$, and over eight times as long as in pure KCl. Increasing the proportion of potassium increases the length of life.

The same relation is seen more completely in the next table (Table V). Decreasing the amount of Mg causes increased growth up to a certain point (100 K + 10 Mg). Still further decrease of the relative amount of Mg beyond this point is unfavorable. The optimum relation is therefore not far from 10 Mg + 100 K.

TABLE V

LIVERWORTS

All quantities given are cubic centimeters of .0375*m* solutions

CULTURE SOLUTION	GROWTH IN 150 DAYS: GEMMAE OF LUNULARIA	
	Length of thallus in mm.	Percentage of gain in length of thallus
KCl	0.5	0
100 KCl 5 $MgCl_2$	3.30	560
100 KCl 10 $MgCl_2$	3.41	582
100 KCl 25 $MgCl_2$	2.6	420
$MgCl_2$	0.5	0
Distilled water	0.60	1220

It will be noticed also that the gemmae made no growth whatever in pure $MgCl_2$ or pure KCl, while in mixtures of the two a good growth occurred.

For the study of flowering plants wheat was chosen. The seeds were supported in the solutions on strips of filter paper as described in a previous paper.[4] The results agree with those already given. Table VI shows that certain mixtures of potassium chlorid and magnesium chlorid are much more favorable than either of the pure salts (see also *figs. 2, 3*).

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[4] OSTERHOUT, BOT. GAZETTE **44**:259–272. 1907.

Turning now to the experiments with sulfates and nitrates, we see entirely similar results, save that the mixed solutions, while better than pure magnesium salts, are not better than pure potassium salts. The question might then arise whether the favorable result is due in this case to mere dilution of magnesium salts with less poisonous ones. This, however, is not the case. We are dealing with a true antagonistic action. This is shown by the fact that addition of the potassium salt in solid form likewise produces a favorable result, and also by the fact that the addition of pure water does not produce anything like the improvement seen on the addition of the same amount of a solution of a potassium salt.

FIG. 2.—Growth of wheat roots during 40 days in .0937*m* solutions: *1*, $MgCl_2$, aggregate length of roots 10^{mm}; *2*, 100^{cc} KCl $+ 25^{cc}$ $MgCl_2$, aggregate length of roots 153^{mm}; *3*, KCl, aggregate length of roots 110^{mm}. ×⅔.

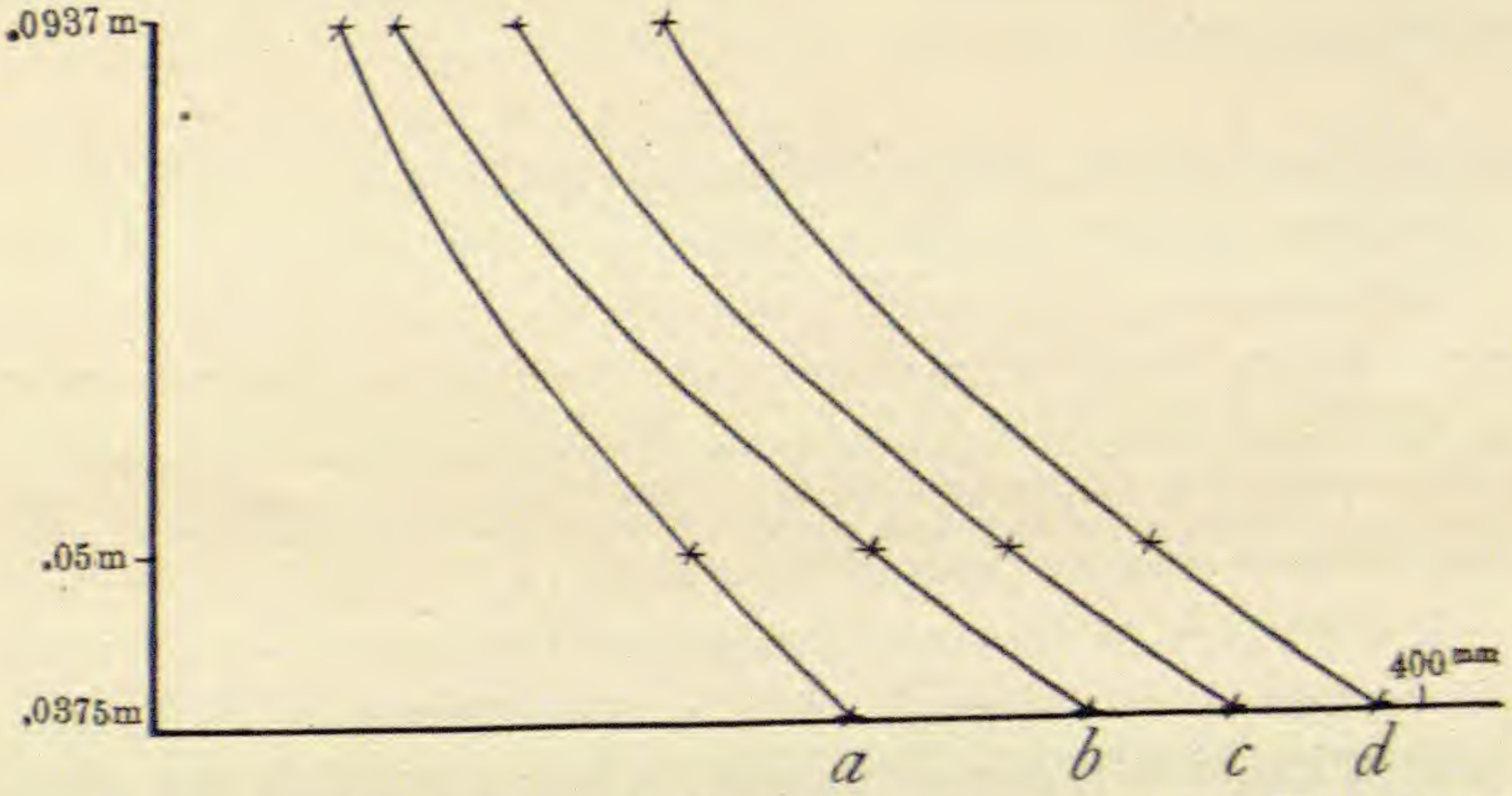

FIG. 3.—Curves showing growth of wheat roots in salt solutions. The ordinates represent concentrations (parts molecular); the abscissae represent the aggregate length of roots per plant in millimeters. *a*, 100^{cc} KCl + 100^{cc} $MgCl_2$; *b*, 100^{cc} KCl + 50^{cc} $MgCl_2$; *c*, KCl; *d*, 100^{cc} KCl + 25^{cc} $MgCl_2$.

TABLE VI

Wheat

Culture solution quantities in cc.	Growth during 60 days aggregate length of roots per plant in mm.		
	In .0937 m solutions	In .05 m solutions	In .0375 m solutions
KCl	110	268	340
100 KCl 100 $MgCl_2$	66	170	220
100 KCl 50 $MgCl_2$	72	224	294
100 KCl 25 $MgCl_2$	153	312	388
$MgCl_2$	10	20	28
50 K_2SO_4 50 H_2O	80	216	276
50 K_2SO_4 100 $MgSO_4$	36	112	144
50 K_2SO_4 50 $MgSO_4$	48	148	190
50 K_2SO_4 25 $MgSO_4$	60	166	240
$MgSO_4$	4	10	24
KNO_3	114	275	345
100 KNO_3 100 $Mg(NO_3)_2$	12	76	104
100 KNO_3 50 $Mg(NO_3)_2$	32	144	198
100 KNO_3 25 $Mg(NO_3)_2$	80	224	290
$Mg(NO_3)_2$	3.5	8	10
Distilled water	740		

Since each molecule of K_2SO_4 yields two K ions, half as much K_2SO_4 is used as KCl or KNO_3. The figures for 50 K_2SO_4+50 H_2O are comparable with the corresponding figures for KCl and KNO_3, though the concentration of the solution is only half as great. For example, the roots reach a length of 80^{mm} in 50^{cc} K_2SO_4 .0937 m+50^{cc} H_2O; a length of 216^{mm} in 50^{cc} K_2SO_4 .05 m+50^{cc} H_2O; and a length of 276^{mm} in 50^{cc} K_2SO_4 .0375 m+50^{cc} H_2O.

It will be noticed that these antagonistic effects are less marked as the concentration is lowered. This is of course true of all antagonistic action, since as the concentration is lowered toxicity diminishes and the effect of its inhibition is consequently less striking.

It is observed that those parts which are in direct contact with solutions always show their effects much more plainly than those

(e. g., leaves and stems) which are raised above them. It seemed desirable therefore to find out how sections of stems and roots would behave in the solutions. The answer to this question is given in Table VII. Transverse sections of the stem of Tradescantia and the root of the common red beet were employed. They were cut on a microtome and were of considerable but uniform thickness.

TABLE VII

CUTTINGS AND SECTIONS

All quantities given are cubic centimeters of .0937*m* solutions

CULTURE SOLUTION	DURATION OF LIFE IN DAYS		DEVELOPMENT
	Microtome sections of stem of *Tropaeolum majus*	Microtome sections of root of *Beta vulgaris*	Cuttings 15^{cm} long of Tradescantia
KCl	20	14	No roots
100 KCl 40 $MgCl_2$	28 +	27	Short roots
$MgCl_2$	20	18	No roots
Distilled water	28 +	28 +	Long roots

A plus sign indicates that the plants were alive at the end of the experiment.

In both cases the color and microscopic appearance served as the criterion of death. As is seen in Table VII, the results agree with those already obtained. The table likewise shows the results obtained from cuttings of Tradescantia (about 15^{cm} long) which were placed with their lower ends in the solutions.

In view of the striking agreement of results obtained from such a variety of material, it seems useless to seek for further proof. The experiments of LOEW and ASO also show antagonism between potassium and magnesium, as far as they go. They do not, however, employ sufficient potassium (nor sufficiently strong solutions) to bring out the results clearly. The use of percentage solutions (rather than molecular solutions) likewise obscures their results. More fundamental is their confusion of physiologically balanced solutions with ordinary nutrient solutions.[5]

As for the theory of LOEW and ASO that the inhibitory action of potassium on magnesium is due to the formation of a double salt, I

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[5] Cf. OSTERHOUT, On nutrient and balanced solutions. University of California Publications, Botany 2:317. 1907; also, BOT. GAZETTE 44:259–272. 1907.

need merely say that it cannot be true because this inhibitory action is seen in mixtures of potassium nitrate and magnesium nitrate where no double salt is formed. Moreover, even in the chlorids and sulfates the formation of a double salt cannot much affect the result, since the double salt, at the concentrations here used, dissociates and sets free magnesium and potassium ions to almost the same extent as the simple salts.

RESULTS

Magnesium salts and potassium salts, used separately, are poisonous to plants, but when mixed together (in suitable proportions) the poisonous effects more or less completely disappear. These results are of importance in soil investigations.

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NEREOCYSTIS AND PELAGOPHYCUS

WILLIAM ALBERT SETCHELL

Nereocystis Luetkeana (Mert.) P. & R. is one of the most striking of all the algae of the western coast of North America, yet very varied statements are made concerning its dimensions and duration. This matter has been brought up again recently by Professor T. C. **FRYE**, who in an article in this journal (**42**:143-146. 1906) states certain facts and suggestions from his observations on this species as it occurs in Puget Sound.

In the first place, **FRYE** doubts that this species ever reaches a length of over 21^m, although in no case does he mention that he has made any measurements, and he regards a length of 90^m as beyond the bounds of credibility. It is to be regretted that so few authentic measurements are on record for plants of this species, especially for those of the greater lengths. It is difficult, however, to obtain reasonably perfect plants out of the water and in such a position that they may be measured with accuracy. The statement of 90^m rests, so far as I know, upon that made by **MERTENS** (Linnaea **4**:48. 1829) that the stipes reach a length of 45 *Faden* or fathoms, making in all something approaching 90^m, and that the Aleuts use them for fish-lines, one of which he himself bought. It seems likely that **MERTENS'** statement about the length of these lines must have been made with care, as are his statements in general. Of course it may be that there is a misprint here for 15, which, from all experience, seems likely. **RUPRECHT**, however, says (Neue oder unbek. Pfl. a. d. nördl. Th. des stillen Oceans, p. 22. 1852) that Nereocystis, in the neighborhood of Bodega, California, reaches a length of 25 English feet, but that according to trustworthy natives in the employ of the N. A. Company at Fort Ross, it reaches a length ten times greater in the neighborhood of Sitka and the Aleutian Islands. This would correspond with **MERTENS'** figures. This impressed **RUPRECHT** to such an extent that he suggested (*l. c.* 22) that the southern smaller plant might belong to a different species. I have had an opportunity of observing this species at different localities of its growth from Kadiak Island on the coasts of the Alaskan Peninsula down to Port Harford in California. I have never seen any specimens which approximated to the dimensions mentioned by MERTENS and RUPRECHT. All along the west coast, however, it grows out into water having a depth of 12 to 15 fathoms, and this, which is about 30^m, represents the length of the solid portion of the stipe of the adult plant. The hollow portion, both apophysis and bulb, commonly floats on the surface, as do also the long leaves. Off the entrance to Yakutat Bay, Alaska, while at anchor during a fog, I saw soundings taken close to and among Nereocystis plants, showing that the solid portions of the stipes were 10 to 12 fathoms in length. Altogether the plants thus observed must have been in the neighborhood of 45 to 50^m total length, or nearly, if not quite, twice as long as the maximum credited by FRYE. On the coasts of California, I have measured many specimens cast ashore. The longest specimen, in reasonably perfect condition, I ever found cast ashore was on the beach at Carmel Bay in Monterey County, California. Not having a tape line, I resorted to pacing. Its greatest length was 41 good paces, which by measurement of my average pace fully equals that number of meters. All these figures from my own experience come from plants growing in the open. From these and from the experience of the great length attained by the various plants growing in swift currents, I am not prepared to reject the greater lengths recorded by MERTENS and RUPRECHT.

FRYE makes some point of the rock attachment of *Nereocystis Luetkeana* and it certainly is commonly attached to the rocks or to stones. On the Californian coasts, however, I often find it anchored firmly by its holdfasts to other members of the Laminariaceae, particularly to *Pterygophora californica.* I have seen stipes of Pterygophora to which as many as five or six holdfasts of Nereocystis were attached.

The origin of the numerous blades of *Nereocystis Luetkeana* has been made plain through the investigations of a number of writers. At first there is a single blade when the plant is in the primitive laminarioid condition. Soon, and at about the time that the bulb is beginning to form, this splits into two, which by the expansion of the bulb are separated considerably at the base. These in turn split again and are also in the same way separated at the base. The first two or three splittings, thus taking place when the bulb is rapidly expanding, become widely separated and form four to six centers for further splitting and growth. This is well shown in the fine plates of POSTELS and RUPRECHT (Ill. Alg. *pls. 8, 9.* 1840), in that of SAUNDERS (Algae of the Harriman Expedition. *pls. 58, fig. 1,* and *59, fig. 8.* 1898), and less accurately in that of MACMILLAN (Bull. Torr. Bot. Club **26**:*pl. 361. fig. 5.* 1899). The normal certainly seems to be four centers thus separated and which then proceed to form blades by further splittings, but without any considerable separation. I have also described this process in my paper "The elk-kelp" (Erythea **4**:183. 1896). The type of division in this case is a regular dichotomy but, as happens just as considerably in Lessonia, the absolute regularity is interrupted in minor details. However, *Nereocystis Luetkeana* has the regularity of its dichotomy as complete as do the species of Lessonia, and has no such scorpioid unilaterality in its branching and in the succession of divisions leading up to it as does its near relative Macrocystis. Between the two, but nearer, as it seems to me, to Macrocystis in this respect is Pelagophycus. SKOTTSBERG (Zur Kenntn. d. Subantarkt. u. Antarkt. Meeresalgen **1**:137, 138. 1907) seems to take the opposite view and to consider that after the first splitting in *Nereocystis Luetkeana,* the other splittings are unequal as in Macrocystis. An investigation of a large number of older and younger individuals convinces me that such is not the case. In Postelsia also the splitting is largely equal, but finally there comes something of unilateral splitting, so that while Postelsia really stands next to Lessonia and Nereocystis, it approaches slightly also Pelagophycus. This point will be returned to below.

Since the time of MERTENS, it has been generally received and all evidence has seemed to show that *Nereocystis Luetkeana,* in spite of its large size, is an annual plant. In his paper FRYE has distinctly discredited that belief, and while he does not say so in so many words, he intimates very strongly that it is a biennial plant. He says (*l. c.* 143): "Fishermen and pilots . . . . say that it disappears in winter," and later (p. 143) he says: "The fishermen are partly right. Except for stragglers here and there, the kelps are gone; while those remaining were nearly all decayed and loose, with their fronds mostly torn away." This observation was made in March to supplement observations made the previous June. Also in March, however, he finds plants 3 to 9^m below the surface which have reached a length of 1.25 to 2.5^m, in consequence of which he makes the significant statement that "it seems that they do not reach the surface the first year but remain out of reach of waves, pushing up rapidly in the second season only to die when winter overtakes them." He does not tell us what period of time is included in what he calls the first season, but he does call the second season also the second year. It is proper to assume, then, as it seems to me, that he believes that in the first year, spring and summer probably, the growth is slow and only plants up to 2.5^m or thereabouts are produced, while in the second year the rest of the growth of 18 to 20^m is produced. Growth during the winter season also must be, according to his idea, very slow or stopped altogether. This assumption seems necessary in order to arrive at the conclusion that the growth of 1.25 to 2.5^m had been accomplished in the previous year instead of a few previous months. Yet it does not seem that the assumption is justified by known facts, since evidently the date of the germination and season of the sporeling stages have not been observed. That the plants of colder waters, especially Phaeophyceae, can carry through their entire life-processes at a temperature which never rises above the freezing-point has been demonstrated by KJELLMAN (Algae of the Arctic Sea, p. 31. 1883), since he says: "At that depth where the richest marine vegetation is to be found, it [the temperature] does not rise in general above 0° C. at any time of the year." From what we know of the temperature of the surface waters of the region of Puget Sound, it seems safe to infer that the temperature of the waters within a few fathoms of the surface cannot be lower than somewhere between 5° and 10° C. at any time of the year. Furthermore, one cannot judge of the temperature of the water by the temperature of the air, and especially of the water 3 to 9^m below the surface. Consequently, even if the surface of the ground were frozen (cf. FRYE, *l. c.* 144) it is not necessarily true that the water at the depths stated might have been anywhere near the freezing-point. Farther, KJELLMAN adds (*l. c.* 31 ff.) that the temperature of the air affects only the algae of the littoral zone, for it is only those algae which are exposed to the air. Finally, it seems to me, after considering all the facts upon which FRYE's conclusion is based, at least so far as they are presented, that it is not necessary to assume that the initial growth of 2 or 3^{m} must have taken place in the previous year, especially when it is clear that a growth of 18^{m} can occur in two months and a half, viz., from the middle of March to the first of June. There certainly seem to be no theoretical impossibilities in the way of its having all taken place in the same year.

Nereocystis Luetkeana is fairly abundant on that portion of the coast of California lying to the north of Point Conception and I have followed its growth with some care. My conclusions are different from those of FRYE. Many plants arise in pools and in the deeper tideways among the reefs, and some persist through to the fruiting stage. Such plants are easy of observation and the results may be checked by more limited observation of the plants of the deeper waters. I find that they appear in February and March, for the most part, and in places where none are to be seen in December or January, and that they pass through their different stages of growth and fruiting by November, and for the most part disappear in December or January of the same year. The young plants with and without bulbs are in greatest abundance in March, although in some years they are present early in February, and this occurs in the same locality year after year. Belated specimens appear later, but as stragglers, the general rule being as stated above. It has been possible, therefore, to follow the same group of individuals through their life-history, and the result shows that the period of active existence is about nine or ten months and within the period of one year. Exceptional times of germination may occur and I find some, but in such cases the plants are usually tide-pool plants and do not develop beyond a certain stage. MACMILLAN mentions (*l. c.* 274) a set of sporelings of 0.5^{mm} and upward in size, in two feet of water in June, off San Juan Island, Washington. I have never found any young plants so late in the year, but I have found adult fruiting plants as late as April and in prime condition, but they are decidedly out of season, and the cases can be paralleled among annual flowering plants, so that they do not really affect the general truth of the statement as to the annual character of these plants.

The floating seaweeds of the group of the Phaeophyceae have long attracted attention, especially from the early navigators, and while attention in the Atlantic Ocean has been directed particularly to the species of Sargassum or gulfweed, in the Pacific several of the larger members of both Fucaceae and Laminariaceae were early used as indications of the approach toward land. Of all of these, perhaps no one was of such usefulness in this connection as the so-called *Porra* of the Spanish navigators approaching the coast of Lower California on their voyages from the Philippine Islands. RUPRECHT (*l. c.* 22–24) has given a fairly full discussion of this and other names applied by the Spaniards to these floating algae, but he did not have access to all the books, nor is the case different with myself. I have one point, however, to add to the account of RUPRECHT, which I think will clear up the more important part of the subject.

One of the earlier references is that of ANSON, whose voyage round the world in the years 1740 to 1744 was written from his papers and other materials by RICHARD WALTER, chaplain of one of the ships of the expedition, and passed through many editions. On p. 335 of the fourth abridged edition (London, 1748), and in the same language in the larger or so-called Admiralty editions (p. 243 in 1st, 1748; same page in 12th, 1767, and in 15th, 1776), occurs the following, regarding the "Manila ship":

> . . . . and when she has run into the longitude of 96° from Cape Espiritu Santo, she generally meets with a plant floating on the sea, which being called "Porra" by the Spaniards, is, I presume, a species of sea-leek. On the sight of this plant they esteem themselves sufficiently near the Californian shore, and immediately stand to the southward; and so much do they rely on this circumstance that on the first discovery of the plant the whole ship's company chaunt a solemn *Te Deum*, esteeming the difficulties and hazards of their passage to be now at an end; and they constantly correct their longitude thereby, without ever coming within sight of land.

ANSON's account gives us no further details concerning the nature of the plant. LEGENTIL, however, goes more into detail in his *Voyage dans les mers de l'Inde*, published at Paris in 1781. The expedition was made in the years 1768 and 1769, to observe the transit of Venus, and made many observations of a scientific nature in other lines than astronomy. On p. 215, he quotes ANSON in regard to *Porra*, translating into French the passage quoted above. LEGENTIL, however, goes on to give a figure of the *Porra* (cf. Vol. II, *pl. 3*) from a drawing made by Father DON ESTEVAN ROXAS Y MELO. The drawing is an excellent likeness of our Californian *Pelagophycus giganteus* Aresch., and will be discussed later. LEGENTIL consulted M. GUETTARD of the Royal Academy in regard to the nature of this plant and obtained the following opinion (*l. c.*):

> Cette plante . . . . est une espèce de celles qu'on appelle en France du nom de *varech*, goëmon, et par les Botanistes de celui de *fucus:* cette espèce est singulière, en ce que le haut de la tige est terminé par un tubercule ou une vessie, dont les branches partent, et, en ce qu'il paroît, que les pedicules des feuilles sortent d'un même côté. Il me semble qu'elle n'a pas encore été gravée; je ne l'ai pas reconnu parmi celles dont il est parlé dans l'ouvrage de Linné, intitulé *Espèces des Plantes;* elle n'est pas non plus dans l'ouvrage de SAMUEL GOTTLIEB GMELIN, qui en a fait graver un grand nombre d'espèces; on le voit point parmi les plantes de Mexique gravées dans l'ouvrage de HERNANDÉS sur ce pays; il n'est pas plus fait mention dans *l'Histoire des Barbades* par HUGUES, ni dans la *Collection des Plantes* de MORISON. Il me paroît que cette plante est nouvellement découvert par les Européens, et qu'il est bon d'en donner une figure gravée.

GUETTARD also supplied a Latin diagnosis, as follows: "Fucus ramis ex tuberculo rotundo exientibus, foliis planis, profunde crenatis, pediculatis, pediculis uno versu dispositis."

There has been some discussion as to the nature of *Porra* and other floating seaweeds of the western coasts of the Americas, and the best résumé thus far given is that of RUPRECHT (*l. c.*) who, although he had not seen the plate of LEGENTIL, came to the conclusion that it must be a species of Nereocystis, noting, however, the discrepancies between the descriptions and this *N. Luetkeana,* the only species known to him. A statement to the same effect is to be found in POSTELS and RUPRECHT'S *Illustrationes Algarum* (p. 1), with the reference, however, to their species—*N. Luetkeana*—which is probably the *Porra* of some of the Spanish writers. There is also a *Porra* of the South American coasts, for RUPRECHT says (*l. c.* 22) that BORY identified the *Porra* with Macrocystis, and that the *Laminaria porroidea* of LAMOUROUX, as represented by the type specimen, is *Durvillaea utilis.* Again, MAURELLE, according to RUPRECHT, speaks of floating seaweeds found in lat. 38° 14′ N. of two sorts: *Cabeza de Naranja,* or sea oranges, and *Zacate del mare.* The former are likely to be *Nereocystis Luetkeana,* while the latter, RUPRECHT argues with seeming probability, may be Macrocystis. I find on p. 98 of

Vol. I, of the English translation of LAPEROUSE'S *Voyage round the world* (London, 1799), MAURELLE'S observation that in lat. 47° 40′ N. "they observed that the sea was colored as in soundings, and they saw numbers of fish, reeds twenty feet long, and a species of seaweed with a head resembling an orange." In spite of the suggestion that this may have been the *Alcyonium aurantium* Pallas, it seems most probable that it was *N. Luetkeana.* Later in the same volume, LA PEROUSE speaks of the same plant as follows: "As we advanced to the north and approached America, we observed seaweeds of a species entirely new to us. A ball of the size of an orange terminated a stalk forty or fifty feet long, resembling an onion run to seed, but much superior in size." This was in the latitude of Mt. St. Elias on the Alaskan coast, where floating *N. Luetkeana* is common.

Following the suggestion derived from RUPRECHT'S account just alluded to, I have been able to find, by the help of Librarian F. J. TAGGART, a copy of the rare first edition (1780?) of MAURELLE'S *Journal of a voyage in 1775 to explore the coast of America, northward of California* (reprinted in 1781 in PARRINGTON'S *Miscellanies*), in the Bancroft Library of the University of California. MAURELLE'S observations on the floating seaweeds, as there set forth, are as follows (p. 12):

> June 1, 1775. On the same day we observed some seaweeds, the top of which much resembled an orange (*una naranja*), from the upper part of which hung large and broad leaves. At the extremity of this plant is a very long tube, which fixes it to the rocks on the coast until it is loosened by the sea, when it often floats to the distance of 100 leagues. We named this plant the "orange head." The next day we saw another plant with long and narrow leaves like a ribband, which is called *Zacate del Mar;* we also saw many sea-wolves, duck, and fish.

This observation was made when they were apparently well north of Point Conception and the "orange heads" were probably *Nereocystis Luetkeana.* Again he says (p. 25): "We observed that the sea was colored as in soundings; many fish, reeds 20 feet long, and the 'orange heads' likewise appeared." This was on July 9, apparently in the neighborhood of the Straits of Juan de Fuca. He goes on to say (p. 34): "On the 13th of July, we perceived ourselves to be in soundings from the color of the sea; at the same time appeared 'orange heads,' many flags," etc. At this time they were apparently in lat. 55° or 56° N.

Later on, when giving instructions as to approaching the west coast of North America from the Pacific Ocean, he says (p. 56):

> When the coast is 80 or 90 leagues to the E., those sea-plants appear which I have before called "orange heads;" but I must now add, that from the state of them as they float, one may sometimes infer that the land is not so far distant. Its figure much resembles the fistular stalk of garlick; and from the top of its head hang some very long leaves, by which the plant is fixed to the rocks. Now if these leaves are tolerably perfect, they afford a strong presumption that they have not floated far from the coast. On the contrary, those which have been wafted to a considerable distance have generally lost this head, and the stalk becomes more rough, when you may suppose that you are 50 leagues from land. [He further says (p. 57)] When you are 30 or 40 leagues from the coast, ... you will perceive birds, ... together with the plant *Zacate del Mar* before mentioned, which has long and narrow leaves.

It is very evident that both species of the west-coast kelp with the large bulbs were known to the navigators, and it is of interest that the southern species *Nereocystis gigantea* Aresch. or *Pelagophycus giganteus* Aresch. was known so long before it was generally recognized by botanists. The description of GUETTARD, furnished to LEGENTIL, is reasonably explicit, but, when taken into account in connection with the plate referred to above, leaves no doubt as to the identity of the plant with that of ARESCHOUG. The first botanical description, however, was given by LEMAN (Dict. des sciences naturelles **25**:189. Paris. 1822) under the article Laminaria. It reads as follows:

> *Laminaria porra* nob. Stipe très-long, terminé par un renflement fusiforme, portant une grosse vessie sphérique, couronnée de frondes lanceolées, très-élongées et profondément dentées. Cette espèce a été observée dans le mer du Sud par LEGENTIL. Les marins espagnols la nomment *porra*. Elle se fait remarquer par sa longueur qui excède de quarante brasées ou de deux cent pieds. (Voyez LeGentil, Voyage Ind., p. 2. *pl.* 3.)

From this it becomes evident that the earliest specific name for the southern species is *Porra*.

ARESCHOUG first referred the southern or elk-kelp to the genus Nereocystis, but later created the genus Pelagophycus for its reception. The close resemblance between it and Nereocystis, however, has led most writers to retain both in the same genus. This was my own opinion in the paper entitled "The elk-kelp"(Erythea **4**:179–184. *pl.* 7. 1896), but at the same time I called attention (*l. c.* 184) to the unequal splitting and the consequent close relation to Macrocystis.

SKOTTSBERG has given a most careful and valuable discussion of the relationships of the Lessonia group in the Phaeophyceae of his *Subantarktischen und antarktischen Meeresalgen* (Wiss. Ergebn. d. Schwedischen Sudpolar-Exp. **4**:Lief. 6. 1907) and takes up this matter in that connection. His decision is to retain the species in Nereocystis (*l. c.* 139). He seems to incline toward the view that the splitting in Nereocystis is also unilateral. With this view, as indicated above, I cannot find myself in agreement. I have examined many, both older and younger, *Nereocystis Luetkeana*, with the result that I find the splitting to proceed fairly uniformly dichotomously as noted above. In Pelagophycus, on the contrary, the splitting is decidedly unilateral and like that of Macrocystis. A young plant, with characteristic adult form though not of adult dimensions, is represented in the photograph reproduced in connection with my paper. SKOTTSBERG has remarked that there are five leaves on one branch and six on the other. There were six on each, but on one only the petiole remains to show its position. In the specimen figured, the branching has been absolutely unilateral after the first splitting, as is shown by the fact that the leaves are single. In many specimens, however, some of the lower leaves divide a second time, as happens constantly in young specimens of Macrocystis, but not more than one or two usually do this. In this, as in other characters, this plant is intermediate between Nereocystis and Macrocystis. From all the evidence it seems to me best to keep the genus Pelagophycus, and to associate it with the Macrocysteae under the tribe of the Lessoniideae.

As has been shown above, LEMAN'S name of *Laminaria Porra* is the earliest binomial, which necessitates the new combination:

Pelagophycus Porra, comb. nov.

Laminaria Porra Leman. Dict. Sci. Nat. **25**:189. 1822.
Nereocystis gigantea Aresch. Bot. Notiser **1876**: 71.
Pelagophycus giganteus Aresch. Bot. Notiser **1881**: 49.

I desire to thank MR. F. S. COLLINS of Malden, Mass., for transcripts of the reference of LEMAN and also that of LEGENTIL to *Porra*. To F. J. TAGGART, librarian of the Bancroft Library of the University of California, I am indebted for assistance in obtaining access to the works of MAURELLE, ANSON, and LAPEROUSE.

UNIVERSITY OF CALIFORNIA
Berkeley

BRIEFER ARTICLES

NOTES ON CLEISTOGAMY OF GRASSES

(WITH FIVE FIGURES)

The genus *Triplasis* Beauv., so far as we can find, has not been recognized as one of the many genera of grasses producing cleistogamous spikelets. Its nearest relative recognized as having this habit is *Sieglingia decumbens* (L.) Ktze. This and other cleistogamous grasses are discussed by Professor EDUARD HACKEL (Oest. Bot. Zeits. **56**:81–88, 143–154, 180–186. 1906). Specimens of Triplasis in the National Herbarium collected in autumn show reduced panicles wholly or partly included in the sheaths, and bearing few to several cleistogamous spikelets with glumes and awns much reduced, but otherwise like those of the terminal panicle.

Specimens of *T. purpurea* (Walt.) Chapm. collected on the low dunes of the Isle of Palms, S. C., October 18, 1907 (*Chase* 4524), show an additional form of cleistogamous spikelet. This is larger, solitary, and sessile in the base of an indurated prophyllum, in the wings of which it is clasped together with the first internode of the branch, lying between the branch and the back of the prophyllum. The glumes are wanting, repeated dissections failing to show even traces of them. The spikelets are one-flowered, or sometimes have a rudimentary second floret; the lemma and palea are thin, the awn of the lemma and beard of the palea much reduced. (The figures show the difference between this spikelet and one from the terminal panicle, also the comparative size of the two developed grains.) This second form of cleistogene was discovered in the field, and plenty of material for dissection was collected. Most of the culms produced two to eight or nine of these spikelets, beginning usually at the second node. The spikelets in the upper sheaths have smaller florets and rudimentary glumes, and grade into the ordinary form of cleistogene. Since herbarium material cannot be ruthlessly disjointed, only a few sheaths of autumnal specimens, which by their swollen appearance gave promise of containing these cleistogenes, were examined. Of these, one specimen of *T. americana* Beauv. from Florida (*Combs* 871) showed this form, the glumes wanting, the awn, which in this species is 5^{mm} or more long, nearly obsolete. A duplicate type of *T. intermedia* Nash (*Nash* 2426) was found to contain these cleistogenes, and judging by the swollen sheaths they were abundant. This is true also of specimens of the same species from Cape Florida (*Chase* 3959). Of *T. purpurea* (Walt.) Chapm., practically all the late collections contained these spikelets. Hence this habit of producing two forms of cleistogene belongs to the entire genus. Unlike the dichotomous species of Panicum, in which only the cleistogenes perfect their grains, the terminal spikelets are fruitful in Triplasis as well as the two forms of cleistogene. The presence of these cleistogenes at the nodes explains the habit, common to the three species, of disjointing at the lower nodes when dry.

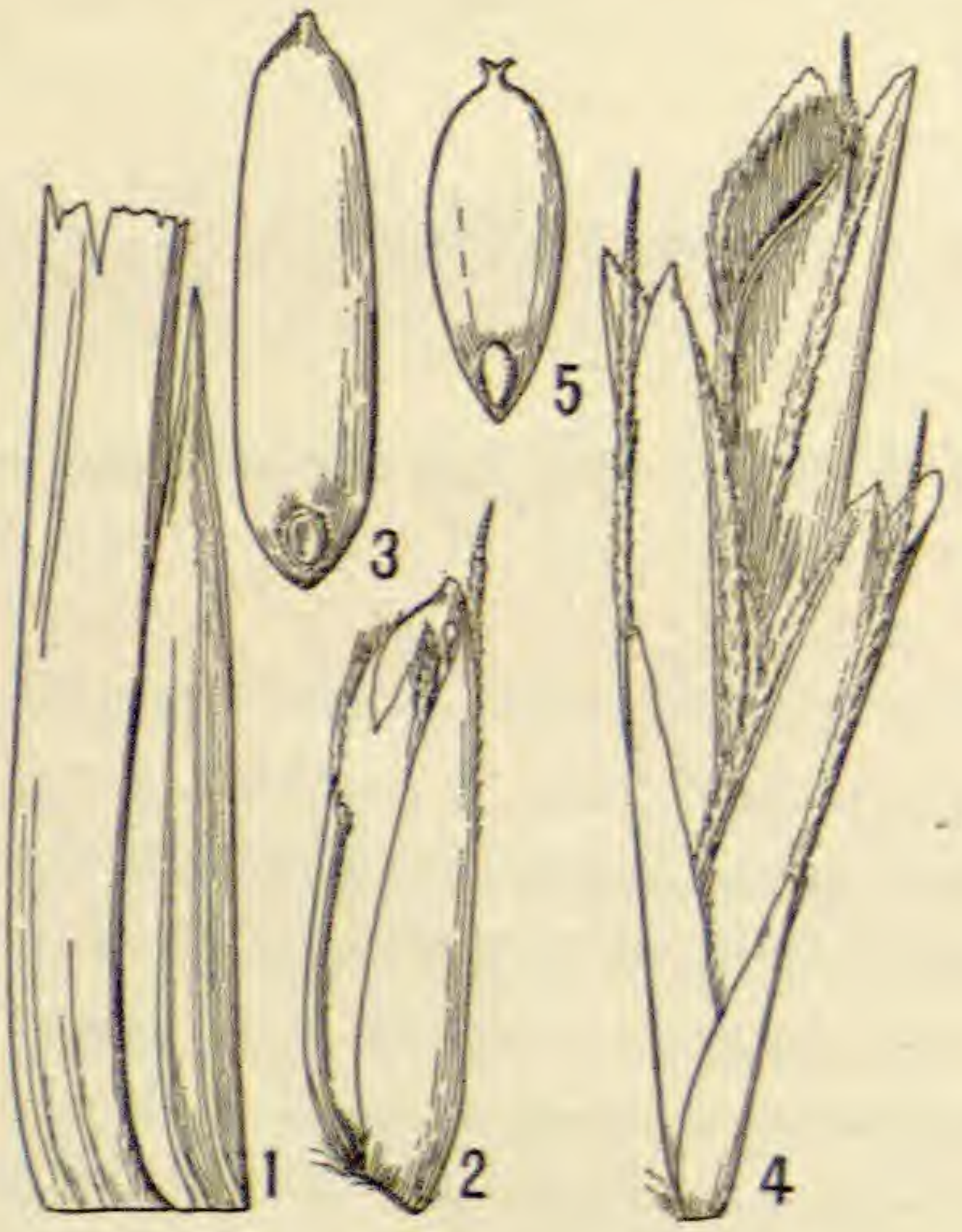

FIG. 1. Base of internode showing prophyllum inclosing cleistogamous spikelet. ×4.—FIG. 2. Cleistogamous spikelet. ×8.—FIG. 3. Grain from same. ×8.—FIG. 4. Spikelet from terminal panicle. ×8.—FIG. 5. Grain from same. ×8.—Both spikelets drawn from the same plant.

Another interesting point in the habit of cleistogamous grasses developed in the field-work of last October. *Amphicarpon amphicarpon* (Pursh) Nash was found with perfectly developed grains in the aerial spikelets. These specimens were collected in the border of a cypress swamp between Wilmington and the eastern coast of North Carolina (*Chase* 4597). After noting these fruitful spikelets, practically all the plants in the small colony were examined, perhaps a hundred, and about fifteen were found with fruiting aerial spikelets. Most of these possessed developed subterranean spikelets also. The aerial spikelets have heretofore been supposed to be sterile. The earlier authors—PURSH, who first described the species under Milium, KUNTH, and BENTHAM—called them staminate and the subterranean spikelets pistillate. It is doubtless very seldom that these aerial spikelets develop their grains. Herbarium material was examined and a single specimen of *A. amphicarpon* from Delaware (*Commons* 18 of 1895) was found with fertile aerial spikelets. No such spikelets were found in *A. floridanum* Chapm.—AGNES CHASE, *U. S. Department of Agriculture, Washington, D. C.*

CURRENT LITERATURE

BOOK REVIEWS

Form and flowering

An interesting discussion of the relations between the vegetative form and the flowering period of plants is presented by Dr. DIELS in a small book,[1] issued more than a year ago, which deserves notice in spite of unavoidable delay.[2]

The questions with which the book deals were raised by the author's travels in West Australia in 1902. After his return he examined the literature and made further investigations to throw light upon the problems of form in the plant kingdom. He has gathered together a considerable number of examples of the relation between form, blooming time, and external conditions. These he presents and discusses in his usual luminous fashion. He has even cited briefly analogous phenomena, not a few from the animal kingdom.

The thesis of the book is that the generative maturity of plants is not connected immutably with a definitive stage of their development, as has been so widely held. A certain minimum of nutritive preparation is presupposed; but once this is passed there is a broad variation zone in which blooming occurs. Its appearance is dependent upon complex, largely unknown conditions, an important part of which, however, are external. The vegetative ontogeny depends upon the cooperation of autogenous and exogenous (an excellent substitute for the awkward term "aitiogenous") factors; for the rudiments of the vegetative organs have many possibilities, and which one is realized is determined by the environment. The mature form of the entire organism is thus a product of vegetative ontogeny and of generative maturity, both of which factors are variable, though their variability is not in the same direction. True, the development of vegetative structures usually ceases at blooming, but this is the only place where the two lines of development, the vegetative and the generative, are inseparably connected. Elsewhere they are free and independent of one another and each varies after its own manner. In this connection of two variable factors lies an important impulse to increase the manifold forms of the plant world. For the conditions which help to regulate the succession of leaf forms and floral maturity change with the changes of climate in space and in time, giving rise to local geographic species and allowing true species to arise in the course of time. Their features attain heritability and become therewith a source of new lines with new possibilities.

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[1] DIELS, L., Jugendformen und Blütenreife im Pflanzenreich. 8vo. pp. 130. *figs. 30.* Berlin: Gebrüder Borntraeger. 1906. *M* 3.80.

[2] The work was sent to a reviewer on its receipt, and was recently returned at our request.—EDS.

A new term, "helicomorphy," is suggested to comprehend GOEBEL's two terms for the two leaf forms in heteroblastic species, the juvenile forms and successive forms. In the course of a short chapter on the phylogenetic significance of helicomorphy, the author pays his respects to the famous "biogenetic law," that ontogeny recapitulates phylogeny, in these terms: "In the botanical field it has absolutely no (nicht einmal immer) heuristic value, and whoever allows himself to be led by it will at most succeed in satisfying the needs of his imagination."

The book is one to be commended to all sorts of botanists; morphologists, physiologists, ecologists, and taxonomists will alike profit by its perusal.—C. R. B.

NOTES FOR STUDENTS

Soil chemics and plant distribution.—Of late the physical character of the soil, and especially the relation of the soil to water, has been more and more regarded as the greatest single factor that determines plant distribution. For this reason the contribution of FERNALD[3] will come to most with something of a shock. FERNALD feels that the dissimilarity of the floras of the alpine districts of New England and adjoining regions cannot be explained by differences in precipitation, exposure, fineness or coarseness of the soil, or water content of the soil. On the other hand, it is possible to divide the alpine regions into three groups on the basis of similar floras. The striking thing that then appears is that the three groups can also be split in the same way on the basis of the chemical nature of the subjacent rocks. One group, including the Adirondacks, Green Mountains, White Mountains, and Mt. Katahdin, is characterized by granites, gneisses, or mica schists, all rich in potassium. The second group, including Smuggler's Notch, Mt. Willoughby, and the Gaspé coast, is characterized by calcareous rocks. The third group includes only the one remarkable area of Mt. Albert, where many plants are found that are known from nowhere else in that part of the continent; this area is one of serpentine. It will thus be seen that FERNALD's groups correspond to those made long ago in Europe by partisans of the chemical theory, except that the European silicicole group is replaced by FERNALD's potassium group. It is likely that in a complex world not everything is to be referred to one cause, and perhaps we have been too ready to see in soil water the answer to all distribution problems. If so, FERNALD's paper will serve an excellent purpose in giving better balance to our views. Yet it must be remembered that this paper is wholly observational, and it is very doubtful if physical and chemical factors can be adequately analyzed in the field. The trend of recent experimental work is mostly toward physical theories or toxic phases of chemical theories, and until FERNALD's theories are substantiated by rigid experimental tests, it is doubtful if they can find acceptance. It is well also to call attention to the idea expressed so long ago by DECANDOLLE in this connection, that what holds

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[3] FERNALD, M. L., The soil preferences of certain alpine and subalpine plants. Rhodora 9:149–193. 1907.

true for one region may be far from true in another. For example, *Shepherdia canadensis*, *Artemisia canadensis*, and *Pellaea atropurpurea* are cited as calcicoles. In the Chicago region, only the latter is commonly a calcicole, and it is also a silicicole. It is doubtful too if the chemical theory can account for the prevalence of alpine plants in bogs, a phenomenon readily explained by the current theories.—HENRY C. COWLES.

Ecological notes from New Zealand.—Some of COCKAYNE'S[4] brief papers should be noticed here. *Coprosma Baueri* is found to show a striking case of leaf variation. In the usual natural habitat the leaves are fleshy and have margins so recurved as to be called "rolled;" in shade the leaves are much larger and quite flat. An accompanying photograph shows how striking is the variation. The second paper discusses the supposed finding of a seashore Celmisia in the mountains; the two forms are very similar, but COCKAYNE believes, as everyone must in these days, that the court of last resort in the determination of the validity of species is no longer the herbarium but the experimental garden. The Poor Knights Islands are small precipitous islands exposed to the open sea and never before visited by a naturalist. The scrub formation, in which *Suttonia divaricata* dominates, differs from any allied New Zealand formation; the luxuriance of the arborescent plants is very remarkable in view of the great exposure of the islands. A Carmichaelia, known previously from but one place and that far away, probably attests that the species was once widespread. In another paper, COCKAYNE[5] has added another to the long list of probable mutants observed in nature. This case is of special interest because the mutation is in a plant well known in the region, and is too conspicuous to be overlooked. *Leptospermum scoparium* is one of the commonest weeds of New Zealand, and possesses white or sometimes pink flowers. The "mutant" consists of a crimson-flowered form, and it is noteworthy that the foliage is so much darker that it is easy to pick out the mutant in the seedling condition. In connection with COCKAYNE'S studies it should be noted that he has published a large amount of ecological literature in popular form in the *Lyttelton Times*, a Christchurch paper. These essays have been well written and must have done much to interest New Zealanders in the remarkable vegetation of their country, and to induce them to take steps to preserve this vegetation in large part through the medium of government reservations.—HENRY C. COWLES.

Plant-breeding in the tropics.—LOCK[6] has published a third report on hybridization experiments carried on at Peradeniya, Ceylon, giving in detail the results

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[4] COCKAYNE, L., On a specific case of leaf variation in *Coprosma Baueri* Endl.; on the supposed Mount Bonpland habitat of *Celmisia Lindsayi* Hook. f.; Notes on a brief botanical visit to the Poor Knights Islands. Trans. N. Z. Inst. **38**:341–360. 1906.

[5] COCKAYNE, L., On the sudden appearance of a new character in an individual of *Leptospermum scoparium*. New Phytologist **6**:43–46. 1907.

[6] LOCK, R. H., Studies in plant breeding in the tropics. III. Experiments with maize. Ann. Roy. Bot. Gard. Peradeniya **3**: 95–184. 1906.

of extensive crosses between various races of Indian corn. In almost every regard the important early work of CORRENS on this species is confirmed, it being again demonstrated that yellow endosperm is dominant over white, starchy over sugary, blue aleurone layer over white, and red pericarp over white. The latter being a character of the mother plant does not appear as "xenia." The same seems to be true, to some extent at least, with regard to the indent character of the grains. It is not clear from the experiments that this character segregates after the Mendelian fashion, but it is pointed out by the author that a certain looseness in the method of pollination used makes it impossible to interpret the behavior of this character. With respect to height of plant, all cases tested seem to indicate that there is a blend in F_1 and no segregation in later generations. The most important contribution to knowledge is the complete confirmation of the view that failure of the Mendelian ratio in the case of blue vs. white aleurone layer is due to defective dominance and not to imperfect segregation. In such crosses the blue appears in ratios almost invariably below expectation, sometimes considerably below, but subsequent breeding of the whites from such a cross shows that enough of them are heterozygotes to make up the deficiency in the number of blue grains in the preceding generation. The author speaks of the "dominance" of the white aleurone layer in this case, but this is not correct, for if the white were really dominant in the accepted sense, the crossing of these white heterozygotes with pure whites would give only white offspring; but this was the method used to demonstrate their heterozygote nature by showing that such a cross produces a considerable proportion of blues. The failure of dominance of blue in these cases is rather to be spoken of as "latency," and the possibility that there may be another factor involved, as in certain other known cases of latency (invisibility), is suggested and needs investigation.—G. H. SHULL.

Anatomy of Lobeliaceae.—The anatomy of the Lobeliaceae has been described by YDRAC,[7] who reaches the conclusion that JUSSIEU'S classification is more natural than that of BENTHAM and HOOKER, and that the family ought to be placed between the Compositae and Campanulaceae, instead of representing only a tribe of the latter. Many genera and species have been examined, and the author has not confined himself to the stem and leaf alone, but gives also many points of interest about the root structure, and a special chapter is devoted to the medicinal species. Characteristic of the family is the occurrence of articulated laticiferous ducts, which are located in the leptome and accompany this tissue in the roots, the stem, and the leaf; but the aquatic *Lobelia Dortmanna* is destitute of latex. None of the Lobeliaceae possesses bicollateral mestome strands, while these are frequently observed in the Campanulaceae and Compositae (Liguliflorae); the mechanical tissue is only represented by collenchyma in the stem, there being no stereomatic pericycle. The leaves are bifacial so far as concerns the distribution of the stomata (on the dorsal face), but the chlorenchyma is mostly of a homogene-

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[7] YDRAC, F. L., Recherches anatomiques sur les Lobeliacées. Thesis. pp. 165. Lons-Le-Saunier. 1905.

ous structure; many species contain inulin. It does not appear as if any decisive conclusion may be drawn from the internal structure alone in regard to the question of classification, and it must not be forgotten that the occurrence of bicollateral mestome strands in the Campanulaceae is not common to all members of the family. Of far greater importance seems to be the morphological structure of the flower, and quite especially of the corolla and the stamens. This same view was held by GRAY, and we find in his *Synoptical flora* that the Lobeliaceae are kept separate from the Campanulaceae.—THEO. HOLM.

Anatomy of Palaeostachya.—In an interesting article HICKLING[8] gives an account of the cone of an important calamitean species. There are approximately eighteen sporangiophores and an equal number of sterile bracts. The former are axillary to the latter and are supplied by traces originating from the same node above the vascular strands, which pass off to the bracts. In spite of the fact that the sporangiophores are apparently in the axils of the bracts, the sporangiophore trace turns sharply upward in the secondary wood of the cone-axis through half an internode, to redescend afterward through the cortex to the sporangiophore. It appears from this investigation that the condition which is present in Calamostachys, in which the sporangiophore is placed high on the axis, is more primitive than that found in Palaeostachya, where it is apparently axillary; but nevertheless the sporangiophore in both these genera is an axillary structure and constitutes the ventral segment of its subtending, divided or undivided, bract. A very interesting statement on the part of the author, in view of the opinions recently expressed by CAMPBELL and SCOTT in regard to the presence of foliar gaps in the equisetal series, is the following: "It (the trace of the sterile bract) arises from the primary wood of that (the main) bundle, just where the carinal canal is obliterated and passes radially outward and slightly upward through the nodal secondary wood. . . . . *No gap is left in the main bundle.*" The italics are the reviewer's.—E. C. JEFFREY.

Seed development in Saxifraga.—JUEL[9] has published a rather lengthy account of seed development in *Saxifraga granulata*, including some reference to and a few figures of *Pyrola minor*. In Saxifraga the nucleus of the megaspore shows an apparently simple, homogeneous chromatin thread in synapsis stage, and the double chromosomes of later stages seem to arise, not from a splitting, but from a doubling of the thread. The number of chromosomes is about thirty. The arrangement of the conducting tissue and the course of the pollen tube are described. No plasma sheath can be demonstrated about the male nuclei in the pollen tube, but a thin sheath is evident after the nuclei are discharged. The male cells pass into a synergid, and probably the tube nucleus also. The definitive

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[8] HICKLING, GEORGE, The anatomy of *Palaeostachya vera*. Annals of Botany 21:369–386. *pls. 32, 33*. 1907.

[9] JUEL, H. O., Studien über die Entwicklungsgeschichte von *Saxifraga granulata*. Nova Acta Reg. Soc. Sci. Upsal. IV. 1:1–41. *pls. 1–4. figs. 6*. 1907.

endosperm nucleus, which is fertilized by the second male nucleus, divides near the lower end of the sac, and following this division the protoplasm is separated by a plasma membrane into a large upper and a small lower cell. After two more divisions in the lower cell, walls appear, and four basal endosperm cells are formed. These elongate and divide again to form eight cells with dense contents which form a cap over the lower end of the more extensive endosperm tissue above. This lower tissue, while not so extensive, is similar in origin to that recently described in the Pontederiaceae. After examining the food-stuffs of the seed, the author closes with a description of a special tissue formed from the funiculus, which serves to separate the seed from the placenta.—W. C. COKER.

Roots of aroids.—LINSBAUER[10] has studied the growth and geotropism of the aerial roots of a large number of aroids. These are often dimorphic; some roots are long and strong, the so-called *Nährwurzel* (here called long roots); others are more slender, usually horizontal and tufted, the *Haftwurzel* (here called short roots). Of these only a few have been sufficiently examined since the rise of the statolith theory of geoperception. He extends SACHS's observation as to the great length of the growing zone; in long roots for most it lies between 20 and 50^{mm} with a minimum of $5-10^{mm}$ and a maximum of 90^{mm}; in short roots, however, it runs from $3-14^{mm}$. Yet the former grow less rapidly than the latter; nor do either grow daily more *in toto* than ordinary soil roots, and their growing zone is not so sharply marked. The typical long roots are mostly geotropic, though only weakly so, for in a whole day they do not curve from the horizontal to the vertical; and many, even under the most advantageous external conditions, are wholly or periodically ageotropic; typical short roots are always ageotropic.

When the roots are not dimorphic (as in Anthurium and some other genera), they are ageotropic. Both sorts have a well-developed columella in the root cap, which is furnished, so long as they are growing, with statolith starch, quite irrespective of their geotropic or ageotropic behavior. This fact LINSBAUER contents himself with stating and does attempt to use it against the theory or to reconcile it therewith.—C. R. B.

Production of diastase.—EISENBERG has attacked anew the problem of the regulatory production of diastase, determining the effect of various factors on its formation.[11] This was estimated by the effectiveness of a given solution in converting into sugar a standard solution of soluble starch. The results coincide in large measure with those of previous observers and strengthen the belief that diastase is formed in variable amounts according as other metabolic factors vary. Thus more diastase is produced in active, less in sluggish, germination. At the optimum temperature for growth diastase production is greatest. In germinating

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[10] LINSBAUER, K., Ueber Wachstum und Geotropismus der Aroideen-Luftwurzeln. Flora **97**:267–297. 1907.

[11] EISENBERG, ELFRIDE, Beiträge zur Kenntnis der Entstehungsbedingungen diastatischer Enzyme in höheren Pflanzen. Flora **97**:347–374. 1907.

wheat in the absence of oxygen, there is no increase in diastase, but the amount formed in air and in pure O_2 is equal. Increasing etherization of seedlings reduces their growth and also the diastase. Traces of acid favor the production of secretion diastase, but not of translocation diastase, while larger amounts hinder in both. In general leaves that readily store starch have much diastase, while those that contain sugar do not; but this is not uniformly true. Insolated starchy leaves have more diastase than starch-free shade leaves of the same plant. No increase of diastase on darkening pea leaves was observable; but the author does not consider the experiments with light satisfactory.—C. R. B.

Nourishment of embryos.—Basing his study upon the conclusions, already well established, that the endosperm is a live tissue which may affect *ipso facto* the development of the embryo, STINGL reports the results[12] of his experiments to determine how embryos were affected by other endosperm than their own. He tested rye, barley, oats, and wheat reciprocally. No embryo freed from the endosperm could be made to develop fully, nor even when replaced after the operation did it develop as well as undisturbed ones. The four sorts were unequally affected. Rye developed about equally with its own and wheat endosperm; not so well with others. Wheat did better with rye endosperm than with its own, but not so well with barley and oats. Barley flourished with wheat endosperm, grew less with its own and rye, and least with oats. Oats embryos developed far more uniformly with strange endosperms than the others did with oats endosperm, though it did best with its own. One must suspect that some neglected factor is accountable for the surprising conclusion that a plant may develop better with foreign endosperm than with its own.—C. R. B.

Alpine plankton studies.—SHANTZ[13] has made a comparative study of the plankton of the lakes of the plains and mountains near Pike's Peak. The different alpine lakes studied differ somewhat largely from each other, but in general their plankton is not abundant, and it is only in the fall that algae become dominant. The plains lakes studied are artificial; their plankton is many times more abundant than is that of the alpine lakes.—HENRY C. COWLES.

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[12] STINGL, G., Experimentelle Studie über die Ernährung von pflanzlichen Embryonen. Flora **97**:308–331. 1907.

[13] SHANTZ, H. L., A biological study of the lakes of the Pike's Peak region—preliminary report. Trans. Amer. Mic. Soc. **27**:75–98. 1907.

NEWS

MR. VERNON H. BLACKMAN, formerly of the department of botany of the British Museum, but more recently lecturer in botany at the Birkbeck Institute, has been called to a professorship in botany at the University of Leeds.

THAT THE International Botanical Congress of 1910 will be held in Brussels has been decided, an appropriation for it having been granted by the Belgian government. The arrangements for the Congress are in charge of TH. DURAND as president, and E. DE WILDEMAN as general secretary.

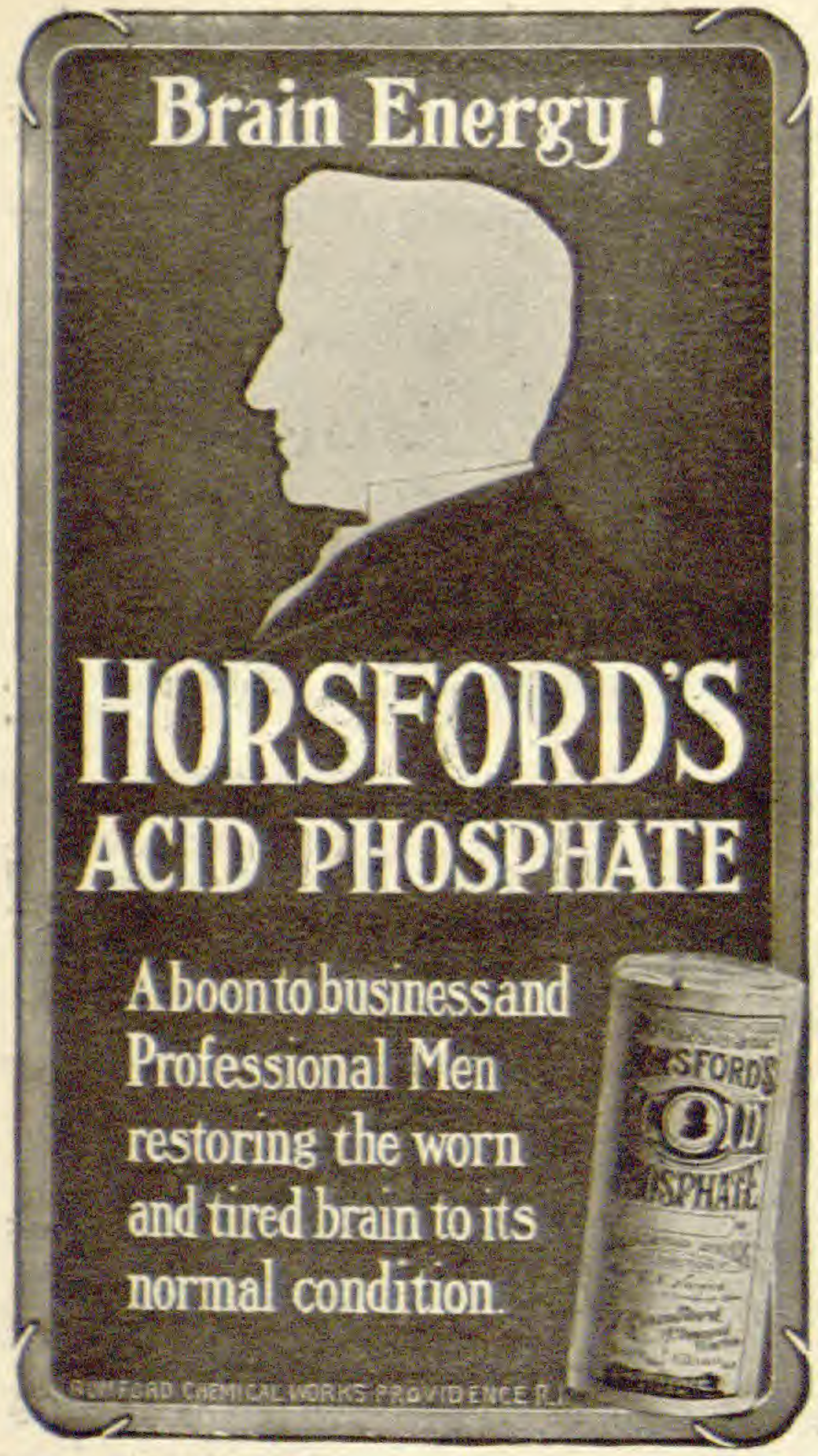
THE BOTANICAL STAFF of the government in the Philippine Islands consists now of seven members: Bureau of Science, E. D. MERRILL, F. W. FOXWORTHY, and C. B. ROBINSON; Normal School, W. R. SHAW; School of Agriculture, E. B. COPELAND; Forest Investigation, H. N. WHITFORD; Collector, A. D. E. ELMER.

A BIOGRAPHICAL SKETCH of the late Mr. A. P. MORGAN has been published by Professor W. A. KELLERMAN in the *Journal of Mycology* (13:233–236. 1907). The bibliographical list contains 46 titles, extending from 1877 to 1907, the first paper having appeared in the second volume of the BOTANICAL GAZETTE. Since 1902 all his papers (26) appeared in the *Journal of Mycology*.

THE FOLLOWING ACTION was taken, during the Chicago meeting of the American Association, by the Botanical Society of America, Section G of the American Association, and the American Society of Naturalists:

> WHEREAS, By the lamented death of Dr. LUCIEN MARCUS UNDERWOOD, late Professor of Botany in Columbia University, science has suffered a severe loss and the American Association, the Botanical Society of America, and the American Society of Naturalists an active and esteemed member, be it
>
> *Resolved*, That these societies place on record their recognition of his fruitful labors along his chosen lines in the field of scientific research and instruction, and their keen appreciation of the stimulating influence of his personal character and scholarly attainments.

THE PHILIPPINE BUREAU OF FORESTRY has been reorganized under Maj. GEO. P. AHERN as director. The field-work has been assigned to two divisions: Forest Administration, under H. D. EVERETT; and Forest Investigation, under H. N. WHITFORD. The work of the latter division is to discover the forest resources and to bring this information to public attention. A detailed system of mapping has been inaugurated, and considerable portions of the islands have been mapped. Special tracts are being studied in detail for working plans, and herbarium and wood specimens are being collected. The herbarium shows 1109 species of trees on the islands, and the number is likely to be increased to 1600 or 1800. During the three months beginning with November, Dr. WHITFORD has been exploring the island of Mindanao.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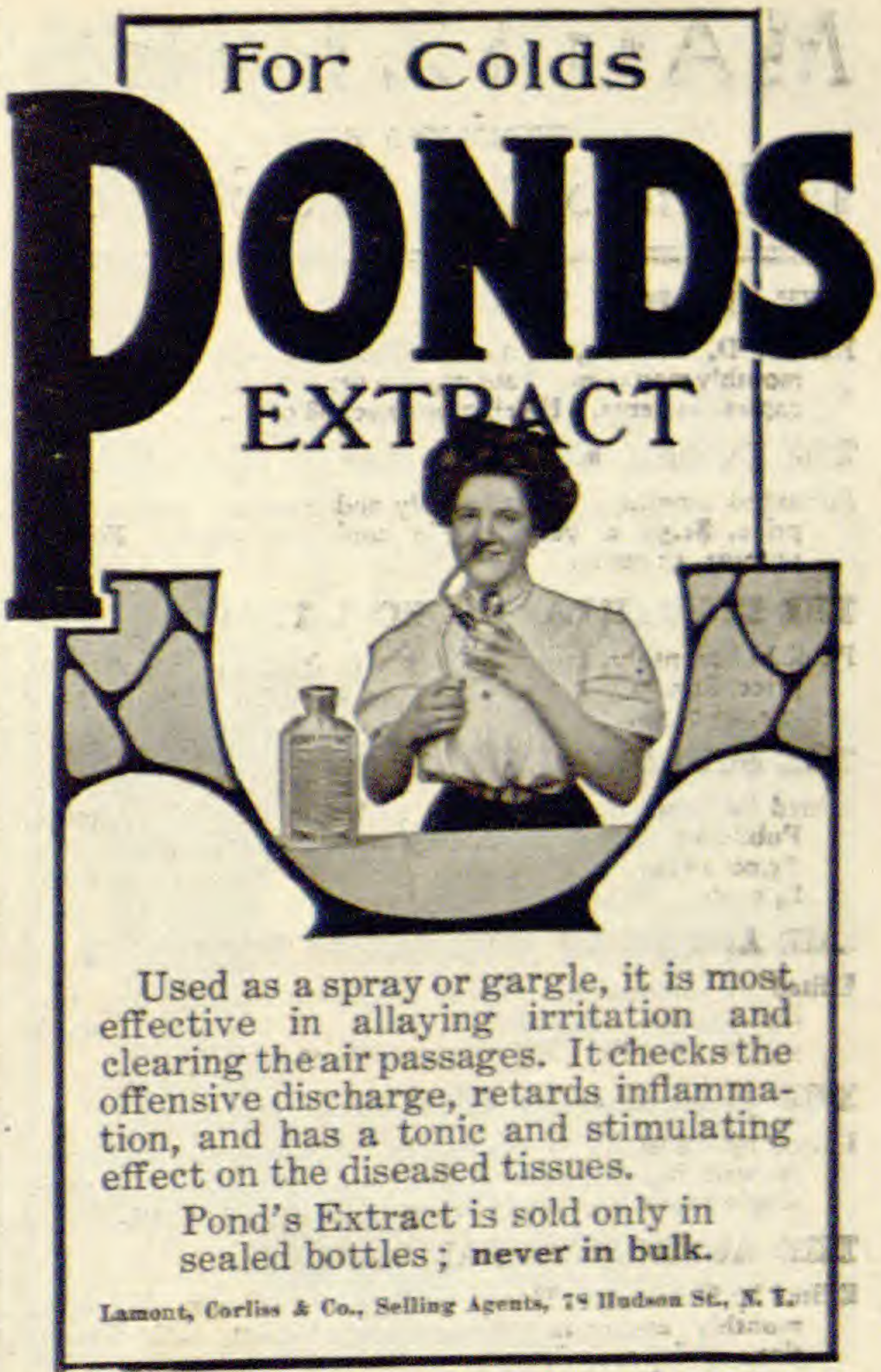
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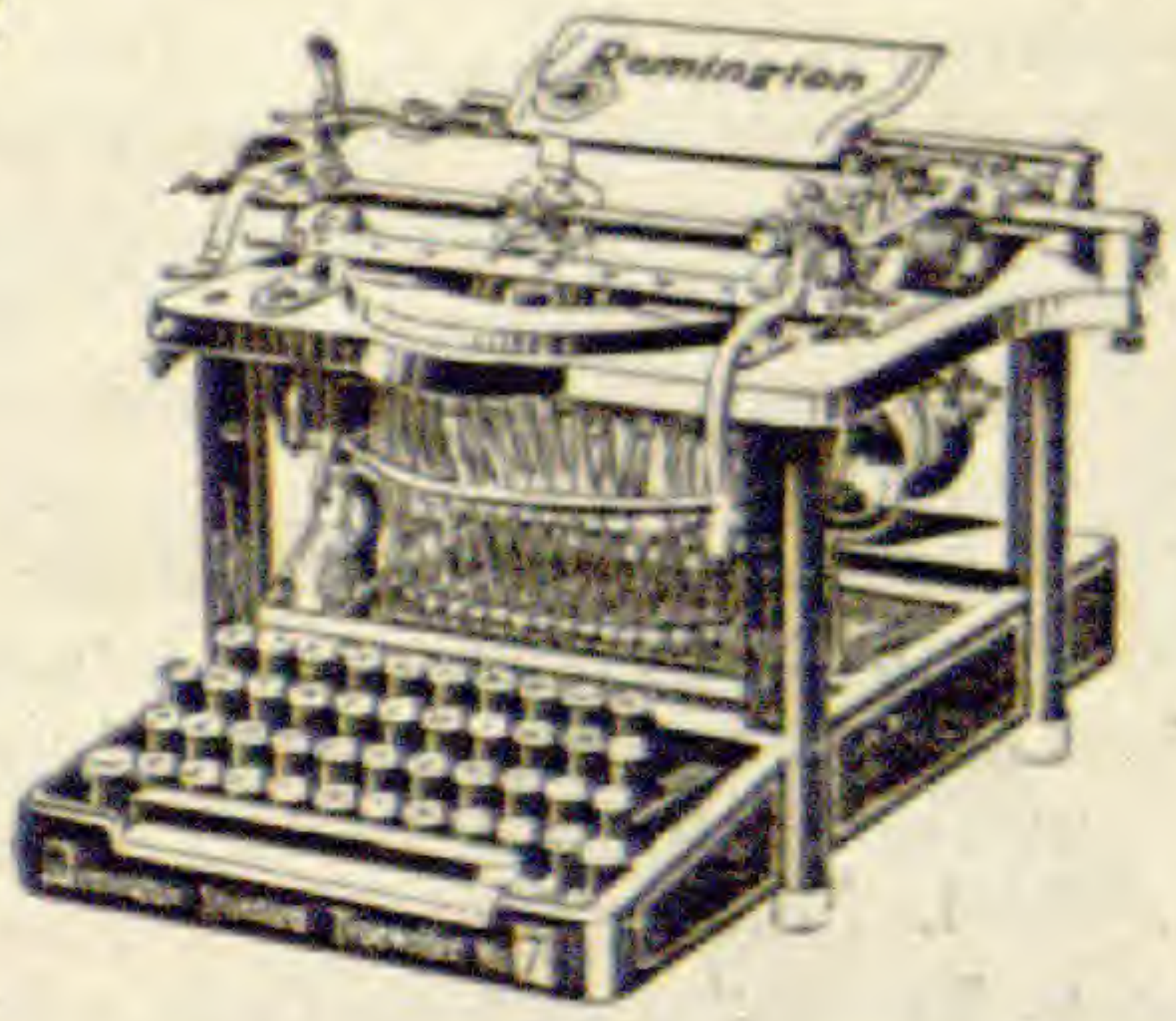
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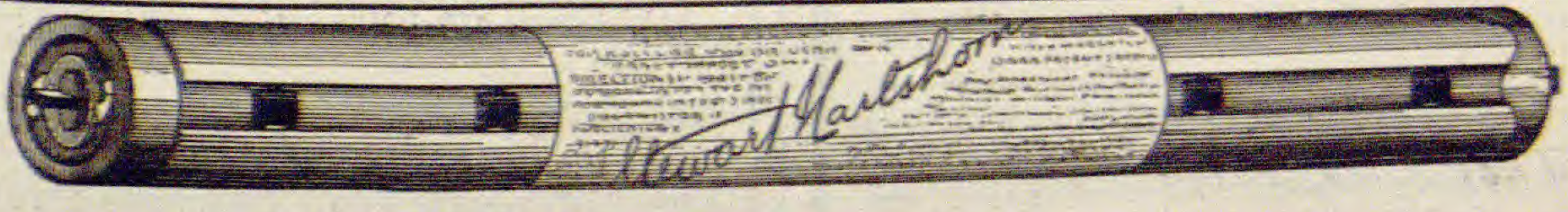
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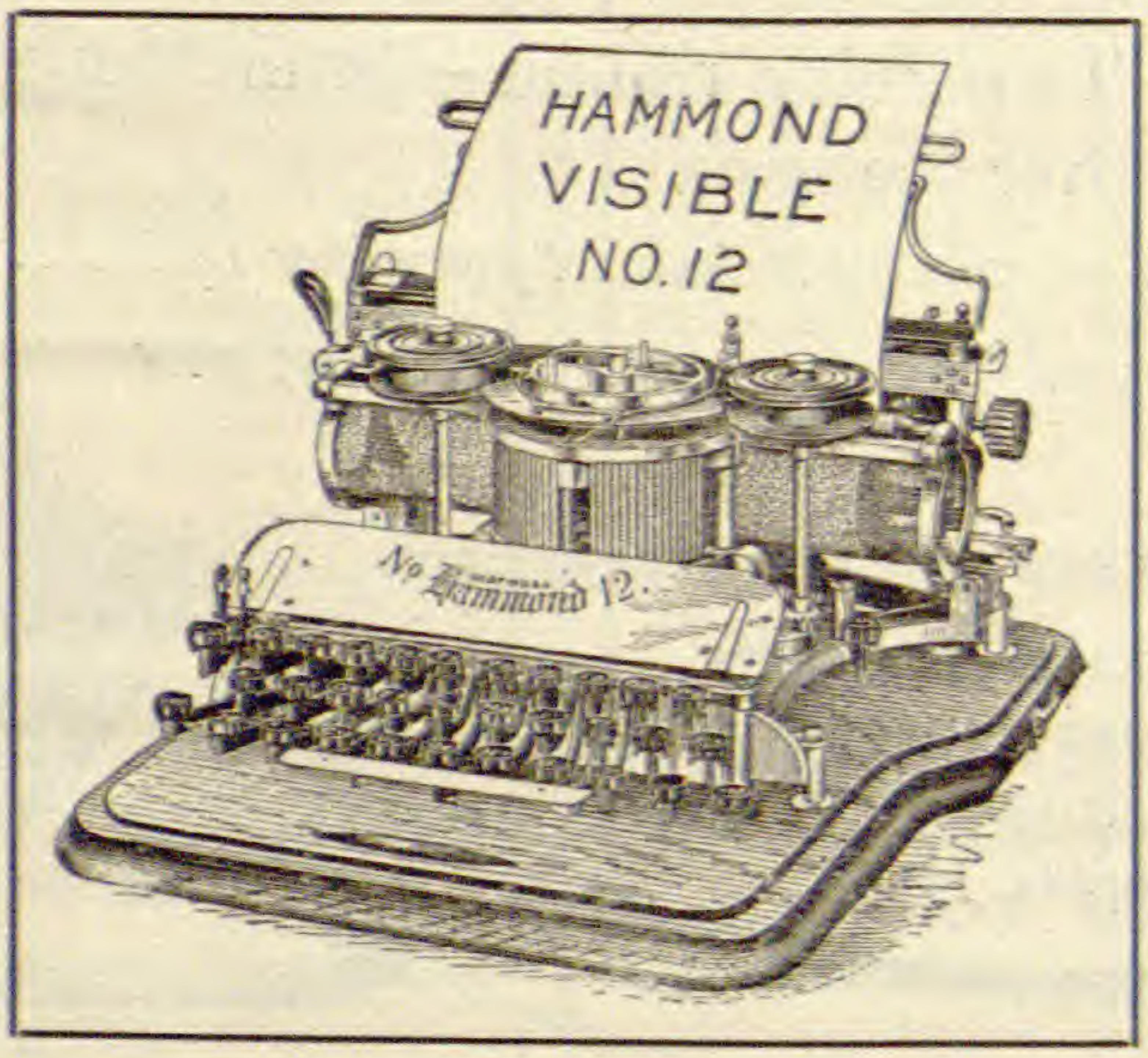
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March 1908

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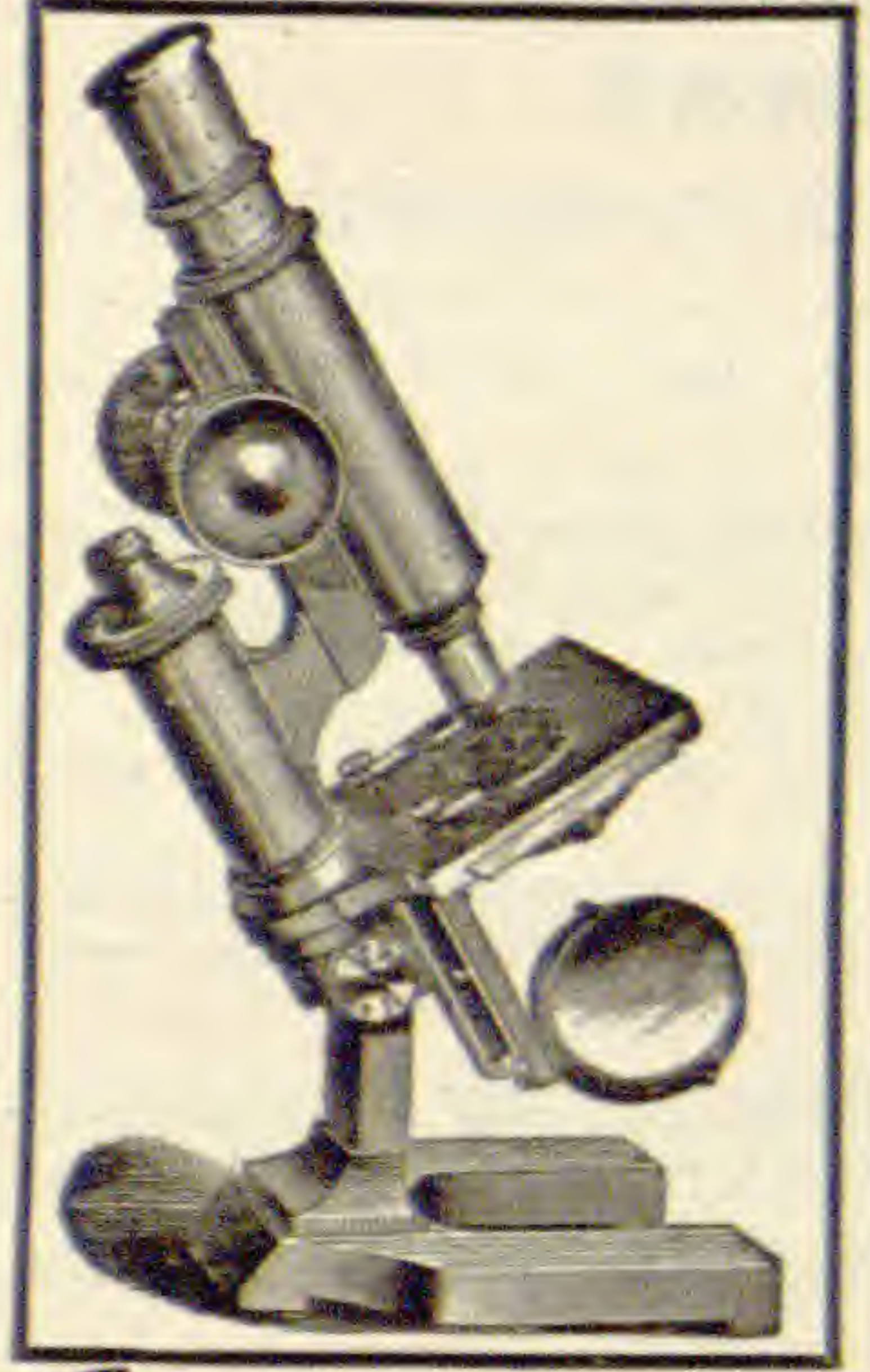

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From the early studies of spermatogenesis in Chara it was generally believed by NÄGELI (**69**), METTENIUS (**62**), HOFMEISTER (**38**), STRASBURGER (**83**), and SACHS (**74**) that the nucleus, before the formation of the sperm, dissolves into cytoplasm to form a homogeneous slimy protoplast from which the body of the sperm is organized. HOFMEISTER, STRASBURGER, and SACHS held a similar view concerning spermatogenesis in some forms of bryophytes and pteridophytes.

The first definite statement that the nucleus does not dissolve before the formation of the sperm was made by SCHMITZ (**77**) from a study of Chara and a number of mosses. He writes that the body of the sperm is produced by a direct change of form of the nucleus, whose peripheral layer becomes thickened to form a spirally coiled band, while the inner part becomes loose and forms a vesicle. The surface layer of the anterior end of the sperm, bearing cilia, consists of the cytoplasm which envelops that part of the nucleus.

Most of the papers concerning spermatogenesis in pteridophytes were published after the appearance of SCHMITZ's study of Chara, by such investigators as CARNOY (**16**, in Hymenophyllum), BELAJEFF (**1**, in Isoetes; **2, 4**, in Gymnogramme and Equisetum), ZACHARIAS (**102**, in Pteris), CAMPBELL (**12**, in Onoclea, Asplenium, Gymnogramme, Adiantum, Alsophila, and Ceratopteris; **13**, in Pilularia; **14**, in Osmunda), BUCHTIEN (**10**, in Equisetum), LECLERC DU SABLON (**61**, in Cheilanthes), GUIGNARD (**31, 32**, in Angiopteris, Pilularia, and Equisetum), SCHOTTLANDER (**78**, in Gymnogramme), and STRASBURGER (**85**, in Marsilia).

These studies begin with the structure of spermatids and are chiefly devoted to details of the events which occur within the spermatids during their transformation into sperms. Since the forms investigated were different, it is natural that the results should diverge in some particulars; but so far as the constituents of the mature sperm are concerned, the views fall into two categories. One group of authors, as ZACHARIAS, LECLERC DU SABLON, and BELAJEFF, believes that the spiral body of the sperm consists of a nucleus always enveloped by a layer of cytoplasm, which becomes the main constituent of the anterior coiled part of the body where cilia are produced; nuclear material does not extend to the anterior part. The other authors mentioned above hold that the nucleus is the main constituent of the body of the sperm, its anterior coiled part being enveloped with cytoplasm on which cilia are borne. Most observers of both groups agree that the cilia are produced from the cytoplasm, and that the vesicle (*Blase*) which is attached at the posterior end of the sperm is cytoplasmic in nature.

Most of the investigation of the origin and nature of the cilia-bearing organs of the sperms of pteridophytes has been done since the discovery of motile sperms and cilia-bearing structure in gymnosperms was made by IKENO (**40, 41, 42, 43**), HIRASÉ (**36, 37**), and WEBBER (**94, 95**). The first paper that dealt with the details of the origin of cilia-bearing structures in the sperms of pteridophytes was that of BELAJEFF. Following his three preliminary notes (**3, 4, 5**), he published in 1898 an account (**6**) of spermatogenesis in Gymnogramme and Equisetum. Two deeply staining bodies (which he called *Nebenkern*) appeared on opposite sides of the nucleus previous to the final mitosis in the antheridium which differentiates the spermatids. At this final division each spermatid receives a blepharoplast which lies close beside the nucleus. The blepharoplast begins to elongate and its elongation is followed by that of the nucleus, so that both structures form two parallel bands which take a spiral form.

SHAW's paper (**80**) on Onoclea and Marsilia appeared almost simultaneously with the foregoing contribution of BELAJEFF. He investigated the cell divisions preceding the formation of the spermatid in Marsilia and found two small bodies, which he called "blepharoplastoids," first in the daughter nuclei after the mitosis which differentiates the spermatid grandmother cell. There is one blepharoplastoid, therefore, for each nucleus of the grandmother cell of the spermatid. This blepharoplastoid divides, but the halves remain close together and pass to one side of the cell. With the next mitosis which occurs in the grandmother cell two new blepharoplasts appear at the pole of the spindle, and they accompany each daughter nucleus after this mitosis into the spermatid mother cell. Then each divides, and after the division of the spermatid mother cell, a blepharoplast is included in each spermatid.

The next year BELAJEFF (**7**) studied Gymnogramme and also the same species of Marsilia which SHAW had investigated. He found centrosome-like bodies at the poles of spindles in various mitoses preceding the formation of the spermatids with their unquestioned blepharoplasts, and he also found the blepharoplasts at the poles of the spindle. From this he drew the conclusion that the blepharoplast of Marsilia holds the same relation to the pole of the spindle as does a centrosome.

THOM (**88**) in the same year observed a blepharoplast in the spermatids of Adiantum and Aspidium as a round body beside the nucleus. He states that the disappearance of nucleoli at the time of the appearance of the blepharoplast would suggest a relationship between them. According to his observation the modification of the blepharoplast into a band structure is considerably delayed after the transformation of the nucleus.

No contribution based upon the original investigation of spermatogenesis in pteridophytes has been published since THOM's paper appeared. However, the problem concerning the nature of the blepharoplast and its relation to the centrosome, not only in pteridophytes but in various groups of plants, has been discussed by STRASBURGER (**87**, with a critical review of the works on this subject), DANGEARD (**21**, on algae), TIMBERLAKE (**89**, on algae), WEBBER (**96**, on Zamia), IKENO (**45**, on Marchantia; **46**), DAVIS (**24**), and some others.

Oogenesis in pteridophytes has not aroused as much interest as spermatogenesis. Oogenesis in Filicales has been observed in various forms by JANCZEWSKI (**49**), GOEBEL (**29**), BRITTON (**9**), CAMPBELL (**15**), and many others.

Morphological investigations of the processes of fertilization in pteridophytes have been published by STRASBURGER (**83**, on Pteris and Ceratopteris), HANSTEIN (**33**, on Marsilia)), CAMPBELL (**13**, on Pilularia; **14**, on Osmunda), SHAW (**79**, on Onoclea), and THOM (**88**, on Adiantum and Aspidium). A few points of cytological interest in their studies concerning fertilization in pteridophytes may be summarized as follows:

In his paper on Osmunda, CAMPBELL writes that in several instances, when the archegonium seemed about ready to open, an appearance was observed that looked very much like the formation of a true polar body, and was not to be compared with the ventral canal cell. He suggests that this structure, like the polar body, may be derived from the division of the nucleus of the egg, his reason for the suggestion being the apparent diminution of the nucleus of the mature egg at the time of the appearance of the structure, although actual division was not observed.

In Onoclea SHAW states that before the archegonium opens, the egg is depressed above, and the nucleus of the egg is flattened, due to the pressure caused by the swelling of the wall of the ventral canal cell. As soon as the archegonium opens and the disorganized contents of the neck canal cells are expelled, the egg becomes turgid. After the sperm has entered the egg at the receptive spot, the egg collapses, and he suggests that this prevents the penetration of more than one sperm. MOTTIER (**67**) expressed some doubt whether the collapsed appearance is really normal.

In Pteris STRASBURGER (**83**) observed the formation of a membrane around the fertilized egg within a very few minutes after the entrance of the sperm, while SHAW in his paper (**79**) on Onoclea found no evidence that a membrane of appreciable thickness was formed immediately after the entrance of sperm.

In his account of Adiantum and Aspidium, THOM finds that when the sperm reaches the nuclear membrane, the nuclear part of the sperm finds or breaks an opening into the egg nucleus and cytoplasmic envelope, while the blepharoplast and the cilia are left out in the cytoplasm of the egg. Whether blepharoplast and cilia are left out in the cytoplasm SHAW leaves in doubt.

That the egg nucleus during the entire process of fertilization is in the resting condition and that the sperm enters the egg nucleus before undergoing any change in form or visible structure, are points of agreement among these authors in their studies on Adiantum, Aspidium, Onoclea, and Osmunda. One peculiar case, different from the foregoing results, was reported for Pilularia by CAMPBELL (**13**), who finds that a sperm nucleus assumes a loose and more granular structure and rounds up before entering to unite with the nucleus of the egg.

No work has been published showing details of the behavior of chromosomes during spermatogenesis, oogenesis, and fertilization in pteridophytes, nor has the organization of the figure of first segmentation division ever been followed.

The present investigation of this subject, with a study of sporogenesis which was considered in my recent paper (**101**), was carried on to trace out the complete history of the chromosomes throughout the life-cycle of *Nephrodium molle* Desv. The former paper, together with the present investigation, may serve as an introduction to the study of apogamy in Nephrodium, which will appear in a subsequent paper.

Material and methods

Gametogenesis and fertilization were studied from prothallia raised from spores in the greenhouse of the Hull Botanical Laboratory during the five months beginning October 1906. The spores for this culture were collected from leaves of the same individuals of *Nephrodium molle* that furnished material for the study of sporogenesis.

Various fluids were used for killing and fixing. The best fixation for the present studies was obtained in the material killed and fixed in the following solution: 1 per cent. chromic acid 25^{cc}, 1 per cent. glacial acetic acid 15^{cc}, 1 per cent. osmic acid 10^{cc}, water 50^{cc}. The methods used after the material was thoroughly washed were similar to those given in the previous paper on sporogenesis (**101**).

Description

VEGETATIVE MITOSIS IN PROTHALLIA

Immediately after the germination of the spore, the nucleus in the prothallial cell is quite large, but shows a tendency to diminish gradually during the further development of the prothallia into many-celled stages. The events of the mitosis in any vegetative cell of a prothallium are not only repetitions of the same type, but they are similar to those of the sporophyte. For this reason no detailed account will be made at this time, but a few essential points deserve mention.

The chromatin reticulum of the resting nucleus of the prothallia, which has a ragged structure like that in a vegetative cell of the sporophyte, becomes transformed into a spirem that runs an irregular course within the nucleus, many tangled parts or loops coming into close contact with the membrane. The spirem then becomes rather smooth and uniform in thickness and very likely there is present a continuous spirem (*fig. 1*). A kinoplasmic cap of conical shape appears at each pole of the nucleus, consisting of a few fibers arranged along the lateral edges of the cone. With the dissolution of the nuclear membrane at the two poles, the fibers push in and attach themselves to the chromosomes which have just segmented from the spirem (*fig. 2*).

The points at which segmentation of the spirem into chromosomes may occur have a certain regularity in relation to location within the nucleus. Although it is impossible to give an accurate explanation of the mechanism at present, the fact that many free ends of the chromosomes, immediately after segmentation, are directed toward the poles may prove that the segmentation took place in bent points of loops. Longitudinal splitting of the chromosomes appeared after the completion of this segmentation.

Contemporary with the entire dissolution of the nuclear membrane, the chromosomes show a tendency to arrange themselves parallel to an axis passing between the two poles (*fig. 3*). The daughter halves of chromosomes either remain in contact side by side throughout their length, or diverge in the middle part, remaining attached only at the ends; but the latter case is of short duration and soon they become reunited in pairs. The chromosomes now shorten and assume an L-shape, and there is established an early condition of an equatorial plate in which the vertical arms of the L-shaped chromosomes are parallel and directed toward the pole, while the horizontal arms lie in the equatorial plane; the spindle threads are attached at the ends directed toward the periphery of the equator (*fig. 4*). A polar view in this stage shows that the horizontal arms hold almost a radial arrangement, while the vertical arms are visible only in the optical section of their ends (*fig. 5*). The shortening and thickening of the chromosomes advance farther until they become short, rod-shaped, and arranged in the equatorial plate.

The separation of daughter chromosomes begins at once (*fig. 6*). As a rule, it takes place similarly in each chromosome, so that the daughter chromosomes proceed toward the pole as a set (*fig. 7*). When the set of daughter chromosomes has reached the pole, they remain for a short time as regularly arranged and straight chromosomes (*fig. 8*), the polar view of which clearly shows that the number of the chromosomes is 66 or 64 (*fig. 9*). The daughter chromosomes grouped at the pole become drawn together tightly and vacuolization begins. When the nuclear membrane is formed, the chromatin structure of the daughter nucleus shows polarity (*fig. 10*). The formation of the cell plate is like that described for the vegetative mitosis of the sporophyte.

SPERMATOGENESIS

A detailed description of the mitoses which take place from the cutting-off of an antheridium initial from a superficial cell until the formation of a spermatid mother cell will be omitted. The number of chromosomes is always 66 or 64. The only point which seems noteworthy is that in the central or primary spermatogenous cell there were observed in the majority of cases threadlike structures imbedded in the cytoplasm, sometimes very densely crowded together near the cell wall. The length of these threads was various, and they were a little more slender than the spirem that is usually present in the mitoses of spermatogenous cells. No such structure was ever observed in the superficial cells or in the antheridium initial from which the central cell is derived, and consequently this structure may originate in the central cell. It does not seem to play any important rôle in the nuclear division. As successive mitoses proceed, it becomes more faintly stained, less conspicuous, and finally becomes impossible to differentiate by staining; but in rare cases it still remains, filling the whole cytoplasm up to the eight- or sixteen-cell stage of the spermatogenous cells. A structure of similar appearance was observed in the central cell of the archegonium (*figs. 41, 41a*).

The spermatid mother cell is characterized by the first appearance of blepharoplasts. In general, the spermatogenous cells, in spite of their small size compared with the vegetative cells of the prothallia, contain large nuclei. The cytoplasm seems generally destitute of plastids and has a very fine fibrillar structure, and the membrane is delicate. The mitoses which take place in the spermatogenous cells are conspicuous on account of the comparatively large amount of chromatin, but otherwise they resemble those of the vegetative cells.

In the telophase of the mitosis, previous to the formation of the spermatid mother cell, the groups of daughter chromosomes, having reached the poles, begin to be vacuolized. Detailed study of this stage shows that the formation of the membrane around the mass of vacuolized chromosomes, or young daughter nucleus, is a little delayed in the polar region, which appears concave from the side view (*fig. 11*). The nucleus when viewed from the pole shows that the chromatin material resulting from the vacuolized chromosomes is very scanty in the polar region and three or four nucleoli are always present (*figs. 12, 13*); such a structure of the nucleus may be regarded as a kind of polarity. If there could possibly be any migration of nucleoli from the interior of the nucleus to the cytoplasm, it would certainly occur in such a critical stage of the nucleus, but there was not observable any peculiar differentiation within the cytoplasm that might warrant the supposition.

As the young daughter nucleus grows in size and assumes a spherical form, the chromatin loses its peculiar arrangement and becomes transformed into the irregular ragged reticulum of the resting condition. Then there are observed by their differential staining two small bodies, the primordia of the blepharoplasts, lying within the cytoplasm at the opposite sides of the nucleus. The first differentiation of the blepharoplasts by stains is very sudden, and they are unexpectedly large, quite surprising in fact, for one would imagine that they should appear at first as very small granules of hardly distinguishable size. The blepharoplasts move toward the nucleus, and during mitosis they hold positions near the poles of the spindle, without any noticeable increase in size (*figs. 14–19*). Sometimes (not often) it is observed that the blepharoplasts lie just at the poles of the spindle, but evidently this does not seem to mean that the blepharoplasts function as centrosomes; instead, the blepharoplasts in this case may have happened to be caught accidentally in the focal centers of the spindle.

In the anaphase of this mitosis 66 or 64 chromosomes are always counted (*fig. 20*). After the telophase each daughter cell of the spermatid mother cell, or each spermatid, contains a single blepharoplast, which now lies very close to the concave side of the nucleus.

When the cell plate is just being completed, a new body appears near the nucleus of the spermatid far away from the blepharoplast (*fig. 21*). For the sake of convenience, I called this body a *Nebenkern* in the preliminary note. It should be understood that this term is used simply as a temporary designation, which must be replaced by a more accurate one. The *Nebenkern* in Nephrodium always appears in the location previously occupied by the central spindle of the last mitosis. This spindle has almost disappeared when the *Nebenkern* appears. Accordingly, it is not probable that the *Nebenkern* originates from the spindle.

The spermatids grow to a certain stage and become almost free from one another, so that the antheridium now contains eight to thirty-two polyhedral or spherical spermatids, with very delicate membranes. During the growth of the nucleus of the spermatids the nuclear reticulum becomes transformed into a ragged network, very rich in irregular chromatin clumps, but with scanty branched strand structures. One or two nucleoli are always present (*fig. 22*). The blepharoplast, a round granule by this time, becomes applied to the nuclear membrane, and the *Nebenkern*, also a round body a great deal smaller than the blepharoplast, lies in various locations within the cytoplasm.

Now the modification of the blepharoplast begins. While the spermatid does not grow further, the nucleus and the blepharoplast increase in size during this metamorphosis, and the space occupied by the cytoplasm consequently gradually diminishes. The blepharoplast, now round in shape, lies very close to the nucleus and elongates along its spherical surface (*figs. 23, 24*), so that the surface view of the blepharoplast at this time shows a rhomboidal outline (*fig. 23*) and its cross-section is crescent-shaped, the concave surface being in close contact with the nuclear membrane (*fig. 24*). The elongation of the blepharoplast now proceeds farther. *Fig. 26* shows the section of the blepharoplast at this stage, cut parallel with the axis of elongation. The cross-section of the blepharoplast is shown in *fig. 27*, and a part of the structure viewed from the surface is given in *fig. 25*.

Until this time the blepharoplast is alike at both ends, and no modification was ever observed in the structure of the nucleus; but soon after the blepharoplast has elongated into a semicircular band, a very complicated modification follows in both nucleus and blepharoplast. One end of the blepharoplast becomes wedge-shaped and loosely laid along the spherical surface of the nucleus (*fig. 28*), while the other end, which still remains pointed, comes into close contact with the nucleus and finally coalesces with it (*figs. 28, 29*). The elongating blepharoplast now takes a spiral direction and has a tendency to form a coiled ribbon-like structure.

While the foregoing modification is occurring in the blepharoplast, the nucleus undergoes a characteristic transformation of form. The distribution of the ragged chromatin clumps within the nucleus becomes irregular; at certain regions of the nuclear cavity they become grouped very densely; while in other parts the chromatin substance is scattered. The nuclear membrane, very delicate at this time, seems to be easily influenced by any change which occurs in the interior of the nucleus, so that the region where the chromatin clumps are densely crowded may protrude above the spherical surface of the nucleus, while the region with scanty chromatin material may form a depression or furrow. This unevenness in the form of the nucleus, brought about by the irregular aggregation of the ragged chromatin clumps in different regions of the interior, develops in such a direction that the nucleus becomes metamorphosed into a coiled structure.

When the nucleus has almost assumed the coiled form, one end of the coil takes on a round sausage-shape (*fig. 30*), which gradually passes into a band structure (*fig. 29*). This band becomes more and more narrow, with a gradual diminution of its width, but this diminution is compensated for by a coalescence with the tapering end of the band-shaped blepharoplast; so that now the tapering end of the blepharoplast runs with the nuclear band as far as the latter structure goes, and finally the blepharoplast ends in the wedge-shaped band. Then the outer surface of that part of the coiled band, which is composed of the blepharoplast and nucleus by their parallel coalescence, becomes covered by the blepharoplast, which grows extensively.

The structure of the coiled nuclear region becomes more and more compact, during this metamorphosis of the nucleus, by the gradual condensation and aggregation of clumps of the chromatin reticulum. Thus the general structure of the coiled sperm is organized within the spermatid. By this time, numerous cilia are observed growing on the surface of the coiled band of the blepharoplast (*fig. 31*). During this modification of the nucleus and blepharoplast, the *Nebenkern* is always present in a definite position close to the nucleus, and when the coiled form of the sperm is established, the *Nebenkern* is observed near the blunt end of the sausage-shaped portion without entering into the construction of the body of the sperm (*fig. 32*).

The mature sperm when observed within the spermatid consists of 2.75 coils, 1.5 of which are composed of the blepharoplast with the nuclear substance lining a certain distance, and the rest exclusively of the nucleus. When the spermatids are discharged from the antheridium the anterior part of the sperm, which consists of the blepharoplast with cilia, first protrudes from the membrane of the spermatid, the membrane being so delicate at this time that it looks like a thin film of cytoplasm. As soon as the sperm begins to swim freely, the cytoplasmic substance with the *Nebenkern* is attached to the posterior coil of the sperm as a vesicle (*fig. 33*). The number of coils in the swimming sperm varies, but an average in material quickly killed is observed to be 2.75 coils. Numerous cilia grow from the whole of the surface of the band-shaped blepharoplast, their length sometimes surpassing more than twice the length of the body of the sperm.

OOGENESIS

The nuclei in the cells of the cushion region are generally larger than those of the rest of the prothallium. At first, successive mitoses that occur in the region to become the cushion divide cells anticlinally, so that there result a number of narrow parallel cells in a single row. In one of these cells, the next mitosis is periclinal and gives rise to an archegonium initial (*fig. 34*). From the archegonium initial, by two successive mitoses, there are cut off a basal cell, a primary neck cell, and a central cell, or primary oogenous cell (*figs. 35, 36, 37*). Up to this differentiation of the central cell, the critical stage of each mitosis showed that the number of chromosomes is 64 or 66.

The central cell grows in size even after its nucleus begins to divide and becomes characteristically asymmetrical in form, due to the influence of the surrounding cells. The cytoplasm in the central cell consists of a fine fibrillar structure in general, which passes into an alveolar structure toward the periphery (*fig. 38*). There are observed in the central cell thread structures of various lengths, scattered throughout the whole cell, especially near the cell wall. Their staining capacity and general aspect recall similar structures which are observed in the central cell of the antheridium.

The resting nucleus in the central cell increases in size considerably (*fig. 38*). The nuclear reticulum consists of ragged chromatin material and three or more nucleoli are present. There now takes place a mitosis to divide the cell into neck-canal and ventral cells (*figs. 39–42*). The mitosis is typically vegetative; in the prophase the spirem is continuous and a single nucleolus is present, perhaps resulting from the union of the nucleoli in the resting stage. The appearance of longitudinal fissions in the chromosomes, their shortening and their arrangement in the equatorial plate, were exactly similar to those which were described in the vegetative mitoses of the prothallia. In the anaphase the two sets of daughter chromosomes show no difference in the amount of chromatin material (*figs. 41, 41a*), but after the telophase the two daughter nuclei become unequal in size (*fig. 42*). The inequality of these two sister nuclei has resulted neither from unequal distribution of the chromatin mass nor from the number of chromosomes, but is a matter of nucleo-cytoplasmic relation, i. e., on account of the position of the nucleus of the central cell, the cell plate being laid down so as to divide the cytoplasm into two unequal cells. The immense amount of food material in the ventral cell causes its nucleus to grow more rapidly than that of the neck canal cell. At this time these two nuclei contain 66 or 64 chromosomes.

Then follows one more mitosis (simultaneous or successive) in each of these cells. The mitosis which divides the neck canal cell into two daughter cells is typical vegetative (*figs. 43–45*). In the telophase, when the daughter nuclei are formed, a cell plate is laid down as usual, but it soon begins to disorganize and finally disappears entirely. The direction of this mitosis is various, being sometimes parallel to the long axis of the archegonium, sometimes perpendicular to it, and sometimes oblique.

The ventral cell sooner or later divides into a ventral canal cell and an egg cell (*figs. 46–48*). No peculiarity is observed in this mitosis except in the metaphase (*fig. 46*), when there is almost always present a single dark staining body near the spindle, which possibly may be a persistent nucleolus, but its origin was not traced. The peculiar thread structure of unknown substance which is differentiated in the central cell is also continuously observed in the ventral cell, without any visible connection with the mitotic figure. In the telophase, the two daughter nuclei show a marked difference in size and shape, perhaps due to a nucleo-cytoplasmic relation, similar to the case previously observed (*fig. 47*). Several nucleoli appear in the young daughter nucleoli (*fig. 48*).

The characteristic curvature of the nucleus of the egg cell begins at the telophase of this division, and there is laid down by this time a dome-shaped cell plate which is of longer duration than that between the neck canal cells. The egg nucleus grows to an immense size and is very irregular in outline, whereas the neck canal cell and the ventral canal cell collapse and become mucilaginous, together with their disorganizing nuclei.

FERTILIZATION

The egg cell when ready for fertilization lies in the bottom of the archegonial cavity. Some of the material resulting from the disorganization of canal cells remains in the neck and ventral region of the archegonium as a slimy substance, even after the wide opening of the neck admits free access of water. The dome-shaped cell wall which separated the egg cell from the ventral canal cell seems almost to disappear at the time of the disorganization of the ventral canal cell, so that the protoplast of the egg cell is exposed at the neck side to the dilute slimy substance, but this exposed surface retains its irregular dome-shape.

The cytoplasm of the egg cell has a fine fibrillar structure, very dense around the nucleus and vacuolate toward the periphery of the cell. The upper part of the nucleus becomes depressed, so that its concave side is directed toward the neck, and it presents a crescent form in the side view (*figs. 48, 49*). The chromatin material within the nucleus becomes transformed completely into a system of anastomosing and branching fine strands and small ragged clumps, characteristic of the resting condition. One, two, or more small nucleoli are present.

Several sperms enter the neck of the archegonium and reach the surface of the exposed protoplast of the egg cell at the so-called receptive spot (*fig. 50*). Normally one of these sperms penetrates the cytoplasm of the egg. The cytoplasmic layer which surrounds the egg nucleus at this time is rather shallow on the exposed surface in the direction of the neck, so that the sperm has to pass through only a shallow layer of the cytoplasm. When the sperm reaches the nucleus, the coil becomes shortened and the penetration of the sperm into the nucleus begins from the anterior region, followed gradually by the posterior region (*fig. 51*).

During the penetration of the sperm the chromatin reticulum of the egg nucleus persists as it was before, but a peculiar irregularity of the reticulum is observed to have occurred near the intruding sperm, which in penetrating has presumably caused a movement of the contents of the egg nucleus. After the sperm has completely penetrated into the egg nucleus, this disturbance of the homogenous arrangement of the chromatin reticulum is still observed so long as the sperm within the egg nucleus maintains its individual outline (*figs. 52a, b*).

It is also observed that there is present a clear region directly surrounding the body of the sperm within the egg nucleus, and this seems to be a transient stage preceding the disintegration of the body.

The disintegration takes place in the body of the sperm in the very situation it occupied within the egg nucleus. The chromatin material, which was aggregated into a compact and condensed structure up to this time, now begins to return into a loose anastomosed complex of numerous chromatin clumps and branched fine strands, similar to those which were observed during the metamorphosis of the nucleus to organize the sperm. Small new nucleoli make their appearance near or in the disintegrated region (*figs. 53a, b*). Whether these nucleoli result from the disintegrated blepharoplast or nuclear region of the sperm or from some different source was not determined. If the blepharoplast originated indirectly in the interior of the nucleus from nucleoli which might have escaped during the reconstruction of the nucleus of the spermatid mother cell in the last telophase (*fig. 11*), and by a temporary change of staining capacity may have escaped observation until they first become differentiated by stains as two bodies, it might be expected that it should now reappear or return again into nucleoli as it disintegrates. Thus close relationship between the blepharoplast and nucleolus might be established. However, this is entirely a speculative view, which lacks evidence sufficient to make the suggestion probable.

Disintegration of the body of the sperm proceeds still farther, the anastomosing chromatin material showing presently the ragged reticular structure characteristic of the resting condition (*figs. 54a, b*), and finally the chromatin material of the sperm nucleus becomes entirely anastomosed with that of the egg nucleus whose membrane now surrounds the chromatin of both egg and sperm (*fig. 55*).

The fusion nucleus in the resting condition contains two or more nucleoli, some of which have appeared at the time of disintegration of the sperm nucleus, while others have probably resulted from the union of nucleoli which already existed in the egg nucleus (*fig. 55*). Figures of the first segmentation division are obtained in material fixed a week after the entrance of the sperm had been observed. The spirem which is organized at the expense of the chromatin reticulum of the fusion nucleus has a continuous structure, without any indication of two chromatin constituents of maternal and paternal origin. Whether there exists one or two spirems was not determined, but even if there be present more than one spirem they are exactly similar in thickness (*fig. 56*). Then the same process occurs as was described in vegetative mitosis. On account of the large number of chromosomes, the best stage for an accurate counting is obtained in the late anaphase, in which two sets of daughter chromosomes are regularly arranged near the poles (*fig. 57*). The polar view of this stage showed 128 or 132, or in rare cases 130, chromosomes (*fig. 58*).

Mitoses that occur following this first segmentation division, during the development of the embryo, were traced up to the appearance of the first leaf. The process is essentially similar, and 128 or 132 chromosomes are invariably present.

Discussion of cytological phenomena

The origin of the blepharoplast.—There have been many contributions concerning the origin and structure of the blepharoplast, not only in sperms of gymnosperms and pteridophytes, but also in zoospores of thallophytes.

In Cycas (IKENO **41, 42, 43, 48**), Ginkgo (HIRASÉ **36, 37, 48**), and Zamia (WEBBER **94, 95, 96**), two blepharoplasts first are formed *de novo* in the cytoplasm of the body cell at some distance from the nucleus, and one of these blepharoplasts is included in each spermatid; accordingly the origin of the blepharoplast is cytoplasmic.

In Equisetum and Gymnogramme, BELAJEFF (**6**) observed the blepharoplasts as two deeply stained bodies on opposite sides of the nucleus previous to the final mitosis which differentiates the spermatid. In Marsilia SHAW (**80**) discovered two blepharoplastoids in the telophase of the mitosis which differentiates the grandmother cell of the spermatid. The blepharoplastoids disappear in the spermatid grandmother cell, in which two new blepharoplasts are formed at the poles of the spindle. BELAJEFF (**7**) examined the same form which SHAW had studied, and he found two centrosome-like bodies at the poles of the spindle of the mitosis which gives rise to the spermatid grandmother cell. These centrosome-like bodies are probably structures similar to the blepharoplastoids of SHAW; however, according to BELAJEFF'S accounts, these structures do not pass into the cytoplasm to disappear, but accompany each daughter nucleus in the telophase and in the prophase of the next mitosis which occurs in the spermatid grandmother cell. This centrosome-like body divides and the two resulting ones become situated at the poles of the spindle. The process is repeated until there is formed a spermatid in which this centrosome-like body remains and becomes the blepharoplast.

THOM's account (**88**) for Adiantum and Aspidium differs from those of the last two authors. In regard to the origin of the blepharoplast he states that the disappearance of nucleoli at the time of the appearance of the blepharoplast in the cytoplasm of the spermatid would suggest a relationship between them.

According to IKENO's account (**45**) of Marchantia, a centrosome is formed within the interior of the nucleus of the entire series of spermatogenous cells up to the spermatid grandmother cell. This body moves to the nuclear membrane and is thrust out into the cytoplasm. It then lies outside of the nucleus and becomes the functioning centrosome, dividing to form two centrosomes that separate and occupy the poles of the spindle. After the mitosis that gives rise to the spermatids, the centrosome remains to become the blepharoplast of the sperm.

Regarding the origin and structure of the blepharoplast of thallophytes, STRASBURGER (**85, 87**) expressed the following view, chiefly based on his study of the zoospores of Vaucheria, Cladophora, and Oedogonium. In all of these forms, he states, the blepharoplast arises from the outer plasma membrane (*Hautschicht*), the nucleus lying close to the plasma membrane at the time when the blepharoplast is formed. Another view, based upon the zoospores of Hydrodictyon, was advanced by TIMBERLAKE (**89**), who found that after nuclear multiplication had ceased, segmentation proceeded until uninucleate masses of protoplasm become separated from one another as zoospores. Then a blepharoplast was formed, lying in contact with the plasma membrane. But before the appearance of the blepharoplast, he adds, a granule may sometimes be observed close to the nucleus and it is possible that this is the first appearance of the blepharoplast. DANGEARD's study (**21**) on Polytoma also suggests some possible relationship between the blepharoplast and nucleus. In zoospores of this form, he finds that the blepharoplast is situated directly under the plasma membrane and that a delicate threadlike structure extends from it into the cytoplasm and sometimes ends at the side of the nucleus in a granule. DAVIS (**24 a**) traced the origin of the blepharoplast in Derbesia, in which the blepharoplast arises from the granules observed close to the nucleus. There is a time previous to the differentiation of the zoospore when the nucleus lies close to the cleavage furrow that finally separates the adjacent zoospore rudiments. Close to the nucleus he observed a number of granules which migrate toward the surface region of the protoplast of the zoospore and assume a ring-shaped arrangement. By the fusion of these granules the blepharoplast is established.

According to these results, excepting STRASBURGER'S on Vaucheria, Cladophora, and Oedogonium, the blepharoplast seems to originate in the interior of the cytoplasm (Cycas, Ginkgo, Zamia, Equisetum, Marsilia, Gymnogramme, Onoclea), sometimes from a position close to the nucleus (Adiantum, Aspidium, Hydrodictyon, Polytoma, Derbesia), and in still another case from the interior of the nucleus (Marchantia).

In Nephrodium, as has just been described, the mitoses which occur within the antheridium were all investigated, from the first spermatogenous cell until the final differentiation of the spermatid. The blepharoplasts were demonstrated by the differentiation of stains as two small deeply staining bodies appearing first within the cytoplasm of the spermatid mother cell. The nucleus of the spermatid mother cell is in the resting condition when these bodies appear, and during the mitosis which differentiates the spermatid the blepharoplasts are situated near the pole of the spindle. After the telophase one blepharoplast accompanies each daughter nucleus and there is established a spermatid with a single blepharoplast. Sometimes blepharoplasts appeared in spermatogenous cells of the four-celled stage of the antheridium, but it is certain that these four cells do not represent spermatogenous cells in an early stage previous to the formation of the spermatid mother cells; on the contrary, they are in this case real spermatid mother cells, the number of sperms produced in the antheridium then being only eight.

The relationship of the blepharoplast and the centrosome.—The centrosome in pteridophytes was figured first by HUMPHREY (**11**) and then by CALKINS (**39**) in the spore mother cells of such forms as Osmunda, Psilotum, Adiantum, and Pteris. One year after CALKINS' paper appeared, SHAW'S account (**80**) of Marsilia and Onoclea was published, according to which the blepharoplast has nothing to do with the mitosis as a center of the mechanism of nuclear division, but simply lies near the pole of the spindle. He does not regard the blepharoplast as a centrosome.

BELAJEFF'S conclusions (7) in reference to Marsilia oppose those of SHAW; for he believes that the blepharoplast always occupies the pole of the spindle and functions as a centrosome during mitosis. He claims that the blepharoplast in pteridophytes is homologous with the centrosome. He figures the division of the centrosome or blepharoplast in the spermatid mother cell previous to the division of the nucleus, and between the two separating daughter centrosomes or blepharoplasts a small central spindle is developed just as in certain animal cells.

IKENO (45) considers the blepharoplast of Marchantia to be actually a centrosome, as shown by its behavior during mitosis. He homologizes the *Nebenkörper*, the deeply staining body in the cytoplasm of the spermatid, with the blepharoplastoid of SHAW. Again he (46) discusses the homologous nature of the blepharoplast and centrosome in his paper entitled "Blepharoplasten im Pflanzenreich." The *Nebenkern* of BELAJEFF, he suggests, is homologous with the deeply staining body (the *Körperchen*) in the spermatid of animals. Last year IKENO (47) reasserted his belief that the blepharoplast is a centrosome. He thinks that the bodies now called blepharoplasts may not all be homologous structures, but he holds that the blepharoplasts of pteridophytes, gymnosperms, liverworts, and myxomycetes are of centrosome origin either ontogenetically or phylogenetically.

The foregoing accounts of IKENO confirm BELAJEFF'S view regarding the homology of centrosome and blepharoplast. STRASBURGER (87) wrote at length on this subject seven years ago, and does not accept BELAJEFF'S view. He emphasizes the kinoplasmic character of the blepharoplast, whether it be a differentiated region of the plasma (as he believes for the zoospores of Vaucheria, Cladophora, and Oedogonium), or a special development in the interior of the cytoplasm (pteridophytes and gymnosperms). He thinks that all kinoplasmic structures, be they centrosomes, centrospheres, or blepharoplasts, hold a very close physiological relation to the structure of the nucleolus, so that the blepharoplast might occupy the position of centrosome without being genetically connected with that structure.

In Nephrodium there is present no centrosome in the whole life-history, and the blepharoplasts which arise *de novo* in the cytoplasm of the spermatid mother cell do not play the part of a centrosome. Since centrosomes are not found in this form it is impossible of course to discuss any relationship between them. If any genetic relationship really exists between the two structures in the plant kingdom, evidence might be sought in some of the lower forms, such as Sphacelaria and some other species in Phaeophyceae, in which centrosomes are known and motile spores are produced.

Fusion of male and female nuclei.—The condition of sexual nuclei at the time of fertilization has been studied in numerous forms of different groups of plants.

Among thallophytes the fusion of gamete nuclei was observed in Closterium and Cosmarium (KLEBAHN **52**), Rhopalodia (KLEBAHN **54**), Cocconeis (KARSTEN **50**), Sphaeroplea (KLEBAHN **55**; GOLENKIN **30**), Oedogonium (KLEBAHN **52**), Vaucheria (OLTMANNS **70**; DAVIS **23**), Coleochaete (OLTMANNS **71**), Fucus (FARMER and WILLIAMS **25**, **26**; STRASBURGER **86**), Dictyota (WILLIAMS **97**), Batrachospermum (SCHMIDLE **76**; OSTERHOUT **72**), Nemalion (WOLFE **98**), Polysiphonia (YAMANOUCHI **100**), Basidiobolus (FAIRCHILD **27**), Albugo (WAGER **92**; STEVENS **81**, **82**; DAVIS **22**), Peronospora (WAGER **93**), Pythium (MIYAKE **63**; TROW **91**), Achyla (TROW **91**), Araiospora (KING **51**), Sphaerotheca (HARPER **34**), Pyronema (HARPER **35**). No matter whether a condition of heterogamy is established or not in these forms after the union of gametes or gametangia, the male nucleus or nuclei pass into the cytoplasm of the egg or oogonium and union takes place between the male and female nuclei in the resting condition.

Similar conditions regarding the union of the sexual nuclei in angiosperms were described by STRASBURGER (**84**) for Monotropa, by SCHAFFNER (**75**) for Sagittaria, by COULTER (**19**, **20**) for Lilium and Ranunculus, by MOTTIER (**66**, **67**) for Lilium, by LAND (**57**) for Silphium, and by others. MOTTIER gives the most detailed account of chromatin at the time of the union, and figures the gamete nuclei as uniting with their chromatin in the resting condition. In the forms mentioned above, the gamete nuclei, with a few exceptional cases, are of almost equal size at the time of union, and fusion of the chromatin of the two gamete nuclei takes place after the dissolution of the portions of the nuclear membranes which are in contact.

There are described among gymnosperms cases in which there is a marked difference in size between male and female nuclei, as Thuja (LAND **58**), Picea (MIYAKE **64**), Abies (MIYAKE **65**), Torreya (ROBERTSON **73**), Sequoia (LAWSON **59**), Cryptomeria (LAWSON **60**). In these species the sperm nucleus, being considerably smaller, becomes imbedded in a depression of the egg nucleus. However, the process of their union does not differ essentially from the cases observed in thallophytes and angiosperms, because the chromatin material of both gamete nuclei in resting condition fuses after the disappearance of the contiguous part of the nuclear membrane.

According to the accounts given by a number of authors there is still another case: in Larix (WOYCICKI **99**) and Taxodium (COKER **18**), the gamete nuclei which come into contact do not fuse, but the chromatin contents of both nuclei are kept in distinguishable maternal and paternal groups; while in Pinus (BLACKMAN **8**; CHAMBERLAIN **17**; FERGUSON **28**) and Tsuga (MURRILL **68**) the chromatin of sperm and egg nuclei remains separate, forming two spirems, and only after their segmentation into chromosomes are the two sets of structures brought together in the first cleavage spindle. In these cases there is never present a resting nucleus including both maternal and paternal chromatin within a common nuclear membrane.

In the case of Nephrodium, as already described, the sperm which entered into the egg nucleus was observed during a certain period without any visible change, entirely imbedded within the chromatin reticulum of the egg nucleus. The chromatin material which constituted the body of the sperm begins to disintegrate, and the final result is a reticular structure similar to that which we have noticed in the nucleus of the spermatid before the formation of the sperm. The reticular structures of both sperm and egg nuclei become anastomosed and mixed together entirely within the membrane of the egg nucleus. The spirem of the first segmentation division is organized from this reticulum as a continuous homogenous structure.

This process of disintegration of the body of the sperm within the egg in Nephrodium accords with THOM'S and SHAW'S accounts. The presence of the resting nucleus including both maternal and paternal chromatin within a common nuclear membrane as described in Nephrodium was also described by IKENO (**43, 44**) in Cycas and Ginkgo, these two being the only forms among Cycadales and Ginkgoales in which fertilization has been traced to the complete union of the two gamete nuclei.

There is only one paper that gives details of fertilization in a bryophyte, a contribution by KRUCH (**56**) on Riella. In this form he states that after the sperm enters the egg, a male nucleus is organized which increases in size until it is about equal to the egg nucleus. This differs from the condition in Nephrodium, for the sperm does not enter into the egg nucleus, but two nuclei fuse side by side with their chromosomes fully organized.

With respect to the cytoplasmic structures of the sperm, all investigations among cycads and in Ginkgo agree that they are left behind in the cytoplasm of the egg before the gamete nuclei unite. A similar condition is reported in both Adiantum and Aspidium. In Nephrodium the author cannot confirm the foregoing view, since cases were observed in which the blepharoplast was not left behind in the cytoplasm. However, such cases do not of course necessarily mean that this structure takes part in what may be regarded as an essential part of the process of fertilization.

Conclusion

Since the present investigation on spermatogenesis, oogenesis, and fertilization, together with the preceding study on sporogenesis, has been made to trace out the complete history of the chromosome in the normal life-cycle of Nephrodium as preparatory to the study of apogamy, the general conclusions will be given in the subsequent paper. However, the two principal points established by the present investigation may be stated here: (1) the counting of the number of chromosomes is possible in the gametophyte; (2) the number of the chromosomes in the gametophyte is constant (64 or 66).

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EXPLANATION OF PLATES VI–VIII

The figures were drawn with the aid of an Abbé camera lucida, under Zeiss apochromatic objective 1.5^{mm} N. A. 1.30, combined with compensating ocular 18, except *figs. 34–38, 41, 42, 46–50* drawn with compensating ocular 4, and *fig. 33* drawn with compensating ocular 18 under higher magnification obtained by extending the tube. The plates are reduced to one-half the original size.

PLATE VI

Mitosis in the vegetative cells of the prothallium

FIG. 1. Prophase; nucleus with a continuous spirem.

FIG. 2. The nucleus after segmentation of spirem into a number of chromosomes, most of them before the stage of longitudinal splitting; two kinoplasmic caps with a few fibers are present.

FIG. 3. The nucleus after disappearance of membrane; chromosomes which are split longitudinally show a tendency to become arranged parallel to the axis passing between the two poles.

FIG. 4. Late prophase; daughter halves of chromosomes are in close contact with each other; they have shortened to L forms and are regularly arranged in an equatorial plate; the vertical arms of the L's are directed to the poles, while the horizontal arms are lying in the plate.

FIG. 5. Polar view of the stage shown in *fig. 4;* the horizontal arms of the L's are in regularly radiating arrangement and the optical section of the vertical arms appear like dots.

FIG. 6. Early anaphase; daughter chromosomes separating in a group.

FIG. 7. Late anaphase; two sets of daughter chromosomes near the pole.

FIG. 8. Early telophase; daughter chromosomes regularly arranged at poles.

FIG. 9. Polar view of the stage shown in the previous figure; 64 chromosomes present.

FIG. 10. Telophase; two daughter nuclei and a cell plate formed; arrangement of chromatin substance in daughter nuclei showing polarity.

Spermatogenesis

FIG. 11. Telophase preceding formation of spermatid mother cells; nuclei of spermatid mother cells just organized; two nucleoli present in one of the nuclei.

FIG. 12. Condition of nucleus later than stage shown in *fig. 11;* chromatin material very scanty in polar region, where nucleoli are present.

FIG. 13. Still later stage than that in *fig. 12;* arrangement of chromatin material still showing polarity.

FIG. 14. More advanced stage than that shown in *fig. 13;* nucleus of spermatid mother cell almost in resting stage; no polarity in distribution of chromatin material; two blepharoplasts within cytoplasm on opposite sides of nucleus.

FIG. 15. Nucleus of spermatid mother cell with spirem; two kinoplasmic caps with a few fibers; blepharoplast near pole of fibers.

FIG. 16. Spirem segmented into chromosomes; nuclear membrane begins to disappear at poles of nucleus; blepharoplast near the pole.

FIG. 17. Late prophase; nuclear membrane has completely disappeared; chromosomes split longitudinally; blepharoplast near center of spindle.

FIG. 18. Anaphase; two sets of daughter chromosomes on the way toward the poles.

FIG. 19. Early telophase; daughter chromosomes grouped at pole with regular arrangement; blepharoplast retains same position as before.

FIG. 20. Polar view of stage shown in *fig. 19;* 66 chromosomes evident.

FIG. 21. Late telophase; spermatid mother cell has divided into two spermatids, each containing a single blepharoplast near concave side of young nucleus; *Nebenkern* near each nucleus.

FIG. 22. One of the spermatids after the cellular connection has become loose and the regular arrangement has been lost; nucleus larger; chromatin reticulum composed of ragged clumps and two nucleoli present; blepharoplast applied very close to nuclear membrane; *Nebenkern* a little apart from the membrane.

FIGS. 23, 24. Two different views of same stage; *fig. 23* shows surface of blepharoplast; *fig. 24* the cross-section; *Nebenkern* moved near nucleus after blepharoplast has begun to grow and elongate.

FIGS. 25–27. Three different views of the same stage; *fig. 25* shows part of surface view; *fig. 26* longitudinal section; *fig. 27* cross-section.

FIG. 28. Nucleus of spermatid has become spiral; free end of blepharoplast wedge-shaped, the other end gradually tapering and along one edge a tapering end of the nucleus runs from the opposite direction and coalesces.

FIG. 29. Stage of spermatid nucleus later than that in *fig. 28;* coiled form of nucleus farther advanced; one end of nucleus, consisting chiefly of the blepharoplast, clearly visible; the other end, terminating in a sausage-shaped structure, slightly recognizable in the center below the *Nebenkern*, which is drawn lightly.

FIG. 30. Stage similar to that shown in *fig. 29*, viewed from different direction; end of nucleus terminating in sausage-shaped structure visible.

FIG. 31. Almost mature sperm within a spermatid; whole structure of 2.75 coils, of which 1.2 coils are blepharoplast, the rest the nucleus that terminates in a blunt end; long cilia borne on outer surface of blepharoplast; *Nebenkern* not drawn.

FIG. 32. Optical section of side view of stage shown in *fig. 31; Nebenkern* larger and near blunt end of sperm.

FIG. 33. Sperm fixed in free swimming condition; anterior end consists of blepharoplast which gradually covers only outer surface, and is replaced by band structure of nuclear substance which finally ends in a sausage form; *Nebenkern* present within vesicle.

PLATE VII

Oogenesis

FIG. 34. Cross-section of part of prothallium where cushion region arises later; nucleus in telophase of mitosis which forms archegonium initial.

FIG. 34*a*. Nucleus shown in previous figure under higher magnification.

FIG. 35. Nucleus of archegonium initial in metaphase of mitosis which cuts off basal cell.

FIG. 35*a*. Nucleus shown in *fig. 35* under higher magnification.

FIG. 36. Division into central cell and primary neck cell.

FIG. 37. Central cell or primary oogenous cell.

FIG. 38. By successive divisions of primary neck cell and growth of central cell, a characteristic archegonial projection is formed; cytoplasm of central cell with peculiar thread structures.

FIG. 39. Nucleus of central cell in early prophase; spirem continuous; a single nucleolus.

FIG. 40. Early metaphase; chromosomes, daughter halves in pairs, have shortened.

FIG. 41. Anaphase; cell growth continuing during mitosis.

FIG. 41*a*. Same mitotic figure shown in *fig. 41* under higher magnification; two sets of daughter chromosomes almost similar in amount; thread structures in cytoplasm.

FIG.. 42. Telophase; two daughter nuclei unequal in size; ventral nucleus considerably larger than nucleus of neck canal cell.

FIG. 43. Prophase of mitosis in a neck canal cell.

FIG. 44. Anaphase of same.

FIG. 45. Telophase; a cell plate laid down between two neck canal cell nuclei; cytoplasm with vacuoles of various sizes.

FIG. 46. Section of archegonium after formation of two neck canal cells; the two nuclei in contact, the cell plate having already disappeared; nucleus in ventral cell in metaphase.

FIG. 46*a*. Nucleus of ventral canal cell shown in *fig. 46* under higher magnification; deeply staining body near spindle.

FIG. 47. Section of archegonium; ventral cell nucleus in telophase; two nuclei of neck canal cell in contact.

FIG. 47*a*. Two daughter nuclei shown in *fig. 47* under higher magnification; size and shape quite different; both contain a number of nucleoli; dome-shaped cell plate laid down separating egg and ventral canal cell.

FIG. 48. Section of archegonium after completion of egg, ventral canal cell, and neck canal cells; nucleus of egg larger than others.

FIG. 49. Section of archegonium with open neck; canal cells becoming mucilaginous, with disorganizing nuclei; egg nucleus irregular; single nucleolus present, due to union of several nucleoli seen when nucleus was organized.

PLATE VIII

Fertilization

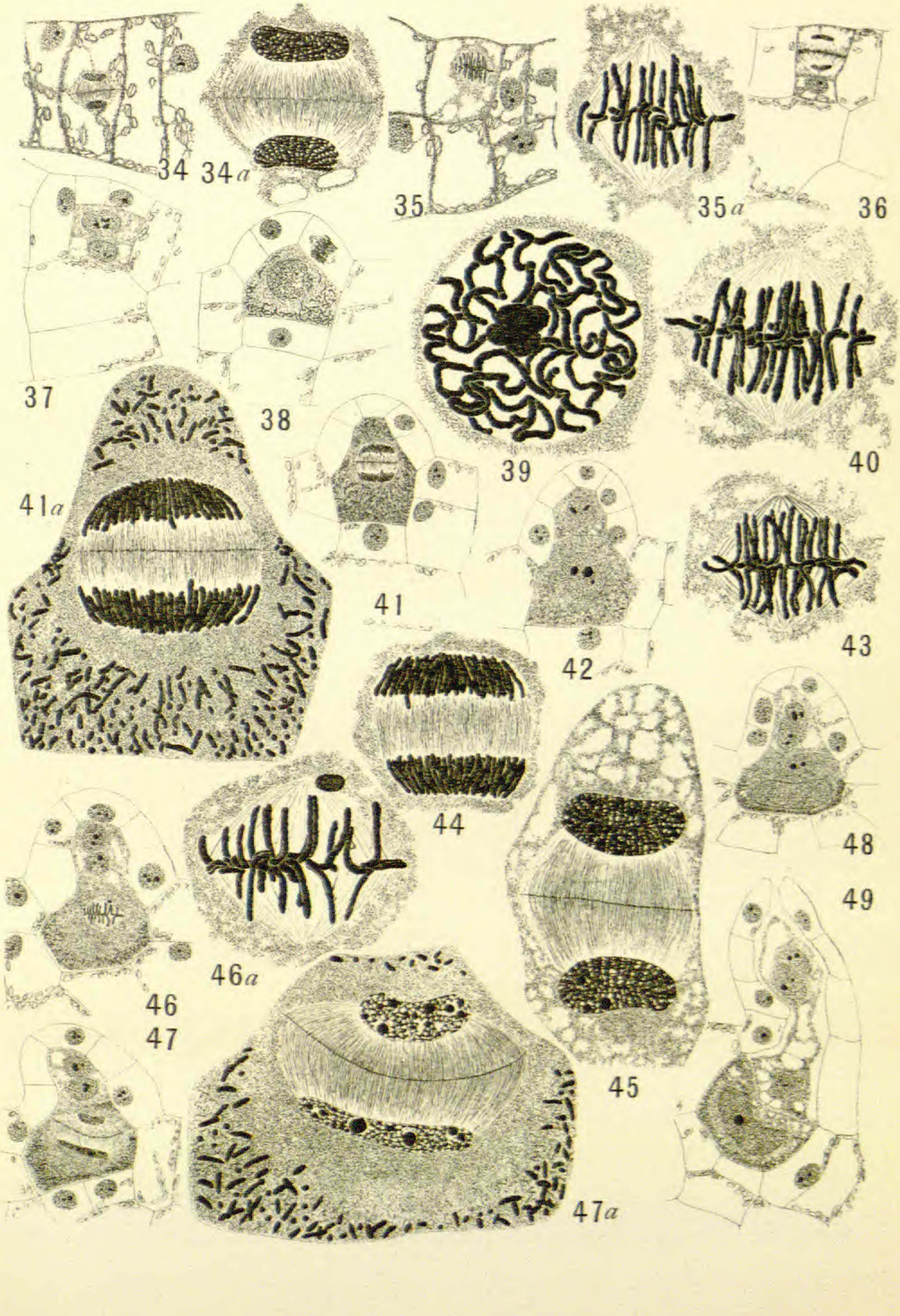
FIG. 50. Entrance of a number of sperms; many of them have already reached the egg.

FIG. 51. Union of sperm and egg; sperm partly within egg nucleus; cytoplasm of egg enveloping nucleus very shallow toward neck.

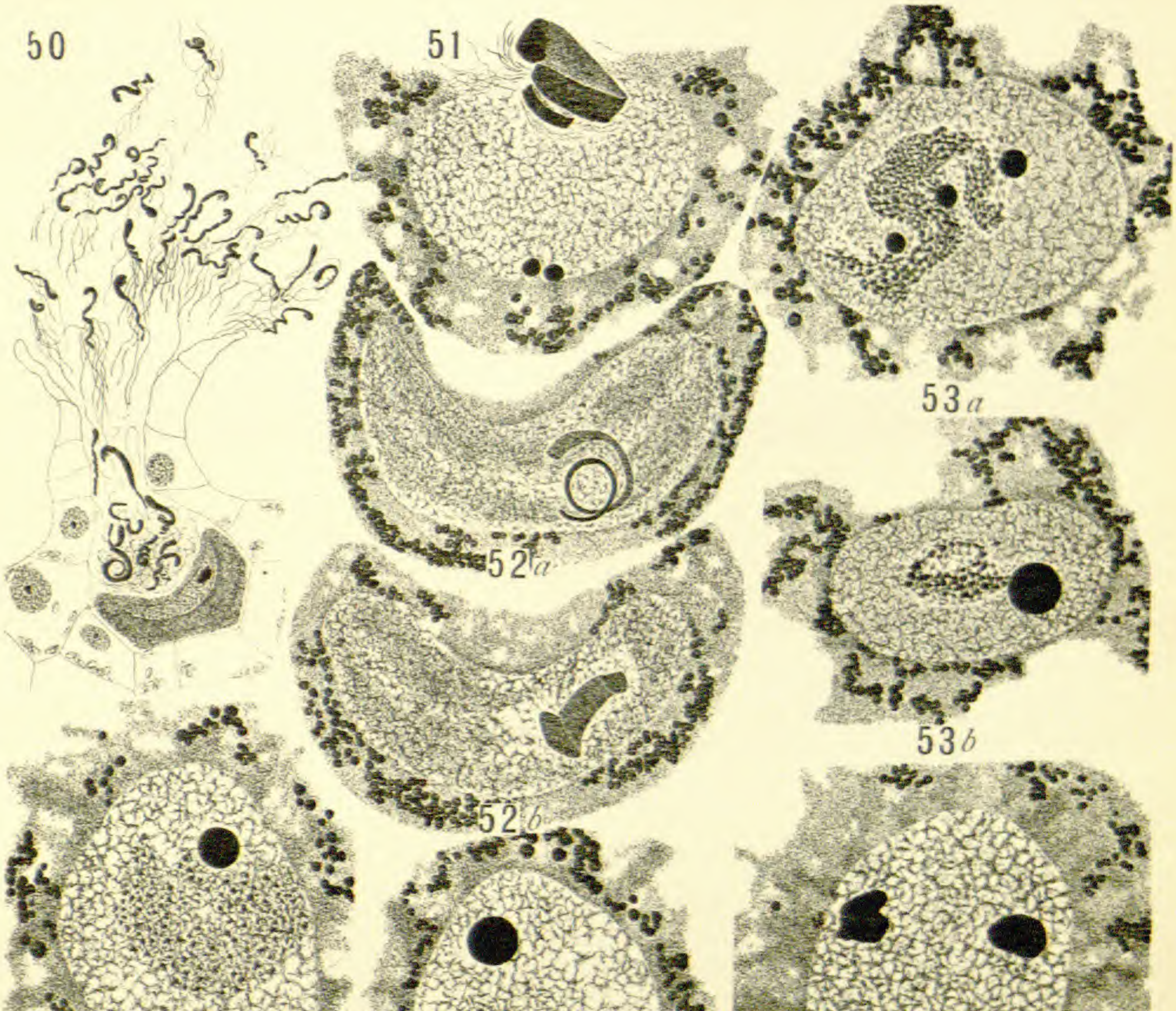
FIGS. 52*a*, *b*. Two sections of same egg nucleus containing sperm, which has not changed; clear region surrounding sperm; upper side of figures toward neck.

FIGS. 53*a*, *b*. Two sections of same egg nucleus containing sperm in later stage than that shown in previous figures; disintegration begun; compact body

YAMANOUCHI on NEPHRODIUM



YAMANOUCHI on NEPHRODIUM



YAMANOUCHI on NEPHRODIUM

of sperm loosened into complex structure of chromatin clumps and fine strands; three nucleoli in region of disintegrating sperm; upper side of figures toward neck.

FIGS. 54*a*, *b*. Two sections of egg nucleus containing a disintegrating sperm in stage later than in last figures; neck toward the left.

FIG. 55. Fusion nucleus after complete union of the two chromatin reticulums of sperm and egg; ragged reticular structure homogeneous throughout; three nucleoli present; upper side of figure toward neck.

FIG. 56. Spirem of first segmentation division of fusion nucleus shows a continuous structure.

FIG. 57. Anaphase of same mitosis; the two sets of chromosomes regularly arranged at the poles.

FIG. 58. Polar view of stage shown in *fig. 57;* 128 chromosomes present.

THE CARBON ASSIMILATION OF PENICILLIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 108

HEINRICH HASSELBRING

Since PASTEUR first showed that the lower fungi (yeasts in this case) could be grown on nutrient solutions containing organic substances other than those derived from plant and animal tissues, a large number of organic compounds have been examined with regard to their nutritive value for molds. It has been found that the most varied and widely different compounds are suitable in one case or another for supplying carbon and nitrogen to those organisms. All the fungi, however, cannot utilize the various compounds with equal facility. Thus, while Penicillium is almost omnivorous, thriving on alcohol, organic acids, sugars, and many other substances, *Mucor racemosus* and *M. javanicus*, according to WEHMER,[2] and yeast, according to LAURENT,[3] do not assimilate alcohol. In like manner, two very closely related compounds, such as the optically active modifications of tartaric acid, as well as chemical isomers without an asymmetric carbon atom,[4] may possess very different nutrient value.

The knowledge that a great number of organic compounds of the most varied structure could supply fungi with the necessary food and energy for all of their activities, soon led to attempts to establish some relation between the structure of organic compounds and their nutritive value. One of the first of these attempts was that of NÄGELI,[5] who came to the conclusion, as the result of the study of a large number of substances, that food value depended upon the specific linkage of

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[1] PASTEUR, L., Les corpuscules organisés qui existent dans l'atmosphère. Ann. Chim. et Phys. III. 64:5–110. 1862.

[2] WEHMER, C., Ueber das Verhalten der Mucor-Arten gegen verdünnten Alcohol. Ber. Deutsch. Bot. Gesells. 23:216, 217. 1905.

[3] LAURENT, E., Nutrition hydrocarbonée et formation de glycogène chez la levure de bière. Ann. Inst. Pasteur 3:113–125. 1889.

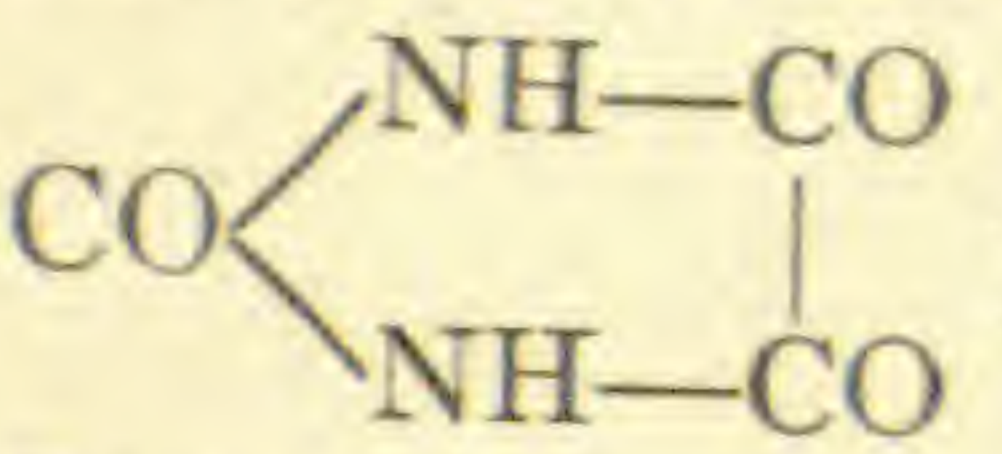
[4] BUCHNER, E., Notiz aus der Gährungschemie. Ber. Deutsch. Chem. Gesells. 25:1161–1163. 1892.

WEHMER, C., Beiträge zur Kenntnis einheimischer Pilze 87–104. 1903.

[5] NÄGELI, C., Ernährung der niederen Pilze durch Kohlenstoff- und Stickstoffverbindungen. Bot. Mitth. 3:395–485. 1881.

certain atomic groups. According to Nägeli, carbon is assimilated from the groups $=CH_2$ and $\equiv CH$, but in the latter case only when it occurs in a chain of two or more C-atoms to which H is bound. Benzoic acid is assimilated, but formic acid is not. On the other hand, carbon is not assimilated when it is directly united only with O or N but not with H. Accordingly, the CN group, oxalic acid, urea, and similar substances, are not suitable sources of carbon.

Subsequent researches showed that the conclusions of Nägeli were no longer tenable, since it was found that the molds were not restricted to such specific groupings as he supposed. Reinke[6] greatly extended the list of substances which could be assimilated, and showed that some of the particular groupings which Nägeli excepted could be utilized. Thus, for example, parabanic acid,



proved an efficient source of carbon for Penicillium. Diakonow[7] demonstrated that carbon from urea and from formic acid could also be utilized by this fungus.

All of these facts indicate that no general relation can at present be established between the atomic structure of a substance and its food value. The mechanism of assimilation probably differs with each individual case and is as much dependent upon the nature of the plant as upon the chemical reactions of the compounds used; for, as already stated, every substance that has nutritive value for one plant will not serve as a food for all plants.

It was with a view of gaining, if possible, some knowledge of the complex problem of the chemistry of the assimilation of some of the simpler compounds that the work reported here was undertaken. It was thought that by studying the effect of a number of related compounds on the growth of the mold fungi, and noting the variations caused by different stimulating agents, it would be possible to gain some knowledge of the probable chemical reactions by which nutrient substances combine with the constituents of the living cell.

This proved to be a most complex problem, whose solution is made

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[6] Reinke, J., Unters. Bot. Lab. Göttingen. (Rev. in Bot. Zeit. **41**:551. 1883, and Just's Bot. Jahresb. **11**:55. 1883.)

[7] Diakonow, N. W., Organische Substanz als Nährsubstanz. Ber. Deutsch. Bot. Gesells. **5**:380–387. 1887.

more difficult on account of the narrow limits of experimentation to which living organisms can be subjected. Although no finally satisfactory conclusions have been reached, a few series of cultures have been worked out with great care. As I am for the present compelled by circumstances to discontinue the work, it is desirable to present the results thus far obtained, with a few final remarks on their possible significance.

METHODS

The form selected for this work was a strain of *Penicillium glaucum*. A number of other molds were tried, but were rejected because they did not thrive well under the conditions of the experiments. Penicillium, which has been used in innumerable physiological investigations, is especially suitable for this work on account of its omnivorous habit, and because many compounds have been studied with regard to their nutritive value for this fungus. The same strain of the mold was used throughout. The stock-cultures were always grown on sterilized bean-stems, so as to avoid any possible temporary influence of the substratum on the strain.

The culture medium used was a solution of the necessary inorganic salts, of the purest grades obtainable in the market. Except where specially noted, the culture fluid always contained the mineral salts in the same concentration, namely, 1^{gm} NH_4NO_3, 0.5^{gm} KH_2PO_4, and 0.25^{gm} $MgSO_4$, per 100^{cc}. Potassium chlorid was added in some cases, and the concentration of the magnesium salt was varied in others. These changes are noted in the respective tables. It was the purpose in this work to have the mineral solution alike in all the cultures in order to make the different series exactly comparable. This was carried out, except where special problems occurred which demanded a change from the standard.

As a source of carbon the following substances were used: alcohol (C_2H_5OH), potassium ethyl sulfate ($C_2H_5KSO_4$), ethyl nitrate ($C_2H_5NO_3$), ethyl acetate ($CH_3COOC_2H_5$), potassium acetate (CH_3COOK), and acetic acid (CH_3COOH). These compounds are all closely related, and have certain radicles in common. The combination of those radicles with various groups has a definite effect upon the mode of reaction of the compounds. By a comparative study of a large number of compounds of this nature with regard to their assimilation by the molds, it was deemed possible to throw some light on the mechanism of their assimilation. The simpler compounds are most suitable for studies of this nature; first, because their reactions are well understood, and second, because it is known that the complex compounds, such as polysaccharides, glucosides, and certainly in some cases even the hexoses, undergo decomposition before they are taken into the cell.[8] The solution of the mode of assimilation of the simpler compounds will therefore enable us to approach an interpretation of this process with the more complex compounds.

Cultures were made in 200^{cc} flasks, of the Erlenmeyer form, loosely stoppered with cotton to permit the free exchange of air. In each flask 50^{cc} of the culture medium were used. The mineral solution was first put into the flasks and sterilized for 20 minutes in flowing steam, after which the organic solution was added. The concentration of all the substances given is the final concentration after the addition of the organic solution, without allowing, however, for error caused by evaporation in the sterilizer, which averaged a little less than 0.5^{gm} per flask. In cases where acid was added the error caused by this addition was also not considered. These two errors compensated each other. Moreover, it was found that much greater differences than these produced no effect on the growth of the fungus, and a variation in the concentration is furthermore unavoidable on account of a slight evaporation in the incubator, which could not be prevented. The cultures were grown in an electrically regulated and electrically heated incubator, which permitted the maintenance of a constant temperature of 28° C. The duration of the cultures, except where noted, was 10 days.

Considerable effort was made to work out the best method for inoculating the flasks. On account of some observations of DUCLAUX,[9] indicating that some media which were suitable for nourishing

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[8] PURIEWITSCH, K., Ueber die Spaltung der Glycoside durch die Schimmelpilze. Ber. Deutsch. Bot. Gesells. **16**:368–377. 1898.

BRUNSTEIN, A., Ueber Spaltungen von Glycosiden durch Schimmelpilze. Beih. Bot. Centralbl. **10**:1–50. 1901.

KOHNSTAMM, P., Amylolytische, glycosidspaltende, proteolytische und cellulose-lösende Fermente in holzebewohnenden Pilzen. *Ibid.* **10**:90–121.

[9] DUCLAUX, E., Sur la nutrition intracellulaire. Ann. Inst. Pasteur **3**:97–112. 1889.

grown mycelia were not suitable for the germination of spores, the spores used in the first cultures were germinated before inoculation. This method was extremely unsatisfactory, because the germinating spores could not be evenly distributed in the culture liquid. Moreover, it was found that spores germinated well in all the media used. Attempts to inoculate the flasks by means of the platinum wire also proved failures, because it was impossible to obtain an even distribution of the dry spores. The method finally adopted, which proved entirely satisfactory, was as follows: A stock-culture on beans, which was well covered with spores, was thoroughly shaken up with the liquid in which it was growing; the liquid was then poured on a screen of fine-meshed muslin in a funnel, and strained into a sterilized flask. This gave a liquid turbid with spores, most of which had been shaken apart and floated free in the liquid. Very little other material passed into the flask. For inoculation three drops of this liquid were dropped into each flask from a sterile pipette. This gave an abundance of spores, perhaps some thousands, which were uniformly distributed in the culture fluid.

The yield of dry material produced was used as an indicator of the effect of the food given. The yield was determined by adding 10^{cc} of a 10 per cent. solution of chemically pure hydrochloric acid to the culture to kill the growth and dissolve any precipitates that had been formed. After this the culture was filtered on a hard filter paper, from which the fungous material was washed into a tared Gooche crucible, and dried at 100–110° C. The variation in the temperature at which the yields were dried was not preventable, since no regulated drying oven was available. All the yields of each series were dried at the same time, however, so that they were subjected to the same conditions in drying. The different sets of each series are therefore comparable with each other.

TABLES AND EXPLANATIONS

In the following tables are given the results of the cultures, with the necessary explanations relating to each series. In the course of the work many hundreds of cultures were made before finally satisfactory details of manipulation were worked out, and for the purpose of determining the concentrations of the various substances that permitted the best growth of the mold. None of these preliminary series is included in this report, although the facts gained from them served in a general way to substantiate the results of the other cultures.

Alcohol.—One table, made for the purpose of determining how long the minimum quantity of alcohol used in some of the cultures could provide sufficient food for an increase in growth, may not be without interest and is here given as *table I.* The yield is given in milligrams in all cases.

TABLE I

Each flask received 0.46^{gm} alcohol = concentration of 0.2 GM. per liter.

Time in days (24 h.)	1	2	3	4	5
1	0.0	0.1	.....	...	...
2	1.4	1.8	1.9	...	...
3	13	14	15	16	17
4	.....	.....	.....	...	...
5	38	39	43	53	58
6	59	61	65	79	92
7	80	91	95	104	110
8	118	119	120	122	124
9	118	121	124	128	128
10	110	113	114	115	120

All the cultures in each horizontal row are duplicates. An increase in weight took place up to the tenth day, after which there was a gradual loss, due to respiration.

TABLE II

Each flask received 0.69^{gm} alcohol = concentration of 0.3 GM. per liter.

Number	I. No acid	II. 0.004nHCl	III. 0.004nHNO$_3$	IV. 0.004nH$_2$SO$_4$	V. Check. No organic compounds
1	53	90	106	113	4
2	57	108	120	116	
3	86	114	125	131	
4	89	115	127	139	
5	93	118	144	141	

TABLE III

Each flask received 0.46^{gm} alcohol = concentration 0.2 GM. per liter.

Number	I. No acid	II. 0.004nHCl	III. 0.004nHNO$_3$	IV. 0.004nH$_2$SO$_4$
1	68	91	100	88
2	70	93	106	89
3	71	93	106	91
4	73	102	108	93
5	77	103	110	95

In about 200 preliminary cultures it was found that the addition of small quantities of acids such as HCl, HNO_3, and H_2SO_4 had a stimulating effect on the growth of Penicillium. This is only an instance of the general rule that poisonous substances act as stimulants when given in sufficiently dilute concentration. These two series were carried through with great care and uniformity, in order to get, if possible, a more accurate quantitative statement of the facts noted in the preliminary cultures. There is considerable individual difference in the cultures, as is noted by reading the figures in each column, the five cultures being duplicates of one another. Nevertheless, they are of the same general magnitude. The addition of mineral acids produces a decided stimulation of growth. The nitric acid in each case gave a greater stimulation than the hydrochloric. The sulfuric acid in one case (*table II*) gave a greater increase than hydrochloric, while in the other case (*table III*) its effect was about equal to that of the HCl. These observations agree with those made on the preliminary cultures. Many of the cultures seemed to show that nitric and sulfuric acids produced greater stimulation than hydrochloric acid. It was thought possible that HCl might begin to act as a poison at the concentration used, but a series of cultures (*table IV*) showed that the optimum for stimulation was much higher than 0.004*n*. Therefore HCl at that concentration cannot act as a poison, but is actually a milder stimulant than HNO_3, and perhaps also weaker than H_2SO_4. In addition to the usual mineral nutrients, the cultures in *tables II, III*, and *IV* were given 0.25^{gm} KCl per 100^{cc} of culture fluid, to avoid the introduction of a new ion, Cl, in the cultures to which HCl was added. None of the alcohol cultures produced spores.

TABLE IV

Each flask received 0.69^{gm} alcohol = concentration 0.3 GM. per liter.

Number	CONCENTRATION OF ACID								
	1	2	3	4	5	6	7	8	9
	0.000	0.002*n*	0.004*n*	0.006*n*	0.008*n*	0.012*n*	0.016*n*	0.024*n*	Check
1..............	208	172	146	266	183	193	236	163	3.6
2..............	209	227	185	268	255	239	239	171	3.6
3..............	209	232	195	284	257	246	240	173	
4..............	214	239	266	285	260	255	246	181	
5..............	224	242	280	295	273	255	257	181	

Esters of alcohol with mineral acids.—The cultures with esters of alcohol with mineral acids gave the results expressed in the following tables. The actual quantity as well as the concentration of the organic substances in each flask are given in *tables V* and *VII* in the horizontal lines, and in *tables VI* and *VIII* at the head of the columns giving the yields for each set.

TABLE V

Numbers	Amount of $C_2H_5KSO_4$	Yield
1–10.......	0.82^{gm} (=0.1 GM. per l.)	none
11–20.......	1.64^{gm} (=0.2 GM. per l.)	none

TABLE VI

Number	0.82^{gm} $C_2H_5KSO_4$ (=0.1 GM. per l.) 0.69^{gm} C_2H_5OH (=0.3 GM. per l.)	0.82^{gm} $C_2H_5KSO_4$	0.41^{gm} $C_2H_5KSO_4$ (=0.05 GM. per l.) 0.69^{gm} C_2H_5OH	0.41^{gm} $C_2H_5KSO_4$	Check 0.69^{gm} C_2H_5OH
1.........	152*	None	200	None	193
2.........	184	None	210	None	
3.........	207	None	214	None	
4.........	219	None	228	None	
5.........	219	None	238	None	

* Contaminated.

TABLE VII

Numbers	$C_2H_5NO_3$*	Yield
1–10...............	0.46^{gm}	None
11–20...............	0.91	None

* The concentration of the ethyl nitrate is not stated, since only a part of the amounts given goes into solution in 50^{cc} of the culture fluid.

TABLE VIII

Number	0.23^{gm} $C_2H_5NO_3$ 0.69^{gm} C_2H_5OH (=0.3 GM. per l.)	0.46^{gm} $C_2H_5NO_3$ 0.69^{gm} C_2H_5OH	0.69^{gm} C_2H_5OH		Check. No organic matter
1............	103	52	68	117	2
2............	106	56	94	118	
3............	112	65	112	141	
4............	115	72	113	143	
5............	126	89	116	146	

The cultures of potassium ethyl sulfate (*table V*) had a barely visible film on the surface, similar to cultures to which no organic matter had been added. Microscopic examination showed that practically all the spores had germinated and had produced germ tubes 50 to 100 μ long. This is clear proof that the potassium ethyl sulfate is not poisonous in the concentrations used, but also that it is not a suitable source of carbon. This is more fully brought out in *table VI*. The cultures with potassium ethyl sulfate behaved as before, while those with sulfate to which alcohol was added gave a yield comparable with that obtained when alcohol alone was given. These cultures, like the alcohol cultures, remained pure white and produced no spores.

Ethyl nitrate in various concentrations ranging from 0.46^{gm} per 50^{cc} to 0.91^{gm} per 50^{cc} proved absolutely valueless as a source of carbon, although like potassium ethyl sulfate it did not inhibit germination at these concentrations. In a series in which the ethyl nitrate was given as the sole source of carbon and nitrogen, no growth took place. Neither is the fungus able to break up the nitrate by means of energy derived from alcohol, as *table VIII* shows, for the addition of ethyl nitrate failed to produce an increase of yield over pure alcohol. On the contrary, the higher concentration depressed the yield, showing that ethyl nitrate is mildly toxic. This is also shown by the fact that spores in cultures with ethyl nitrate germinated two to three days later than the alcohol cultures. The depression of yield may be due partly to the delay in germination, and partly to the toxic effect of the nitrate. The cultures produced no spores.

Ethyl acetate.—While the esters of alcohol with mineral acids proved valueless as a source of carbon for Penicillium, the ethyl ester of acetic acid in dilute solution is an efficient source of carbon; but in stronger solutions it becomes a poison, as appears from *tables IX* and *X*. The mineral solution used here was of the same composition as in the alcohol cultures.

TABLE IX

Each flask received 0.22^{gm} $CH_3COOC_2H_5 = 0.05$ GM. per liter.

Number	No mineral acid	$0.004n HNO_3$	$0.004n H_2SO_4$	$0.004n HCl$
1............	13	12	10	11
2............	14	12	11	11
3............	14	12	12	11
4............	14	12	12	12
5............	15	12	12	13

TABLE X

No.	I 0.88^{gm} $CH_3COOC_2H_5$ (=0.2 GM. per l.) 0.69^{gm} C_2H_5OH (=0.3 GM. per l.	II 0.88^{gm} $CH_3COOC_2H_5$	III 0.44^{gm} $CH_3COOC_2H_5$ (=0.1 GM. per l. 0.69^{gm} C_2H_5OH	IV 0.44^{gm} $CH_3COOC_2H_5$
1	None	None	59	19
2	None	None	74	20
3	None	None	79	21
4	None	None	89	22
5	None	None	90	22

Ethyl acetate is easily soluble in water and forms a suitable source of carbon for fungi. It is poisonous, however, in comparatively low concentrations. The fungi in this medium grew for a time and then ceased growing, owing to the accumulation of acetic acid in the cultures. The addition of mineral acids had no very evident effect on the growth of the mold, but seemed to depress rather than increase the yield. It is important to note that the cultures containing alcohol in addition to ethyl acetate (*table X*, *col. III*) gave a yield about equal to that produced by alcohol alone (*table II*, *col. I*). It is probable that the alcohol, which is the better food, is largely responsible for this growth, and that the acetate was left largely intact. If it had been consumed in the usual way, the accumulation of acetic acid would have stopped the growth of the cultures. The cultures containing alcohol failed to fruit, while those containing only ethyl acetate fruited abundantly. The spores from sets I and II, *table X*, were killed, as no growth appeared when they were transferred to bean-stems.

Acetic acid.—Acetic acid in its free state forms an efficient source of carbon, but is so highly toxic that it must be used in extremely dilute solutions. Nevertheless, two series of cultures placed the fact of its assimilation beyond doubt. These are given here.

TABLE XI

Acetic acid in each flask 0.012^{gm} ($=0.004n$).

Number	No mineral acid	$0.004nH_2SO_4$	$0.004nHNO_3$	$0.004nHCl$	Check
1	2	1	2.5	1	1
2	3	2	3	2	
3	3	3	3	2	
4	3	3	3.5	3	
5	3	3	4	3	

The addition of mineral acids seems to have no depressing effect, although the yields are so small that this would perhaps not be made evident. In the second series the weights were not determined, for the difference in growth is shown more strikingly by the appearance of the cultures than by the difference in weights. These cultures were made as follows:

TABLE XII

1– 5..........	No acid	Bare trace of growth
6–10..........	0.024^{gm} per flask (=0.008*n*)	Good growth forming thin flocculent film
11–15..........	0.048^{gm} per flask (=0.016*n*)	No germination
16–20..........	0.072^{gm} per flask (0.032*n*)	No germination

All the acetic acid cultures fruited, so that even the minute colonies were blue with spores.

Potassium acetate.—A large number of cultures was made with potassium acetate. The data from the most significant series of those are given here.

TABLE XIII

Mineral nutrients per 100^{cc} solution 1^{gm} NH_4NO_3, 0.5^{gm} KH_2PO_4, 0.25^{gm} $MgSO_4$, 0.25^{gm} KCl. Quantity and concentration of CH_3COOK per flask is given at the head of each column.

No.	I 0.245^{gm} CH_3COOK (=0.05 GM. per l.)	II 0.49^{gm} CH_3COOK (=0.1 GM. per l.)	III 0.98^{gm} CH_3COOK (=0.2 GM. per l.)	IV 1.47^{gm} CH_3COOK (=0.3 GM. per l.)
1....	31	41	40	40
2....	31	42	41	40
3....	32	42	41	41
4....	32	43	42	42
5....	32	43	42	51

TABLE XIV

The conditions of this series were the same as those for the preceding, except that no potassium chlorid was used in the mineral solution.

No.	I 0.245^{gm} CH_3COOK	II 0.49^{gm} CH_3COOK	III 0.98^{gm} CH_3COOK	IV 1.47^{gm} CH_3COOK
1....	30	26*	39	40
2....	31	40	40	41
5....	32	41	41	41
4....	32	41	41	41
5....	32	43	42	42

* Contaminated with a bacterium forming a green pigment.

TABLE XV

In this series the amount of CH_3COOK was 0.98gm per flask, but the magnesium content was varied.

Number	I 0.5gm $MgSO_4$ per 100cc	II 1gm $MgSO_4$ per 100cc
1	54	5
2	55	14
3	55	17
4	55	20
5	56	37
6	56	39
7	56	41
8	57	47
9	57	55
11	58	64

The potassium acetate cultures show a remarkable uniformity of yield, which is independent of the concentration of the acetate, except in very dilute solutions and probably also in very concentrated solutions. The lowest concentration in *tables IX* and *X* is too dilute to allow the full development of the fungus under those conditions, but all the other concentrations give the same yield.

With the increase of magnesium in the cultures, the yield is increased but still remains uniform (*table XV, col. I*). A higher concentration of $MgSO_4$ becomes poisonous.

In the growth of all the potassium acetate cultures the medium becomes alkaline, showing that only the CH_3COO- radicle is taken up by the fungus. The excess of KOH (or $KHCO_3$) results in the precipitation of NH_4MgPO_4, thus keeping the culture neutral or only slightly alkaline for a time. When all the Mg is precipitated, the increased alkalinity prevents further growth. The addition of more Mg delays this period, and hence gives a greater yield. The yield is practically quantitative for a certain amount of Mg, and is independent of the amount of CH_3COOK.

The potassium acetate cultures brought out the striking difference in power of resistance to deleterious substances possessed by different spores from the same culture. This was also observed in other cases where deleterious concentrations of substances were used, but it was nowhere as evident as in the potassium acetate cultures. By the method of inoculation, it is likely that more than a thousand spores were sown in each flask. In the favorable media a large percentage germinated so that the surface was covered from the beginning with a film of fungus. In the cultures containing the stronger solutions of CH_3COOK, sometimes less than a hundred colonies were formed, but these were able to grow with sufficient vigor to produce a yield equal to that of the flasks in which more spores had germinated.

All the potassium acetate cultures formed spores, but those in the concentration of 0.05 GM. per liter produced them most abundantly.

DISCUSSION

A general survey of these data shows that alcohol, acetic acid, and the substances from which the acetic acid radicle CH_3COO- is easily derived are assimilated by *Penicillium glaucum*. In the case of alcohol the addition of mineral acids stimulates growth, but HNO_3 produces greater stimulation than HCl. The esters of alcohol with mineral acids are valueless as a source of carbon, and their lack of nutritive value is not due to any toxic properties. The substances which possess the greater food value among the foregoing are, in general, those which are readily oxidized. To what extent will these data enable us to correlate the mode of assimilation of these compounds with the known chemical reactions of the substances?

The first possibility that presents itself is that alcohol enters into combination with substances in the protoplasm by virtue of its dissociation into ethylidene and water

$$CH_3CH_2OH \leftrightharpoons CH_3CH{=}+HOH,$$

which, as NEF[10] has shown, takes place completely at about 650° C. At ordinary temperatures dissociation into ethylidene and water takes place to a very slight extent, probably less than 0.01 per cent. The dissociation, however, is enormously increased by a combination of alcohol with other substances, as with metals or mineral acids, and also by the action of enzymes and other catalytic agents. The alcoholates are dissociated to so great an extent at ordinary temperatures that they burn spontaneously in the air. Ethyl nitrate dissociates at about 200° C. (?) and potassium ethyl sulfate at 250° C. Ethyl sulfuric acid and ethyl sulfate dissociate at low temperatures, so that ether formation begins in a mixture of alcohol with a little sulfuric acid at

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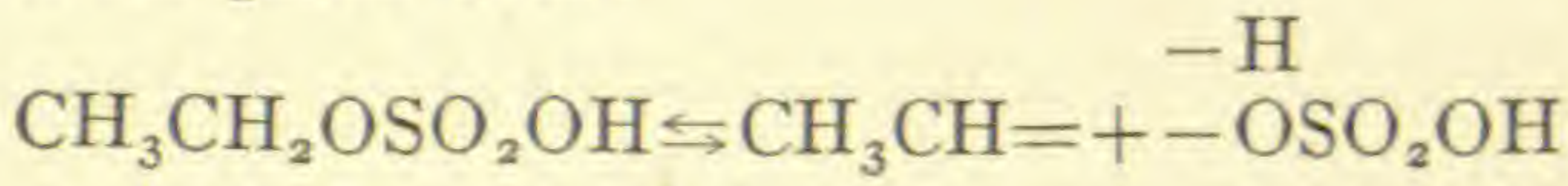
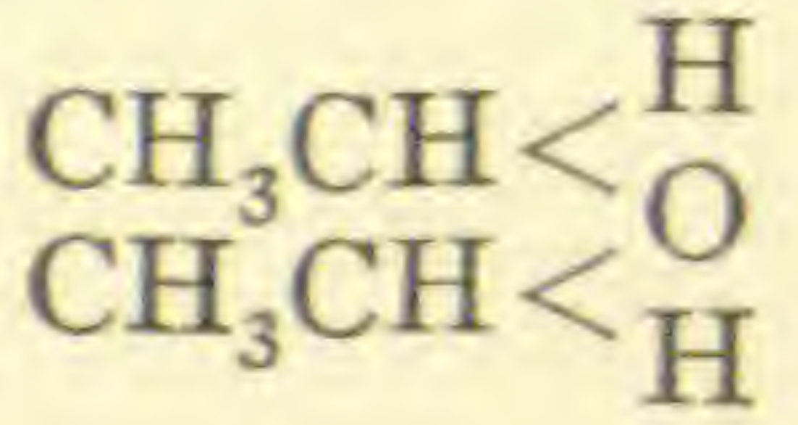
[10] NEF, J. U., On the fundamental conceptions underlying the chemistry of the element carbon. Journ. Am. Chem. Soc. **26**:1549–1577. 1904.

95° C. The reaction is due, as NEF[11] has shown, to the absorption of water by the bivalent carbon of the ethylidene particles. The reaction may be represented as follows. The dissociation of ethyl sulfuric acid takes place thus:



$$CH_3CH_2OSO_2OH \leftrightarrows CH_3CH= + \overset{-H}{-OSO_2OH}$$

The sulfuric acid is regenerated and reacts with more alcohol to give ethyl sulfuric acid, or ethyl sulfate, while the ethylidene decomposes water to form ether thus:



$$\begin{array}{ll} CH_3CH< & H \\ & O \\ CH_3CH< & H \end{array}$$

If, in the first steps of assimilation, alcohol enters into combination with some substance of the protoplasm to form an unknown compound, represented by $CH_2CH=Ppm$, then we should expect substances which increase ethylidene dissociation to affect the rapidity of assimilation. It is immaterial whether the alcohol is first elaborated into sugar, glycerin, or some other substance, before it becomes a part of the protoplasm, or whether it is directly taken up by the permanent constituents of the cell. In either case it must combine with some substance of the cell, and this combination must be regarded as the first step in assimilation.

An examination of the cultures (*tables II, III*) shows an increase in growth due to the addition of sulfuric acid, which, even in dilute solutions, would to some extent combine with alcohol and dissociate into ethylidene; but an equal increase is obtained by the addition of hydrochloric acid, and nitric acid shows an even greater increase in most cases; yet neither hydrochloric nor nitric acid combines with alcohol when mixed with it. This would indicate that stimulation by the acids is not due to any dissociating effect on the alcohol. Furthermore, if assimilation took place by direct combination of ethylidene with some substance of the cell, then bodies which dissociate very easily should be most rapidly assimilated. We find, however, that potassium ethyl sulfate and ethyl nitrate, which dissociate to a high degree, are valueless as sources of carbon when given alone, and in all probability cannot even be utilized by means of energy derived

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[11] NEF, J. U., Dissociationsvorgänge bei den Alkyläthern der Salpetersäure, der Schwefelsäure, und der Halogenwasserstoffsäuren. Liebig's Annalen 318:1–57. 1901

from alcohol when it is given together with the esters. This would seem to argue against the direct absorption of ethylidene by the protoplasm or cell constituents.

Another possibility to be considered is the oxidation of alcohol to acetaldehyde or even to acetic acid. This view becomes the more probable on account of the ease with which alcohol is known in many instances to be oxidized by organisms. This view would perhaps explain also the increased stimulation of nitric acid over hydrochloric acid, on the basis of the oxidizing power of nitric acid. Oxidation to acetic acid, if it takes place at all, does not proceed to such an extent that the acid accumulates in the cultures.

To determine whether acetic acid was accumulated in the cultures, 10 flasks, with alcohol as an organic food, were inoculated in the usual way. After a vigorous growth took place, the culture solution was poured off, and after rinsing was replaced by a 0.3 GM. solution of alcohol. After further 6 days this liquid was tested for acetic acid, but none could be detected. DUCLAUX also was unable to observe the formation of acetic acid from alcohol by Aspergillus, but mentions the fact that alcohol was assimilated with the intermediate formation of oxalic acid. I was unable to show the presence of oxalic acid in the cultures of Penicillium, nor is it likely that this is formed. If alcohol is assimilated by way of acetic acid the oxidation takes place entirely within the cell.

Another fact gained from the ethyl acetate cultures (*table X*) speaks against the oxidation of alcohol to acetic acid. From the general principles resulting from the work on selective power or organisms by PASTEUR, DUCLAUX, and PFEFFER, we know that when two foods of different nutrient value are given to a plant, the one most readily assimilated is used, often to the exclusion of the other. Although the experiments with ethyl acetate have not been carried as far as might be desirable, there is some evidence that acid accumulates in the cultures, consequently that the alcohol radicle is more readily absorbed than the acid radicles. This, it would seem, would not take place if it was first necessary to oxidize the alcohol to acetic acid. If any oxidation takes place it is probable that this stops with acetaldehyde, from which the formation of sugars can proceed. It would then be probable that the acetic acid in all cases was reduced to the aldehyde. This would account for the greater ease of assimilation of alcohol, since the oxidation of alcohol takes place more readily than the reduction of acetic acid.

The cultures of acetic acid and potassium acetate require no discussion, since it is evident, especially from the acetate cultures, that it is the acetate ion that is assimilated. This ion was assimilated, so far as could be determined, with much more difficulty than alcohol, which would be in accord with the belief that the acid must be reduced to aldehyde.

INCIDENTAL OBSERVATIONS

As in any long series of cultures, some facts were observed in this study, which, while not directly concerned with the work, may be of sufficient interest to be worth noting.

DUCLAUX is responsible for the belief, current in textbooks, that some substances which permit vigorous growth of mycelia are not suitable for the germination of spores. Alcohol is one of the substances mentioned by him. Contrary to this statement, it was found that alcohol was not only favorable for growth, but also permitted abundant germination of spores. From the preliminary cultures it seems probable that all concentrations which will permit growth will also permit germination.

In many other instances substances failed to allow mycelia to develop and yet were not detrimental to germination, e. g., $C_2H_5KSO_4$. This whole subject deserves further investigation, with careful study of the effect of different concentrations on growth and germination.

An interesting observation was made on the alcohol cultures, namely, that none of them produced spores during the growth of the cultures. It is difficult at present to offer an explanation of this fact. The fungus grew more vigorously in the alcohol cultures than in any others, and as far as could be observed all the external conditions necessary for the production of spores were present. The mycelia formed dense white mats on the culture liquid, and innumerable hyphae grew into the air—a condition which usually leads to the production of spores—yet none of the aerial hyphae bore spores. It would seem as if this strain of Penicillium was unable to manufacture all of the compounds necessary for spore formation from alcohol alone;

yet such an explanation is improbable when we remember that other compounds easily derived from alcohol, e. g., acetic acid, ethyl acetate, and potassium acetate, furnish material for the development of the fungus and the production of spores. It is also possible that the suppression of spores is due to some deleterious action of the alcohol in the culture fluid, although it is difficult to see how a substance can at one and the same time act as a food of high nutrient value and as a poison. At first sight the cultures containing both ethyl acetate and alcohol would seem to bear out the view that alcohol was deleterious to spore-formation, for cultures with ethyl acetate alone fruited, while those containing alcohol also failed to fruit. In such cultures, however, it is probable that the more nutritious alcohol was absorbed first, and largely to the exclusion of the acetate. Further work is necessary to give a complete explanation of this unusual phenomenon. It may be found that all strains of Penicillium do not act alike in this respect.

Another fact, brought out in the course of this investigation, was the great individual difference of resistance of spores to deleterious agents. In the lower concentrations of all substances favorable for growth, practically all spores germinated, forming a dense matlike growth over the surface of the culture fluid. When, with increase of concentration, the substance becomes deleterious, germination and growth are not stopped abruptly, but the number of colonies becomes fewer and fewer until the final concentration is reached, where germination of even the most resistant spores is inhibited. In most cases, where only a few colonies were formed, these grew with unusual vigor, so that the total weight of the culture was often as great as that of the cultures of lower concentrations.

This was especially well shown in the series with potassium acetate, *tables XIII–XV*. Here comparatively few spores germinated in the higher concentrations, forming isolated floating colonies. Yet the yield from these was equal to the yield from other flasks which were uniformly covered. This seems to indicate that the mycelium from the more resistant spores continues itself to be more vigorous throughout life, although it is not impossible that the belief of DUCLAUX holds true here; that is, while the concentration in any given case may be injurious to germination, it does not interfere with later development.

The spores which are able to germinate then have the whole of the nutrient solution at their disposal and make more vigorous growth.

In conclusion I wish to express my thanks to Professor CHARLES R. BARNES, under whom this work was carried on; and to Professor J. U. NEF of the Department of Chemistry for many helpful suggestions relating to the chemical aspects of the work.

THE UNIVERSITY OF CHICAGO

BRIEFER ARTICLES

A NEW SPECIES OF ACHLYA

(WITH SIX FIGURES)

In October 1906, while studying the Saprolegniaceae of Chapel Hill, we met with a new form of Achlya in a culture taken from a small brook near the east gate of the university campus. *Achlya ferax* and *Saprolegnia diclina* developed in the same collection, but the three species were isolated, and the new form kept growing in pure cultures for over two months. A careful study was made of its growth and structure and some of the observations made are given at the end of this paper. The specific description is as follows:

Achlya hypogyna, sp. nov.—Hyphae slender, tapering gradually toward the apex, at base about 35 μ in diameter, at or near tip about 8 μ, in vigorous cultures reaching a length of 1^{cm}. Zoosporangia nearly cylindrical, sparingly produced. Oogonia generally borne on short branches, racemosely arranged on the main hyphae, but occasionally terminating a main hypha, and very rarely intercalary; globular or rarely oblong, the walls more or less abundantly producing short, rounded outgrowths; yellow when old. Oospores 1–7 (commonly 3–5), centric, diameter 24–36 μ, averaging 27–28 μ. Antheridia cut off from oogonial branches just below the oogonia, very rarely absent. Fertilizing tubes arising from the common septa and penetrating the oogonia from below (hypogynous).

The absence of antheridial branches and the origin of the fertilizing tubes from the septa separating the oogonia and antheridia distinguish this from all other species of Achlya and from all other Saprolegniaceae except the *Hypogyna* group in Saprolegnia. In two or three cases noted a short antheridial branch arose from the main hypha near the oogonium and applied its end to the surface of the oogonium, and in one case such an antheridial branch was of diclinous origin. In all such cases, however, the characteristic sub-oogonial antheridia were also present and no fertilizing tubes were formed from the supernumerary antheridial branches. It is evident that *Achlya hypogyna* is closely related to *A. racemosa* Hildeb., var. *stelligera* Cornu.[1] The general habit, the structure of the oogonia, and the sub-oogonial antheridia are very much the same in both forms;

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[1] HUMPHREY, JAMES ELLIS, The Saprolegniaceae of the United States, with notes on other species. Trans. Amer. Philos. Soc. 17:63–148. *pls. 14–20.* 1892.

but the more slender hyphae, the larger oospores, and the exseptate origin of the antheridial branches are characters of sufficient importance to raise the Chapel Hill form to specific rank.

The presence of hypogynous fertilizing tubes in a species so evidently in the same group with *A. racemosa* and its variety *stelligera*, in which they are absent, must modify to some extent our ideas of the distinctive value of this character. MAURIZIO suggests that sub-oogonial antheridia with hypogynous tubes may be of generic value. He says:[2]

> Vielleicht könnte man das hypogyne Antheridium zu einem Gattungsmerkmal erhöhen, wodurch die offenbar eine natürliche Gruppe bildenden hypogynen Formen von der Gattung Saprolegnia abgetrennt würden. Charakterisirt eine Querwand in der Traghyphe den in das Oogonium eindringenden Fortsatz als einen Befruchtungsschlauch wie dies bei den zwei vorliegenden Arten der Fall ist . . . . ist dieser ein Analogon des Befruchtungsschlauches bei *Pythium ferax* de Bary, so müsste man auch die letztere Species von den Pythiumarten trennen.

Such a suggestion cannot be considered when we find this character present in only one of a group of such clearly related forms as *A. racemosa*, *A. racemosa stelligera*, and *A. hypogyna*. These three forms, which we might call the *Racemosa* group, are distinctly segregated as MAURIZIO'S *Hypogyna* group among the species of Saprolegnia.[3]

In the cultivation of *Achlya hypogyna* it was found best to use small gnats as a medium. The culture could then be made in hanging drops or on slides in Petri dishes and the growth studied without misplacement. A marked peculiarity was the scarcity of zoosporangia. These were found only in young cultures, appearing about 24 hours after infection; two are shown in *fig. 1*. The oogonia, however, were very abundant; their usual arrangement is shown in *fig. 2*. A mature oogonium and antheridium of typical appearance are represented in *fig. 3*. The fertilizing tube in this case is branched near its base. In a very few cases the oogonia are oblong (*fig. 4*), and some intercalary ones are occasionally seen (*fig. 5*). When the stalk is very short, the antheridium may extend some distance into the main hypha (*fig. 6*). It will be noted that one of the oogonia here shown has no projections on its walls; this peculiarity is of very rare occurrence. In one case a hanging drop containing a gnat was infected with a sporangium in which the spores were formed but not yet discharged. The spores remained in the sporangium, but sent out

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[2] Beiträge zur Biologie der Saprolegnieen. Mitteilungen des Deutschen Fischerei-Vereins 7^1:1–66. *figs. 19.* 1899.

[3] Flora 79:109–158. *pls. 3–5.* 1894. Jahrb. Wiss. Bot. 29:75–131. *pls. 1–11.* 1896. Beiträge zur Biologie der Saprolegnieen. Mitteil. Deutsch. Fischerei-Vereins 7^1:1–66. *figs. 19.* 1899.

tubes, which penetrated the sporangium wall and ran over to the gnat. In twenty-four hours after the gnat was reached, sporangia were beginning to discharge in the new culture. In another case when infection was made with a hypha bearing some young oogonia, the gnat was penetrated by a hypha which arose from the stalk of one of the oogonia; not until forty-two hours later was the new growth conspicuous.—W. C. COKER and J. D. PEMBERTON, *Chapel Hill, N. C.*

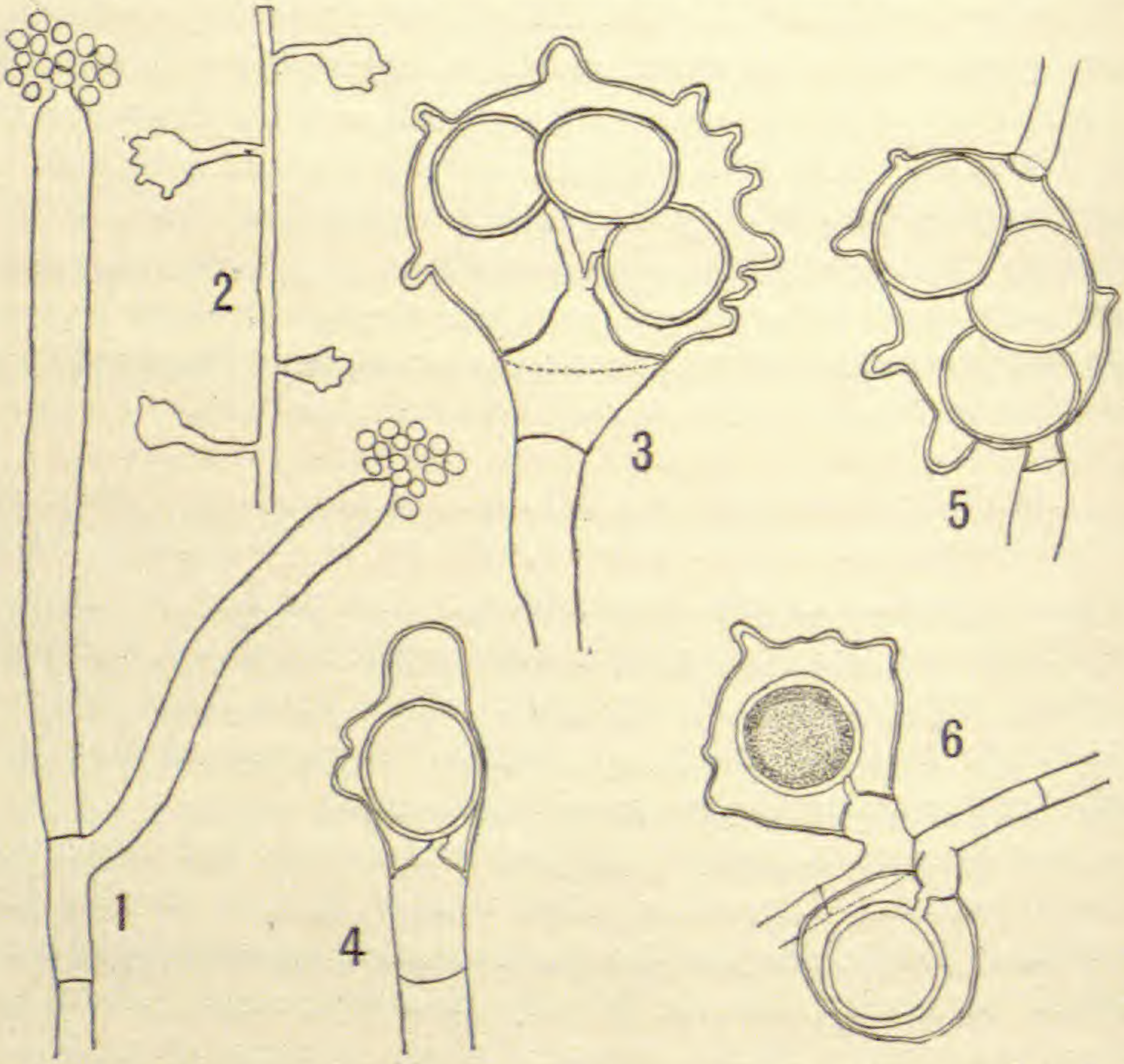

FIG. 1. Two emptied zoosporangia. ×335.—FIG. 2. Immature oogonia on a main hypha. ×75.—FIG. 3. Typical oogonium with three oospores; antheridium below and branched fertilizing tube arising from the partition. ×335.—FIG. 4. Oblong oogonium with antheridium. ×335.—FIG. 5. Intercalary oogonium, without an antheridium. ×335.—FIG. 6. Two short-stalked oogonia, with antheridia extending into main hypha. ×335.

ON THE ORIGIN OF ANGIOSPERMS[1]

In an extensive paper to be published during 1908, I have reached the following conclusions concerning the origin of angiosperms:

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[1] After this paper had gone to press, it appeared (in German) in Ber. Deutsch. Bot. Gesells. **25**:496, 497. 1907. The editors were not aware that it had been sent elsewhere for publication, and regret the unnecessary duplication.—EDS.

1. In Juliania the secretory canals are not wanting in the bark, as JADIN affirms. It is a genus of Rhoideae, exceptional in its 2–4 flowered cupula.

2. The Juglandaceae and also the Anacardiaceae are descendants of such Rhoideae as Juliania and Pistacia, but are still more reduced in the structure of flower and fruit.

3. The Brunelliaceae, Burseraceae, Irvingiaceae, Sabiaceae, Anacardiaceae, ENGLER's Julianiales, Juglandales, and some genera of his Simarubaceae (Picrodendrum, Picramnia, Alvaradoa) must constitute together the old order Terebinthaceae.

4. Also, the Leitneraceae, Aceraceae, Amentaceae (1. Quercineae, 2. Myriceae, 3. Coryleae, 4. Casuarineae, 5. Betuleae), and Urticales, thus comprising most of the chalazogams, are reduced descendants of Pistacia-like Terebinthaceae, and not descendants of Hamamelidaceae or Columniferae (including Euphorbiaceae), the latter of which must be considered as reduced descendants of Buettnerinae.

5. From the preceding orders the Balanopsidaceae (Balanops and Trilocularia) differ much in anatomical structure; they are Hamamelidaceae and are related to Trochodendrum, Tetracentrum, Daphniphyllum, and Rhodoleia.

6. The Salicaceae must be regarded as reduced descendants of Flacourtiaceae, and related to Homalieae and Idesieae; the Lacistemaceae as a tribe of Flacourtiaceae next to Homalieae; and the Piperales (including Lactoris and Myrothamnus) as reduced descendants of Magnoliaceae.

7. The derivation of the Hamamelidales (Platanaceae and Hamamelidaceae), as congeners of the Saxifragaceae, from Magnoliaceae must also be maintained.

8. The chalazogamy of Ulmus, Juglans, and many of the Amentaceae suggests the presence of chalazogamy, and other embryological resemblances to Casuarina, in Myrica, Leitnera, Acer, Juliania, Pistacia, Rhus, and other Terebinthaceae.

9. As descendants of Terebinthaceae, and in accord with WIELAND's discoveries among Bennettitaceae, in spite of WETTSTEIN's opinion to the contrary, the Amentaceae (including Casuarina) and the Urticales are becoming completely out of the question as connecting links between gymnosperms and angiosperms, and cannot interfere longer with my opinion that the Magnoliaceae are descendants of Cycas-like or Bennettites-like gymnosperms.

10. Also the Gnetaceae, which in some respects resemble dicotyledons, and the Conifers, extremely adapted to xerophilous conditions, on account

of their much advanced reduction are out of the question as connecting links between gymnosperms and angiosperms.

11. The resemblances of Loranthaceae to Gnetaceae are not founded on natural affinity, all the Santalales being reduced descendants of Saxifragaceae or of Celastrales.

On account of the great importance of the problem under consideration, I recommend that the following forms be examined during this next season, as to their fertilization and embryology, by the botanists of Europe (Myrica, Acer, Pistacia, and Rhus), the United States (Myrica, Leitnera, Acer, Juliania, etc.), Tokyo (Myrica, Acer, Rhus), Buitenzorg and Peradeniya (Terebinthaceae).—HANS HALLIER, *Botanische Staatsinstitut, Hamburg.*

THE GENERIC NAME GOLDMANIA

DR. J. N. ROSE of the United States National Museum has kindly called my attention to the duplication of a generic name by the publication of *Goldmania* in my recent paper entitled "New or otherwise noteworthy Spermatophytes from Mexico, Central America, and the West Indies" (Field Col. Mus. Bot. Ser. **2**:247–287. 1907). This name having been used for a new genus of the Leguminosae, namely *Goldmania* Rose (Mém. Soc. Phys. et Hist. Nat. Genève **34**:274. 1903), I propose the name **Goldmanella**, gen. nov. of Compositae (Coreopsideae), for the plant which I described as *Goldmania*. The binominal may be formed as follows: **Goldmanella sarmentosa** Greenman, n. comb. (*Goldmania sarmentosa* Greenm. Field Col. Mus. Bot. Ser. **2**:270. 1907).—J. M. GREENMAN, *Field Museum of Natural History.*

CURRENT LITERATURE

BOOK REVIEWS

Injury by smoke

Dr. A. Wieler[1] has written a volume upon the effects on vegetation of sulfur dioxid, the most injurious constituent of smoke.[2] The subject of smoke injury to agricultural and forest crops has long been of importance in Europe, and is now beginning to attract attention in this country, where, with the extension of agriculture into semiarid mining regions and the increased interest in forestry, some means of restraining injury to crops or some basis for adjusting damages becomes more and more necessary.

The first chapter of the book is devoted to the demonstration of the presence of SO_2 in the leaves of plants injured by smoke. For this the author relies on the method of Windisch, which revealed the presence of SO_2 in leaves of ivy even 14 days after fumigation. Numerous analyses from smoky districts showed SO_2 in the leaves in varying amounts. The analyses do not show any definite relation between the amount of SO_2 present in the leaves and distance from the source of smoke.

In the second chapter it is shown that, contrary to the view of von Schroeder and Reusz, SO_2, like other gases, enters only through the stomata. Leaves whose stomatal surfaces were coated showed no injury when exposed for several hours to comparatively strong concentrations of the gas. In very young leaves and in leaves whose stomata are permanently closed, the gas penetrates the cuticle.

The third chapter, which takes up the greater part of the book, discusses the physiological effects of SO_2, which are so complicated in nature that all cannot be explained by the killing action of the gas on the tissues. Probably both its acid nature and its capacity for forming addition products with aldehydes in the tissues come into play. Two kinds of injury are distinguished, the acute and the chronic. The acute form is rare and occurs only in the immediate vicinity of the source of smoke, where the concentrations of SO_2 are sufficiently high to kill the tissues directly. Yet experiments on the resistance of various organs show that the concentration of gas necessary to produce the acute form of injury is much greater than that found in smoke-injured regions, and the great individual differences shown tend to obscure the results. To find an explanation of the chronic injury, which is the more common and more difficult to recognize and account for, the effect of dilute mixtures of the gas were studied. Even under uniform

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[1] Wieler, A., Untersuchungen über die Einwirkung schwefliger Säure auf die Pflanzen. 8vo. pp. vii + 427. *figs. 19*. Berlin: Gebrüder Borntraeger. 1905. *M*12.

[2] The importance of this work justifies the publication of a review long after it was due.—Eds.

conditions respiration was found to be irregular. Photosynthesis was depressed, probably by the direct action of the gas on the chlorophyll, and not by a closing of the stomata (*Betula alba* and Salix); while transpiration is not depressed by the gas in any concentration. The rate of absorption of water is not reduced, the removal of the products of photosynthesis is delayed, and growth is retarded. In spite of all these effects of the gas, the author regards them as insufficient to account for the chronic injury. In a later chapter he reasons that the cause of the chronic injury must be sought in the effect of the gas on the soil, in which a condition similar to that present in cedar bogs is brought about. All the effects on trees point to a death by drouth.

Other chapters deal with the relation of soil conditions to the growth of trees, and with the resistance of various plants to smoke injury. Finally, the methods of determining smoke injury are discussed.

As a whole, the work is a record of careful experiments and observations on this very obscure subject. The great difficulty of properly diagnosing injurious effects due to smoke is repeatedly emphasized. Even in the carefully guarded experiments, the individual differences of different plants, as well as unfavorable factors due to the conditions of the experiments, often tend to obscure the effects of the dilute mixtures of the gases used. All this, however, has served to draw the author's attention to an important phase of the subject which has hitherto probably not been sufficiently considered, that is, the effect of the acids of smoke in the soil. Through changes induced by the continuous accumulation of SO_2 in the soil, the author believes that conditions are brought about which lie at the bottom of all the observed phenomena of smoke injury.—H. HASSELBRING.

MINOR NOTICES

Manual of micrography.—Dr. J. W. MOLL, professor of botany in the Royal University of Groningen, has prepared a manual[3] for the use of beginners in laboratory practice in botany, which he believes is adapted not only to enable them to gain useful information by their own efforts, but also to train them in proper methods of scientific work. The book is the outgrowth of the author's experience, which has led him strongly to deprecate the use in the laboratory of books which tell the student what is to be seen. In the preface he sets forth his views vigorously on the pedagogics of botany, with special reference to the place of practical exercises.

The book is divided into three parts. The first gives a brief account of instruments, reagents, and materials; and the second gives directions for the analysis of microscopic structure and outlines for micrographic descriptions. The third part contains 489 exercises, which are scarcely more than lists of the material and preparations needed. Of course such exercises presuppose verbal

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[3] MOLL, J. W., Handboek der botanische micrographie, ten gebruike bij de practische oefeningen voor aanstaande medici, pharmaceuten en biologen. Small 8vo. pp. vi+356. *figs. 4.* Groningen: J. B. Wolters. 1907. *fl.* 4.25.

explanation as to what is to be done, and written reports of what has been seen. The great number and variety of the exercises would permit selections for a general course in botany, or special courses intended for medical or pharmaceutical students. One section, of some hundred exercises, is devoted to important anatomical and morphological papers, each represented by a single exercise. Another takes up the "simples" of the Dutch pharmacopoeia for the benefit of pharmaceutical students. The language of the handbook will probably preclude its use even by teachers in this country.—C. R. B.

Jost's plant physiology.—The active demand which exhausted the first edition of this valuable work, and the advances in plant physiology since 1903, not to mention the appearance of an English translation, have required the preparation of a second German edition.[4] As we have formerly and recently expressed a highly favorable opinion of the quality of this work,[5] nothing is now needed but to point out changes in the new edition. The title-page indicates the transfer of Dr. JOST from Strassburg to Bonn. In the arrangement of material we note the change in the title of the third section from *Energiewechsel* to *Ortwechsel*, and the transfer of the lecture on the forms of energy in the plant from the third section to the first (*Stoffwechsel*), bringing it into close association with the discussion of dissimilation, where it more properly belongs. The term *Ortwechsel* is a happy designation of the section which deals with movements. The additions, though slight, are numerous and in some cases important. The number of figures is increased nearly a dozen, and some substitutions are noticeable. The bibliography, which formerly followed each chapter, is now gathered together at the close of the volume, where it is much more compactly presented; but the alphabetic arrangement is continued under each topic, and by bold-faced type the possible obscurity of the solid lists is obviated. By this change some twenty pages are gained, and devoted to the new matter, so that the size of the volume remains practically the same.—C. R. B.

Molecular physiology.—In 1903 Professor LÉO ERRERA prepared a course of lectures in what he calls molecular physiology.[6] The manuscript has been arranged and edited by Dr. SCHOUTEDEN and has been published by the Botanical Institute in its *Recueil*. In a preface Professor H. J. HAMBURGER of Groningen, to whom the manuscript was submitted for advice as to its publication, writes "que ce serait un crime de lèse-science de ne pas les publier."

The lessons consist of a presentation of those portions of molecular physics which are particularly applicable to physiology. The properties and behavior of gases, liquids, and solids, such as diffusion, surface tension, cohesion, viscosity,

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[4] JOST, L., Vorlesugen über Pflanzenphysiologie. 2 ed. Imp. 8vo. pp. xiv+693. *figs. 183*. Jena: Gustav Fischer. 1908. *M*14; geb. *M*16.

[5] BOT. GAZETTE **37**:390. 1904, and **44**:309. 1907.

[6] ERRERA, LÉO, Cours de physiologie moléculaire, fait au doctorat en sciences botaniques en 1903. Leçons recueillies et rédigées par H. SCHOUTEDEN. Recueil Inst. Bot. Bruxelles **7**:1–153. 1907.

imbibition, and solution, are discussed, first in the abstract, and then applied, so far as possible, to the explanation of the formation of membranes, protoplasmic movement, cell division, molecular structure, turgor, the movement of water, etc. They form a useful summary, naturally not quite up to date, of facts and theories which it is essential for the student of physiology to command. It is doubtful if elsewhere they can be found more conveniently arranged and effectively presented. All of them have for some years found a place in the writer's lectures on plant physics.—C. R. B.

North American Flora.—The first part of volume IX has just appeared, being the sixth part published. It contains a part of the Polyporaceae, by W. A. MURRILL, 33 genera being presented, the following being new: *Hydnoporia, Fuscoporia* (9 spp., 2 new), *Fuscoporella* (6 spp., 5 new), *Fomitiporia* (17 spp., 16 new), *Fomitiporella* (9 spp., 7 new), *Tinctoporia* (1 new), *Melanoporella*, and *Melanoporia*. The large genera are Coriolus (40 spp., 13 new), Polyporus (35 spp., 2 new), Tyromyces (25 spp., 6 new), and Hexagona (19 spp., 5 new). In other genera 9 new species are described.—J. M. C.

Icones Plantarum.[7]—The species illustrated in the twenty-five plates of this part include 11 new species by HEMSLEY, one of which represents a new Chinese genus (*Sinofranchetia*) of Lardizabalaceae. Five additional new species are described, but not illustrated. These 16 new species are all oriental, chiefly Chinese, and belong to the following families: Sabiaceae, Hamamelidaceae (8), Lardizabalaceae (6), and Triuridaceae.—J. M. C.

Pflanzenfamilien.[8]—Part 229 contains the completion of Hookeriaceae, the Hypopterygiaceae, Helicophyllaceae, Rhacopilaceae, and most of Leskeaceae, by V. F. BROTHERUS. Part 230 contains the completion of Ascolichenes and the beginning of Hymenolichenes, by A. ZAHLBRUCKNER. The fourth part concludes the second supplement (1899–1904) and includes the title-page and index.—J. M. C.

Das Pflanzenreich.[9]—Part 32 contains the tribe Coelogyninae of Orchidaceae by PFITZER and KRÄNZLIN. Of the 15 genera recognized, the following are described as new: *Ptychogyne, Hologyne, Sigmatogyne, Chelonistele*, and *Camelostalix*. The species number 246, the large genera being Coelogyne (103), Dendrochilum (74), and Pholidota (31).—J. M. C.

Eucalyptus.—The ninth part of MAIDEN's revision of Eucalyptus[10] contains the description, synonymy, range, and affinities of eight species. This series, begun in 1903, now includes thirty-one species.—J. M. C.

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[7] HOOKER's Icones Plantarum. IV. 9^2: *pls. 2826–2850.* 1907.

[8] ENGLER, A., und PRANTL, K., Die natürlichen Pflanzenfamilien. Lief. 229 und 228 und Ergänzungsheft II, Lief. 4. Leipzig: Wilhelm Engelmann. 1907.

[9] ENGLER, A., Das Pflanzenreich. Heft. 32. Orchidaceae-Monandrae-Coelogyninae von E. PFITZER und FR. KRÄNZLIN. pp. 169. *figs. 54* (294). Leipzig: Wilhelm Engelmann. 1907. *M*8.40.

[10] MAIDEN, J. H., A critical revision of the genus Eucalyptus. Part IX, pp. 259–294. *pls. 41–44.* Sydney: Published by the State of New South Wales. 1907. *2s. 6d.*

NOTES FOR STUDENTS

Regeneration in mushrooms.—By a series of experimental studies on the regeneration phenomena in mushrooms, MAGNUS[11] has attempted to analyze the principles underlying the organization of the complex fruit-bodies of these plants. It was found that the cultivated mushroom, which was chiefly used in the experiments, possessed a marked capacity for regeneration, which varied with the age of the fruit-bodies and with the character of the tissues involved. In extremely young fruit-bodies, wounds caused by removing sections are quickly filled up by the growth of new tissue, but with increasing age this power of regeneration gradually disappears. The most extensive changes occur when a piece of the pileus in slightly older stages is removed by a perpendicular cut. The wound soon becomes overgrown with a sort of wound tissue, which is covered with an irregular hymenium consisting of sinuous lamellae or spines or irpex-like plates, all intermingled. The hymenium arises without reference to gravity, but it always originates in contact with the old hymenium and then gradually spreads over the normal tissue. Although the reticulations of the new hymenium arise without order, there appears a certain definite regularity in their relation to each other, so that whatever their form may be they always remain at definite distances from each other. It appears that the whole surface is capable of producing lamellae, but by some chance advantage certain portions start first, and these then exert an inhibiting influence over a given area and prevent the formation of new growing points, which would cause the lamellae to be too much crowded. In the normal expanding pileus the lamellae appear to be formed according to the same principles; for when the first-formed lamellae become separated by the growth of the margin of the pileus, new ones are interpolated among them, keeping the number per unit of space constant.

Besides the typical regeneration of parts, various tissues of the mushroom have the power of vegetative sprouting. In the youngest stages all hyphae return readily to a condition of vegetative growth; with increasing differentiation, however, this power is lost to a great extent. The palisade cells of the hymenium and the cells of the cortex soon lose their capacity for vegetative budding, although some of the cortical cells in the lower part of the stem later regain this power. In general it appears that the most highly specialized cells are the first to lose their capacity for returning to the vegetative condition. These facts argue in favor of a progressive differentiation of the hyphae which make up the fruit-body, and is opposed to the view that the character of the hyphae is largely determined by tropisms dependent upon their position in the fruit-body. If the character of the hyphae were merely a function of their position in the fruit-body, we should expect all hyphae to return readily to the vegetative stage. On account of this character of progressive differentiation toward a determinate form, the author regards the fruit-body of higher mushrooms as a definite entity, resembling in

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[11] MAGNUS, WERNER, Ueber die Formbildung der Hutpilze. Archiv. für Biontologie 1:85–161. *pls. 8–13.* 1906.

this respect the animal body and differing thereby from vascular plants of indeterminate growth.

The paper is an excellent contribution toward the solution of the problem of the formation of the fruit-bodies of higher fungi by the correlated growth of apparently independent elements. The problem has been attacked from a new standpoint by a method which has shown a closer correlation of the different parts than has hitherto been demonstrated.—H. HASSELBRING.

Coastal plain vegetation.—R. M. HARPER's studies of the vegetation of the coastal plain (BOT. GAZETTE **40**: 392, 393. 1905) continue to appear in various journals. His most pretentious work, consisting of a phytogeographical sketch of the Altamaha Grit region of the coastal plain of Georgia, has been recently reviewed in this journal (**43**: 225. 1907). This admirable paper should be read by all ecologists. The fulness of citation in the bibliography accompanying this work is most excellent, and may well be copied by authors generally; in addition to the exact citation of the original paper, citation is made of reviews of the paper cited, and a short appreciation of the article is given. In the *Bull. Torr. Bot. Club* (**32**:451–467. 1905) appear accounts of phytogeographical explorations in the coastal plain of Georgia in 1904, in which are noted interesting patches of mesophytic forests rather apart from the region where such forests dominate; also a peculiar case of a pine barren similarly isolated from the great pine barren region. In the same journal are accounts of new and noteworthy plants from the coastal plain of Georgia (**33**: 229–245. 1906), notes on the distribution of some Alabama plants (**33**: 523–536. 1906), and an account of a midsummer journey through the Carolinas and Virginia (**34**: 351–377. 1907). In the latter paper mention is made of the remarkable flatness of the region, and a type of plant society, locally known as "pocosins," is described; a "pocosin" is a sort of heathlike swampy thicket of evergreen shrubs and scattered pines especially characteristic of the Carolinas.

In *Torreya* are several short articles, one of which gives a statistical method for comparing the ages of different floras (**5**: 207–210. 1905), based on the idea that monocotyls develop dominantly in a region before dicotyls; the average of a number of local lists in glaciated and coastal regions gives about 30 per cent. of monocotyls; while similar lists in older regions have about 24 per cent. of monocotyls. In the Altamaha Grit region of Georgia, the relatively young pine barrens have 44 per cent. of monocotyls; while there are but 13 per cent. in the climax forests of the region. Other papers in the same journal give short studies in the North Carolina coastal plain (**6**: 41–45. 1906), the Paleozoic region of Alabama, in which some new coastal plain plants are found (**6**: 111–117. 1906), southeastern Mississippi and eastern Louisiana (**6**: 197–205. 1906), the Altamaha Grit region of Georgia, giving additional notes on this region (**6**: 241–246. 1906), and a Long Island cedar (i. e., Chamaecyparis) swamp (**7**: 198–200. 1907).

In other journals are found papers on the coastal plain plants of New England (Rhodora **8**: 27–30. 1906), the vegetation of Bald Knob, Elmore County, Alabama, competition between oaks, and the forest resources of Georgia. The paper on Bald Knob (Plant World **9**:265–269. 1906) recounts an interesting occurrence of coastal plain plants on the xerophytic summit of a hill. Here there is strong evidence that this is a pioneer plant association, a fact which the author doubtless correctly thinks is in favor of the theory that the coastal pines represent a pioneer formation. The paper dealing with competition in oaks (Plant World **10**:114–116. 1907) recounts the crowding-out of specimens of *Quercus laurifolia* by *Q. Phellos*, when set out near together; the especial interest here comes from the fact that the former oak is a pioneer, and the latter a denizen of the climax forests of the region. The paper on the forest resources of Georgia (Southern Woodlands **1**:4–23. 1907) is the first of a series, and gives an account of the distributional factors involved, and the natural subdivisions of the forests of Georgia, accompanied by a map.—H. C. COWLES.

A tropical study in physiographic ecology.—WHITFORD's paper[12] on Philippine vegetation is a record of the first attempt to test the principles of physiographic ecology in the tropics, and hence is of more than usual interest to ecologists who have worked only in the temperate zone. The area studied embraces the slopes of Mt. Mariveles, from the sea-level to an altitude of 1400 meters. The introduction includes remarks on the importance of determining the available water-content of the soil at all seasons, and some mention is made of the practical application of ecological study. The climate has well-marked wet and dry periods, and yet evergreens are as characteristic of the vegetation as are deciduous trees. The prevailing forest type is thus midway between SCHIMPER's rain-forest and the monsoon forest. Possibly the retention of much water in the soil during the relatively short dry period is a factor in the matter.

The climax forest of the lowland is the Bambusa-Parkia forest, and it is WHITFORD's belief that this is the general type to which other formations of the region are tending. An interesting feature here are the parangs, which are artificial formations, corresponding somewhat to our clearings. There are various types, but all seem to show evidence of gradual displacement by the culminating Bambusa-Parkia forest. A further similar evidence of this is the appearance of natural xerophytic patches resembling the parang within the Bambusa-Parkia forest. Next up the slope comes the Anisoptera-Strombosia formation, in which there is a dominance of the Dipterocarpaceae. Tables show the enormous number of tree species in these forests. Here, as in all of the formations, many details are given of the life-habits of the plants. The buttressed bases are associated with the overtowering trees of the highest forest stratum. Next come the Dipterocarpus-Shorea formation, in which 30 per cent. of the species are dipterocarps, and the Shorea-Plectronia formation. From the first to the last of the formations named above there has been a progressive increase in mesophytism, and in the dominance of the evergreen habit, correlated with a similar increase

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[12] WHITFORD, H. N., The vegetation of the Lamao forest reserve. Philipp. Jour. Sci. **1**:373–431, 637–682. 1906.

in rain and humidity. At the mountain top, however, there is a dwarf or "elfin" forest of Eugenia and Vaccinium; yet the humidity is greater here than anywhere below. In this "elfin" forest is found the greatest development of epiphytes, not alone mosses and lichens, but also liverworts, orchids, and even filmy ferns.

The final sections deal with the strand formations, which have but a meager development in the region studied. The strand vegetation closely resembles that of the Javanese strand, described by SCHIMPER, and need not be reviewed fully. The chief formations are the Pes-Caprae and Barringtonia-Pandanus formations of sandy shores and the Mangrove-Nipa-Acanthus formation of muddy shores. The Bambusa-Parkia formation encroaches rapidly on these shore formations. It is suggested that in other portions of the Philippines the climax forest differs from the dominant Bambusa-Parkia forest of the region described. It is unnecessary to point out the great importance of this study. It might have been anticipated that the broad principles of succession are as applicable to tropical as to temperate regions, but it remained for WHITFORD to show this to be the case.—H. C. COWLES.

Ascocarp of Lachnea.—Miss FRASER[13] has recently discovered another case of supposed reduced fertilization similar to that described for *Humaria granulata* by BLACKMAN and FRASER. In *Lachnea stercorea*, a form in which a functionless antheridium and trichogyne are said to be present, she finds the archicarp arising, as described by WORONIN for *Lachnea scutellata*, from a lateral branch of the multinucleate cells of the mycelium. Five or more cells are formed, the terminal one of which becomes the ascogonium. This ascogonium, which is multinucleate, gives rise to a branch, into which pass several nuclei. When mature this branch, which is regarded as a trichogyne, contains five or six multinucleate cells, the terminal one becoming greatly enlarged and containing many nuclei. The position and degree of development of this trichogyne seems to vary greatly, developing as a terminal or lateral structure, which may or may not be entirely inclosed by the investing hyphae. Miss FRASER regards this trichogyne as intermediate in structure between that of Pyronema on the one hand, where a unicellular functioning organ is well developed, and Physcia or Collema on the other hand, where the trichogyne is multicellular and functioning. Although the position and origin of the antheridium does not appear to have been definitely worked out, the author believes it to arise from the cell next below the ascogonium, that it is a unicellular multinucleate structure, similar to that of Pyronema, and that it fuses with the terminal cell of the trichogyne. In some cases no trace of the trichogyne could be found, and it is believed that it fails to develop, as in Humaria. The nuclei of the antheridium often remain in the cell and degenerate *in situ* or pass into the terminal cell of the trichogyne. Fertilization by the fusion of male and female nuclei in the ascogonium does not occur, there-

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[13] FRASER, H. C. I., On the sexuality and development of the ascocarp in *Lachnea stercorea* Pers. Annals of Botany 21:349–360. *pls. 29, 30.* 1907.

fore, but the nuclei of the ascogonium were observed fusing in pairs, a condition also found in Humaria. This fusion occurs at various stages in the development of the ascogonium, either when it is very young or very old, and not at a certain stage in its development, as described for Pyronema. Since Pyronema possesses a functioning trichogyne and fertilization occurs by the fusion of sexual nuclei, Miss FRASER would regard *Lachnea stercorea* as intermediate between Pyronema and Humaria, in which no trichogyne is present. A much-branched ascogenous hyphal system is formed from the ascogonium in the usual fashion, the asci arising from the binucleate subterminal cells. The two nuclei fuse to form the ascus nucleus, which divides by successive divisions to form the nuclei of the eight ascospores.—J. B. OVERTON.

Polymorphism of Hymenomycetes.—Some interesting results bearing on the polymorphism of the Basidiomycetes were obtained by LYMAN[14] in a series of cultures. It is stated that about 75 species belonging to the Polyporaceae, Hydnaceae, and Thelephoraceae were grown in pure cultures and that about 40 per cent. of these possess some secondary method of reproduction. In the present paper six species are treated, all but one belonging to the Thelephoraceae. The most interesting results were obtained in connection with two *Fungi imperfecti: Michenera artocreas* B. & C. and *Aegerita candida* Pers. Michenera was shown to be the conidial form of *Corticium subgiganteum* Berk., with which it had often been found associated. The spores of *Aegerita candida* yielded a form of Peniophora which had not been described, and consequently becomes *P. candida* (Pers.) Lyman. Several other forms of Corticium were found to have secondary spore forms, either conidia, chlamydospores, or bulbils. A peculiar tendency toward a differentiation of the mycelia of these fungi into juvenile and adult forms was noted in most of the species. The most pronounced example illustrating this tendency is *Corticium alutaceum.* The basidiospores of this form produce a mycelium with slender hyphae without clamp connections which bears conidiophores; and later the adult mycelium with large hyphae having clamp connections but without condiophores. Continuous cultures of conidia produce more and more persistent juvenile mycelium. The adult mycelium is not entirely suppressed, however, if the culture has sufficient nutriment to permit continued growth.

The cultures with *Lentodium squamulosum* are of special interest, for they seem to show that this peculiar and much-discussed fungus is an autonomous plant and not, as many writers have believed, an abnormal form of *Lentinus tigrinus.* In pure cultures the spores of the fungus give rise to a mycelium which finally produces fruit-bodies having all the characters, including the peculiar chambered hymenophore, of the parent. This form produces conidia from the veil and from the margin of the young pileus. Careful studies of this kind throw much-needed light on the biology of the higher fungi, whose life-histories present many obscure points.—H. HASSELBRING.

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[14] LYMAN, GEO. F., Culture studies on the polymorphism of Hymenomycetes. Proc. Boston Soc. Nat. Hist. 33:125–209. *pls. 18–26.* 1907.

African fresh-water algae.—The fresh-water algae collected by the third Tanganyika Expedition (1904–5), under Dr. W. A. CUNNINGTON, have been reported by G. S. WEST.[15] The collections were extensive and consisted largely of plankton obtained from the three great lakes (Nyasa, Victoria Nyanza, and Tanganyika). Some material was collected in swamps and swampy pools, and some of the most interesting algae were obtained from among the finely divided leaves of certain utricularias. The phytoplankton is first presented, and is peculiar in the absence of many genera which are a dominant feature of the European lake plankton. A table shows all the species observed in these African plankton collections, and this is followed by a discussion of the peculiarities of the plankton of each lake. The bulk of the report consists of the systematic account of the algae. The absence of Stigonemaceae is referred to as remarkable, Nostochopsis being the only representative of the family. Species of Hapalosiphon are of general occurrence in swamps and marshes, as well as certain bog-loving and rupestral species of Stigonema. The list of new forms includes 18 varieties, 36 species, and a new genus (*Sphinctosiphon*) of Palmellaceae. The total number of species is 372 (105 genera), and 122 of the 187 Chlorophyceae are Conjugales.

Certain conclusions are as follows: The algal flora of Tanganyika differs very much from that of the other lakes, but its peculiarities could be accounted for by prolonged isolation. The algae of Tanganyika showing marine affinities may have been produced by a gradual increase in the salinity of the water over an extended period. The relatively small proportion of Chlorophyceae in the plankton, as well as the large proportion of Bacillarieae and Myxophyceae (Cyanophyceae), is also an indication that the water of the lake was at one time more saline. In large bodies of fresh water, a single example of plankton from a given locality must not be regarded as representative of the plankton of the entire lake.—J. M. C.

California peach blight.—RALPH E. SMITH describes this disease as occurring in practically every part of California where peaches grow.[16] It was first recorded in 1900 by PIERCE and has since increased largely. All varieties are affected. The buds and fruiting twigs die, the green twigs become spotted, and the leaves and fruit drop off. Gum exudes copiously, especially in wet weather, over the one-year-old fruiting twigs. Twigs which were sound and healthy in December were found to be spotted by the first of February. Spraying with Bordeaux mixture at the usual time was not effective, but spraying in December proved efficient, and the ordinary Bordeaux of the 5-5-50 strength of the lime-sulfur-salt mixture is recommended.

The fungus is referred to *Coryneum beyerinkii*. The mycelium produces spots on the leaves and shoots in winter and spore pustules near the center of these spots. Affected leaf tissue soon drops out, giving rise to "shot-holes." Conidia

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[15] WEST, G. S., Report on the fresh-water algae, including phytoplankton, of the third Tanganyika Expedition conducted by Dr. W. A. CUNNINGTON, 1904–5. Jour. Linn. Soc. Bot. 39:81–197. *pls. 2–10.* 1907.

[16] Agr. Exper. Sta. Calif., Bull. 191. Sept. 1907.

are abundant upon leaf scars and twigs. The life-history is summed up as follows:

> The spores, scattered profusely over the limbs, germinate as soon as enough rain has fallen to wet them up thoroughly, usually in December or January. Spores lying on the green bark of new shoots penetrate the tissue and cause the characteristic spotting. Spores lying about the bud scales produce a mycelium which penetrates and kills outright both the bud and quite an area of surrounding bark, the spot extending from one-fourth to one inch in length. On the spots spore pustules are developed.

The fungus was cultivated in beef agar, in ordinary agar, and on sterilized peach twigs. The colonies are black with distinct zonal arrangement of conidia. No indication of an ascigerous stage was found.—F. L. STEVENS.

Fungus diseases of sugar cane.—BUTLER [17] has given an account of the fungi attacking sugar cane in Bengal. The most serious disease seems to be caused by *Colletotrichum falcatum* Went., which causes a rot of the stalks that spreads from the base upward. In the early stages of the disease the fungus greatly reduces the sugar-content of the cane. With the disappearance of the cane sugar there is a simultaneous increase of glucose. This is attributed to the inverting action of the fungus, and by proper flask experiments it was shown that the fungus has the power of inverting cane sugar. All parts of the cane are attacked by the fungus. On the stem it is often accompanied by a form of Melanconium (*Trichosphaeria Sacchari*), which was once regarded as the cause of the most destructive cane disease of the West Indies, namely the "rind disease." BUTLER believes, however, that the Melanconium is only an accompanying fungus and that *Colletotrichum falcatum* is possibly the cause of the famous "rind disease."

A number of other fungi are more briefly described. *Ustilago Sacchari* Rabenh. transforms the growing axis into a long spore sac. *Diplodia cacaoicola* (P. Henn.), which was originally found on *Theobroma cacao*, is said also to attack the stems of cane. *Cytospora Sacchari* Butl. is described as a new species also found on the stems of cane. *Thielaviopsis ethaceticus* Went. is the cause of the "pine-apple" disease of the young sets, while *Sphaeronema adiposum* Butl. somewhat resembles the latter in its effects. The most serious leaf diseases are the brown leaf spot caused by *Cercospora longipes* Butl., described as a new species, and the well-known ring-spot, found everywhere on cane leaves in the tropics and caused by *Leptosphaeria Sacchari* Br. & H.—H. HASSELBRING.

Cyanogenesis in plants.—TREUB returns to a discussion of the origin and distribution of hydrocyanic acid.[18] Incidentally he adds in one of his tables to the list of plants in which HCN has been found (as given by GRESHOFF at the York meeting of the B. A. A. S.) six genera and sixteen species. HCN as a rule, in hot countries at least, disappears from the leaves before their fall, the contrary

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[17] BUTLER, E. J., Fungus diseases of sugar cane in Bengal. Mem. Dept. Agric. India Bot. **1**: no. 3. pp. 53. *pls. 11.* 1906.

[18] TREUB, M., Nouvelles recherches sur la rôle de l'acide cyanhydrique dans les plantes vertes, II. Ann. Jard. Bot. Buitenzorg **21**: 79–106. *pls. 1, 2.* 1907.

case of Sambucus (GUIGNARD) and Indigofera (TREUB) being exceptions. The amount diminishes regularly with age in most cases, though there are not wanting examples where the amount remains nearly constant until late in the life of the leaf, when it suddenly disappears. Since distillation after maceration yields more HCN than direct distillation, it is evident that a part at least, and as experiments indicate most or all, of the HCN exists in the form of a glucoside which is split up by an enzyme. No matter how quickly the killing and distillation is carried on, the glucoside is hydrolyzed, so that the enzyme acts with "astonishing rapidity." Some study was made of the enzymes concerned, but these must be worked out later. That HCN is a reserve is shown by the fact that in plants put into obscurity sufficient to preclude photosynthesis, HCN diminishes after the third day; and when the same plants are brought out into the light again it increases. Further, the maximum content of HCN occurs at midday. Light is influential only because it provides for the making of glucose, which is necessary to the formation of the glucoside.

In a second short paper TREUB[19] disposes of the contention that the rôle of HCN is that of protection against animals.—C. R. B.

Taxonomic notes.—C. WARNSTORF (Hedwigia **47**:76–112. 1907), in a series of descriptions of new species of Sphagnum, includes 4 from the United States: *S. missouricum* (Missouri), *S. Bushii* and *S. alabamae* (Alabama), and *S. Evansii* (New Jersey).—LEROY ABRAMS (Torreya **7**:217–219. *fig. 1.* 1907) has described a new maple (*A. bernardinum*) from the San Bernardino Mountains of California.—W. A. SETCHELL (Jour. Mycol. **13**:236–241. *pl. 107.* 1907) has published new species of hypogaeous fungi (Secotiaceae) under Secotium and Elasmomyces.—Sv. MURBECK (Lunds Univ. Arsskrift II. **2**: no. 14. pp. 30. *pls. 2.* 1907) has studied the *vesicarius* group of Rumex, recognizing 3 forms under *R. vesicarius* L.; separating *R. planivalvis*, *R. simpliciflorus* (3 forms), *R. vesceritensis*, and *R. cyprius* as new species; and characterizing *R. roseus* L.—W. TRELEASE (Ann. Rep. Mo. Bot. Garden **18**:225–230. *pls. 12–17.* 1907) has described 2 new species and 2 new varieties of Yucca; also (*idem* 231–256. *pls. 18–34*) has published an account of *Agave macroacantha* and allied Euagaves, disentangling an extensive synonomy.—J. R. DRUMMOND (*idem* 25–75. *pls. 1–4*) has published an account of the literature of Furcraea with a synopsis of the known species, recognizing 10 as valid and 6 more as possibly valid but imperfectly known.— A. MAUBLANC (Bull. Trim. Soc. Mycol. France **23**:146–149. *figs. 7.* 1907) has described a new genus (*Ceratopycnidium*) of Spheropsidaceae from the Congo.—A. A. HELLER (Muhlenbergia **3**:133–134. 1907) has reestablished *Chloropyron* Behr with 4 species, heretofore referred to Cordylanthus or Adenostegia. All the species belong to salt marshes near the Pacific coast or to saline soil in the interior.—J. M. C.

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[19] TREUB, M., Notice sur "l'effêt protecteur" assigné à l'acide cyanhydrique des plantes. *Ibid.* 197–114. *pls. 3, 4.* 1907.

Parasitic seed-plants.—FRAYSSE[20] has presented an interesting account of the ecological relations of a number of parasites. Especial attention was given to *Osyris alba*, a green parasite of the Mediterranean region, which grows on many hosts, but particularly on legumes and mycorhizal plants. The seeds germinate with difficulty, and the seedlings can live for nearly a year independently. The complicated structures of the perennial haustoria are quite fully described. The host reacts by forming tyloses and other apparently defensive modifications. Similar studies were made of *Odontites rubra serotina, Euphrasia officinalis,* Lathraea, and *Monotropa hypopitys.* Most botanists regard the last species as a mycorhizal plant, but FRAYSSE seems to find it parasitic on Pinus. In all of the species studied, attention is paid to the starch and its distribution in and around the haustoria; this starch is digested by diastases secreted by the haustoria, and is then absorbed by the parasite. The last portion of the treatise deals with the remarkable characteristics of *Cytinus Hypocistis*, which lives internally for a long time, forming vegetative structures comparable to a thallus. The penetration of this thallus is accomplished by mechanical means in part and in part by the action of diastases.—H. C. COWLES.

A new hymenomycete.—MCALPINE[21] finds that the fungus described as *Isaria fuciformis* in 1872 by BERKELEY, from material collected in Australia in 1854, is a basidiomycete. The fungus grows principally on grasses, but it also attacks other plants. It consists of an effused somewhat gelatinous layer from which rise Isaria-like tufts of hyphae, bearing conidia. On account of these conidia-bearing tufts the fungus was placed in the genus Isaria. MCALPINE finds, however, that the effused part of the fungus bears a basidiosporic hymenium, in consequence of which he places it in the genus Hypochnus as *H. fuciformis* (Berk.) McAlp.

In a note following the foregoing article, VON HÖHNEL and SYDOW point out that the name Hypochnus is no longer tenable, since the species included under it belong partly to Corticium and partly to Tomentella. To include species having tufts of hyphae rising above the hymenium, V. HÖHNEL and LITSCHAUER have elevated PATOUILLARD'S section Epithele to a genus. In this VON HÖHNEL and SYDOW place MCALPINE'S fungus as *E. fuciformis* (Berk.) v. Höhn. et Syd.—H. HASSELBRING.

Embryo sac of Impatiens.—LONGO[22] has described an interesting haustorial apparatus in connection with *Impatiens amphorata.* In the growth of the sac the small nucellus is resorbed, the sac coming into contact with the inner integument, whose inner cells function as a tapetum. From the enlarged micropylar

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[20] FRAYSSE, A., Contribution à la biologie des plantes phanérogames parasites. Montpellier. 1906. See Bot. Cent. **102**: 51–52. 1906.

[21] MCALPINE, D., A new hymenomycete—the so-called *Isaria fuciformis* Berk. Ann. Mycol. **4**: 541–551. *pls. 8, 9.* 1906.

[22] LONGO, B., Nuove ricerche sulla nutrizione dell' embrione vegetale. Reale Accad. Lincei **16**: 591–594. *figs. 2.* 1907.

end of the sac a haustorial tube is developed, which passes through the micropyle, and then enlarges very much and sends out numerous branches which penetrate the funiculus and also the outer integument. The tube is occupied by an endosperm nucleus, which in the enlarged region outside of the micropyle becomes correspondingly enlarged and amoeboid in outline. There is also a short chalazal haustorium. The tapetal layer of the inner integument eventually becomes cutinized, and the vascular connections through the funiculus are imperfect, so that the embryo is fed through the haustoria, especially the prominent micropylar one. Such haustoria are common among the Sympetalae, but the micropylar ones are imbedded in the heavy integument and usually do not wander out of the ovule through the micropyle.—J. M. C.

A parasitic alga on tea.—HUTCHISON[23] has studied the life-history of *Cephaleuros virescens*, the so-called "red rust" of tea. This remarkable alga is the greatest menace to the tea crop in northeastern India; and another observer is cited as stating that it is doing serious damage to mango trees in Bengal as a stem blight. This "blight" alga is one of the Chroolepidae, and attacks the leaves of the tea plant, completely piercing them; but the serious form of attack is upon the cortex of young stems. The alga occurs as orange-yellow, roughly circular patches on the upper surface of the host. It is propagated from these patches either by the discharge of zoospores under conditions that favor swimming, or by the breaking-off of the sporangium and its transportation bodily by the wind. The attack on young stems is intimately connected with their rough surface, all the young algal patches being found in the crevices of this surface. If the young shoot grows rapidly, it may outstrip the alga and "no permanent infection takes place;" but if the young shoot grows slowly, the alga is able to penetrate and destroy it.—J. M. C.

The cereal rusts.—EVANS[24] has undertaken a detailed study of the histology of the "cereal rusts," that is, the species of Puccinia formerly included under *P. graminis*, *P. rubigo-vera*, and *P. coronata*, and more recently broken up into a greater number of species on the basis of both morphological and physiological characters. The investigation was begun at Cambridge in 1903, at the suggestion of the late Professor MARSHALL WARD, and has been continued at Pretoria, at the Transvaal Department of Agriculture, where the author is mycologist. This first paper deals with the development of the uredo-mycelia, and the principal results are as follows: In the early stages of the mycelia, these species exhibit distinctive morphological characters; the sub-stomatal vesicle is of a definite shape for each species; the haustoria of some species are very distinctive; and in some species a well-developed appressorium is present, while in others it is not so evident.—J. M. C.

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[23] HUTCHINSON, C. M., *Cephaleuros virescens* Kunze: the "red rust" of tea. Mem. Depart. Agric. India Bot. 1:no. 6. pp. 35. *pls. 8.* 1907.

[24] EVANS, B. POLE, The cereal rusts. I. The development of their uredo-mycelia. Annals of Botany 21:441–466. *pls. 40–43.* 1907.

Coffein and theobromin.—In a detailed account of his studies on the genera Coffea, Thea, Theobroma, and Cola, WEEVERS answers for the first time these questions:[25] Do coffein and theobromin arise in connection with the proteid metabolism of the plant or not? If so, do they arise through analysis or synthesis of proteids, and can they be used in proteid synthesis? He concludes that both are produced in consequence of secondary processes of dissimilation, that they are stored for a longer or shorter time, and then are again used in proteid synthesis. They constitute a very compact means of storing N, as they contain about 30 per cent. N as compared with 19 per cent. or less in proteids, and this is doubtless the reason why they occur so abundantly in seeds, though they may have also a protective function.—C. R. B.

Desert vegetation in South Africa.—The country of Welwitschia has always seemed fascinating to botanical travelers. Its accessibility is not fully realized; not only is it near the coast, but the heart of the desert is traversed by a railroad which has a station named Welwitsch from the abundance of the remarkable gymnosperm in the region thereabouts. PEARSON[26] has given an account of a journey through a part of German Southwest Africa, in which there is a description of the coastal strip with *Ancathosicyos horrida*, the Namib plains with Welwitschia, and the dry river beds with a less xerophytic vegetation closely resembling the Acacia vegetation farther east. Near Windhuk, the seat of government, there are Acacia park forests, in which *Acacia giraffae* is the predominating species.—H. C. COWLES.

Root-parasitism.—BARBER[27] has continued his studies of the haustorium of *Santalum album*. In the first paper[28] the early stages up to penetration were described; while the present paper deals with the mature haustorium. The penetration of the haustorium, its general structure, and its relation to the host tissues are first described. Then follow detailed descriptions of the cortex and "nucleus," the vascular system and its connections with the host, the character and development of the "sucker" with its double function of secretion and absorption, cases of irregular penetration, the general activity of the haustorium, and the interrelations of host and parasite. The numerous plates serve to make the descriptions remarkably clear.—J. M. C.

Taiwania.—HAYATA described this new genus of Coniferales from the Island of Formosa in 1906,[29] from a few dry branches bearing cones. Since that time he

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[25] WEEVERS, TH., Die physiologische Bedeutung des Koffeins und des Theobromins. Ann. Jard. Bot. Buitenzorg **21**:1–78. 1907.

[26] PEARSON, H. H. W., Some notes on a journey from Walfish Bay to Windhuk. Kew Bull. **1907**:339–360.

[27] BARBER, C. A., Studies in root-parasitism. The haustorium of *Santalum album*. II. Mem. Depart. Agric. India Bot. **1**:no. 1^2. pp. 58. *pls. 15.* 1907.

[28] BOT. GAZETTE **40**:159. 1905 and **42**:317. 1906.

[29] Jour. Linn. Soc. Bot. **32**:330–332. *pl. 16.*

has been able to secure more favorable material for study and has just published[30] his results. The genus belongs to the Taxodineae, and resembles Cunninghamia in the form of its cones, Arthrotaxis in its foliage, and Cryptomeria in its habit. A study of the anatomy of the leaf shows that in this feature it is intermediate between Cunninghamia and Cryptomeria, and perhaps nearest to Arthrotaxis. On the whole, the author concludes that it should be placed among Taxodineae between Cunninghamia and Arthrotaxis.—J. M. C.

Synopsis of Godetia.—W. L. JEPSON[31] has published a synopsis of the North American species of Godetia, which are restricted to the Pacific coast and are represented chiefly in California. After some account of the history of the genus, early type specimens, concept of "species" in the genus, geographic distribution, and hybrids, the 17 recognized species are presented in two groups: the *Amoena* group, comprising the loosely flowered, freely branching forms; and the *Purpurea* group, comprising the spicately flowered, simple stemmed forms. The critical character of the study may be suggested by the statement that there are published 5 new species, 15 new varieties, 6 new forms, and 3 new combinations. The "little-known species, old and recent," number 11.—J. M. C.

Nitrogen fixation by algae.—After a valuable review of the literature of the subject, HEINZE[32] gives an account of his investigation of the fixation of free nitrogen by algae. He had found earlier that fungi, apart from bacteria, do not possess this power. In this paper it is shown that some algae, Nostoc for example, possess this power to some degree; quantitatively the work of algae in this way is not very important. A much greater rôle is suggested by their symbiotic life with such nitrifying organisms as Azotobacter; the work of the latter is doubtless facilitated largely by the carbohydrate contributions of the algal symbionts.—H. C. COWLES.

Anatomy of Equisetum.—QUÉVA'S[33] studies on the histology of Equisetum lead him to the conclusion that secondary growth does not exist even in the nodes, where there is some appearance of cambial activity. Since secondary wood was common in the Calamites, and JEFFREY has observed a cambium in the cone of *E. hiemale* and *E. limosum*, it is probable that we have here to do with a vestigial structure. The cortex arises by tangential divisions of a superficial layer of cells. Differentiation of the xylem is exclusively in the centrifugal direction, and the protoxylem usually disappears owing to rapid elongation of the stem.—M. A. CHRYSLER.

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[30] HAYATA, B., On Taiwania and its affinity to other genera. Bot. Mag. Tokyo 21:21–27. *pl. 1.* 1907.

[31] JEPSON, W. L., A synopsis of the North American Godetias. Univ. Calif. Publ. Bot. 2:319–354. *pl. 29.* 1907.

[32] HEINZE, B., Einige Beiträge zur mikrobiologischen Bodenkunde. Cent. Bakt. 16^2:640–653, 703–711. 1906.

[33] QUÉVA, C., Histogenèse et structure de stipe et de la fronde des Equisetum. Mem. Soc. Hist. Nat. d'Autun 20:4–41. 1907.

Embryo sac of Cassia.—W. T. SAXTON[34] has investigated the South American *Cassia tomentosa*, as growing in private gardens in Cape Town, South Africa. The usual linear row of four megaspores appears, but curiously enough the functioning spore is not the innermost one, but the one next to it. The embryo sac develops a tubular prolongation at the antipodal end, which is filled by a row of antipodal cells, as in certain Compositae. The number of chromosomes in the divisions of sporophytic nuclei is reported to be twelve.—J. M. C.

Functionless chlorophyll.—BONNIER long ago discovered that chlorophyll in some parasites appears to have lost its synthetic power. FRIEDEL[35] finds that in the ovaries of *Ornithogalum arabicum* the abundant chlorophyll has no synthetic power, or at least that the gas exchange is so slight as to be entirely masked by the respiratory gas exchanges. Ovaries of *O. umbellatum*, however, have vigorous synthetic powers.—H. C. COWLES.

Gametophytes of gymnosperms.—PORSCH,[36] in a general review of investigations upon the gametophytes of gymnosperms, has considered more than thirty papers, most of them published since 1903. The material is arranged systematically under Cycadales, Ginkgoales, etc., and is illustrated by a few well-chosen text figures. Some of the comments are very suggestive.—CHARLES J. CHAMBERLAIN.

Codonotheca.—In 1903 SELLARDS described this form-genus from material obtained from the iron-clay concretions of Mazon Creek, Illinois. He has just reprinted[37] the essential features of this description, and expresses the belief that it is the microsporangiate structure of some of the Cycadofilices, probably of the Neuropteris type.—J. M. C.

Apogamy in Rumex.—MURBECK[38] has reported the discovery of apogamy in a form of *R. Acetosella* which he calls *angiocarpus*. This announcement comes by way of *Bot. Notiser* **1907**:238.—J. M. C.

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[34] SAXTON, W. T., On the development of the ovule and embryo sac in *Cassia tomentosa*. Trans. S. Africa Phil. Soc. **18**:1–5. *pls. 1, 2.* 1907.

[35] FRIEDEL, J., Sur un cas d'organe vert dépourvu de pourvoir assimilateur. Compt. Rend. Acad. Sci. Paris **142**:1092, 1093. 1906.

[36] PORSCH, DR. OTTO, Ueber einige neuere phylogenetisch bemerkenswerte Ergebnisse der Gametophytenerforschung der Gymnospermen. Kritisches Sammelreferat. Festschrift des Naturwiss. Vereines an der Universität Wien. pp. 39. *figs. 16.* 1907.

[37] SELLARDS, E. H., Notes on the spore-bearing organ Codonotheca and its relationship with the Cycadofilices. New Phytologist **6**:175–178. 1907.

[38] MURBECK, S., Verh. Naturh. Verein. Preussisch. Rheinl. Bonn. **63**^{2}:—. 1907

NEWS

DR. J. B. FARMER has accepted the position of editor in chief of the *Gardeners' Chronicle.*

THE DEATH is announced of Dr. P. LACKMANN, professor of botany at Grenoble, at the age of fifty-six years.

DR. E. G. DELACROIX, director of the station for plant pathology of the "Institut national agronomique," died at Paris, November 2, 1907.

THE COUNCIL of the Geological Society of London has awarded the Murchison medal to Mr. A. C. SEWARD, professor of botany in the University of Cambridge.

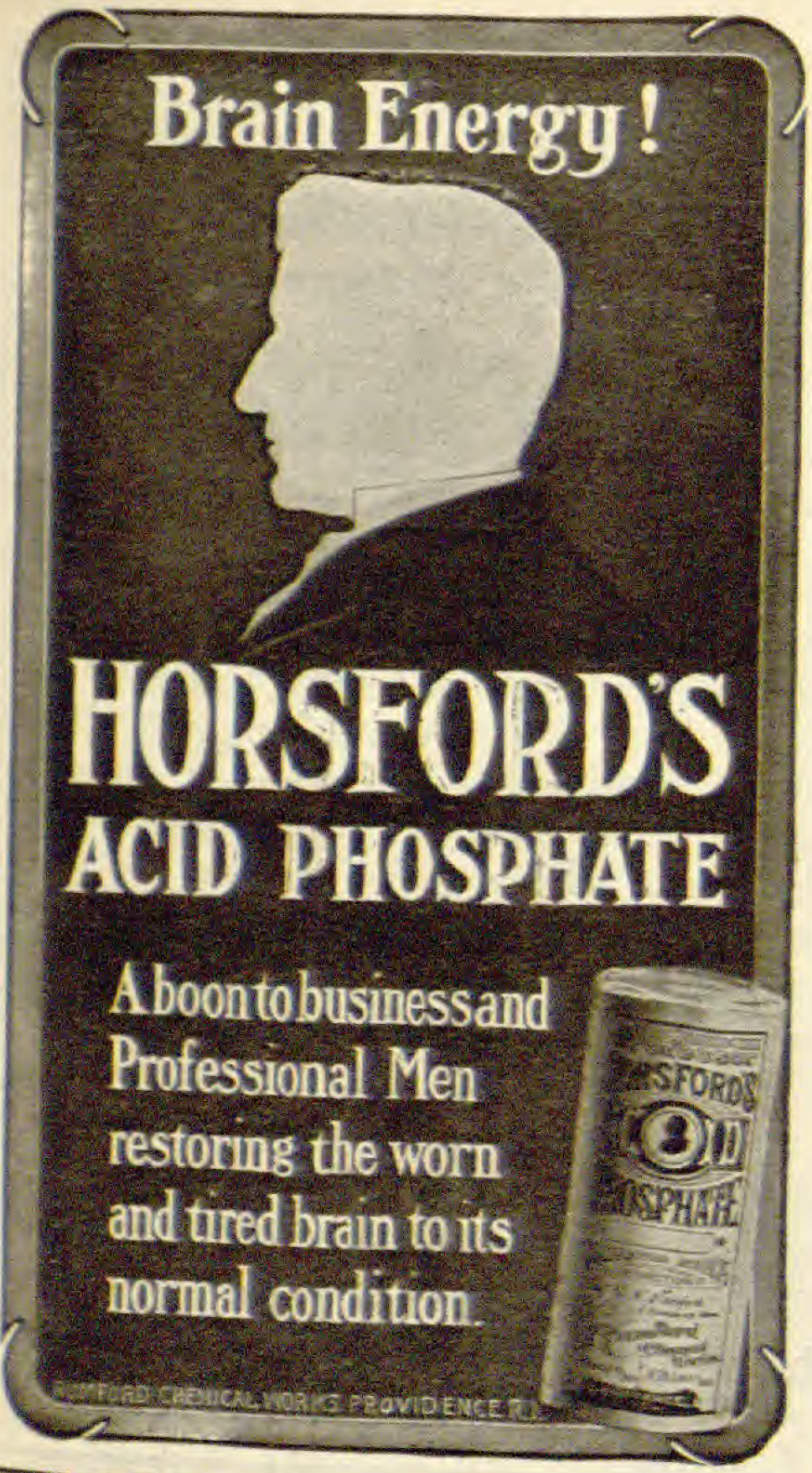
DR. LUDWIG JOST, professor in the Agricultural Academy at Bonn-Poppelsdorf, returns to Strassburg, April 1, to succeed Professor GRAF ZU SOLMS-LAUBACH upon his retirement as professor of botany and director of the Botanical Garden.

THE MACMILLAN COMPANY announce a book by Dr. B. M. DUGGAR, Cornell University, entitled *The physiology of plant production.* As the title implies, it is primarily an application of plant physiology to the requirements of agriculture and horticulture.

DR. FORREST SHREVE, associate professor of botany in the Woman's College of Baltimore, has accepted an appointment in the Department of Botanical Research of the Carnegie Institution, and will enter upon his duties at the Desert Botanical Laboratory, Tucson, Arizona, at the close of the academic year.

AT THE CHICAGO MEETING of the American Association, the following officers of Section G were elected: H. M. RICHARDS (Columbia), vice-president; H. C. COWLES (Chicago), secretary for a term of five years. The Botanical Society of America elected the following officers: W. F. GANONG (Smith), president; D. S. JOHNSON (Johns Hopkins), secretary (continued). The American Society of Naturalists elected as its president D. P. PENHALLOW (McGill).

THE REPORT of the director of the Missouri Botanical Garden for 1907 contains the following items: The number of visitors during the year was 135,497, being the largest number in any year excepting the year of the World's Fair. The incorporated additions to the herbarium were 35,876 sheets. Among the unincorporated material are 20,000 specimens of Texan plants collected by LINDHEIMER between 1849 and 1851. The herbarium, so far as now mounted, contains 595,143 specimens. The library now contains 58,156 books and pamphlets, besides 77 manuscript volumes.

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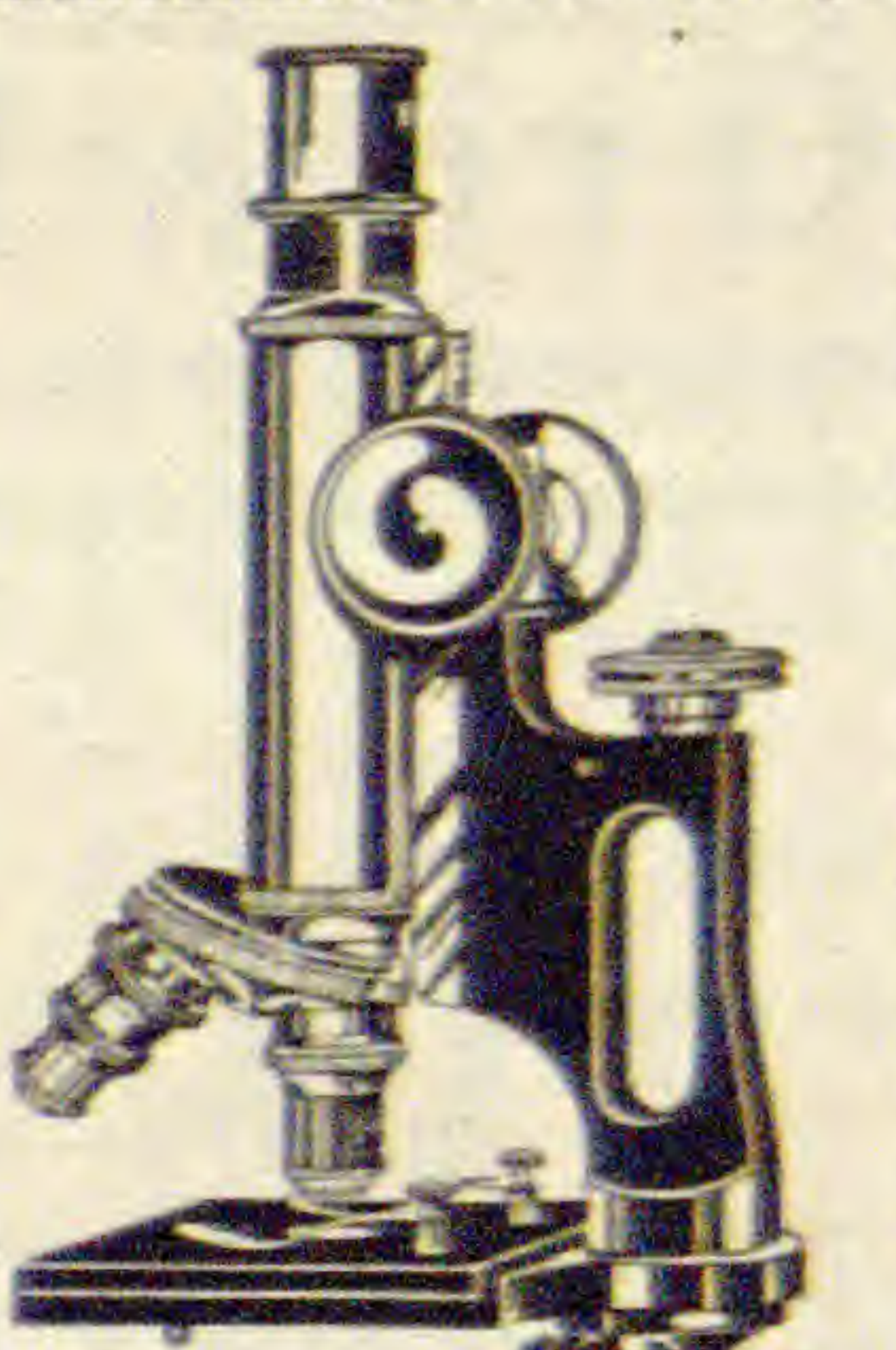

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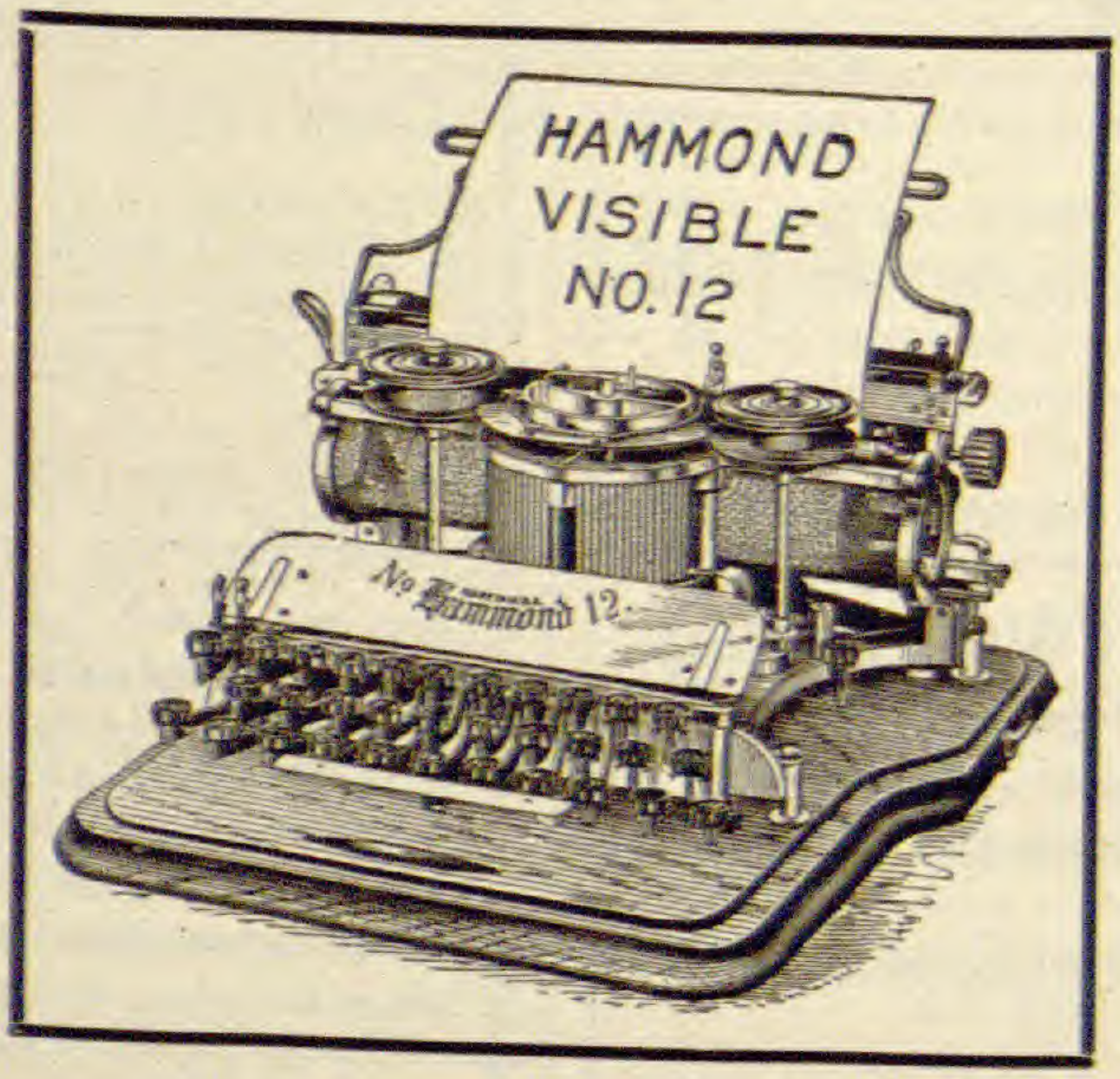
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THE
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April 1908

Editors: JOHN M. COULTER and CHARLES R. BARNES

CONTENTS

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CURRENT LITERATURE

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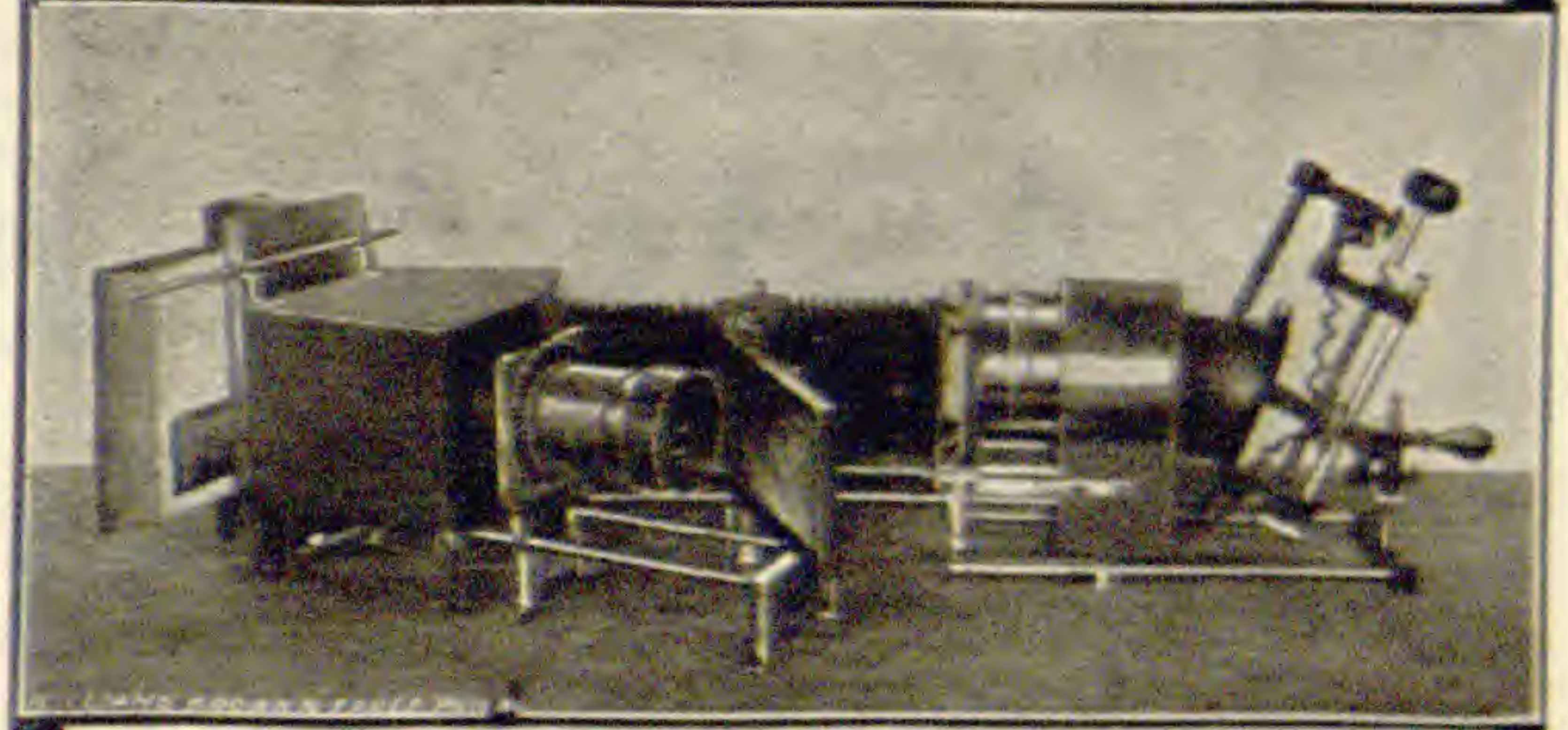

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VOLUME XLV NUMBER 4

BOTANICAL GAZETTE

APRIL 1908

THE RELATION OF PLANT SOCIETIES TO EVAPORATION

EDGAR N. TRANSEAU

(WITH NINE FIGURES)

During the summers of 1906 and 1907 the writer made a study of the plant societies about Cold Spring Harbor, Long Island, N. Y. In this connection an effort was made to obtain quantitative measurements of the various environmental factors upon which depend habitat differentiation and the succession of plant societies. The publication of LIVINGSTON's paper on "The relation of desert plants to soil moisture and to evaporation" in the autumn of 1906 suggested to me that a series of vaporimeters, standardized and exposed in the various plant societies, would give comparative data that would be far more valuable than the usual temperature and relative humidity readings.

Through the kindness of DR. LIVINGSTON I was supplied with several porous cups which were standardized with the vaporimeter at Tucson. They were similar to those afterward sent out from the Desert Laboratory to various parts of the United States. For this reason the readings given in this paper may be compared directly with any obtained by other observers using these instruments.

In the nature of the case, with the small number of instruments at my disposal, it became necessary to establish one instrument as a standard for the region. Because of the desirability of having a record of the rate of evaporation in the garden of the Station for Experimental Evolution, the standard vaporimeter for my work was placed in this garden about 3^{m} east of the weather bureau instrument shelter. With this instrument all others were compared. Whenever a vaporimeter was placed in a habitat, or a reading made, a corresponding reading was made of the standard vaporimeter, as nearly simultaneously as possible (*fig. 1*).

The vaporimeter consists of a porous cup supported about 1^{dm} above a pint jar by a glass tube which extends from the upper end of the porous cup to the bottom of the jar. The tube is fitted air tight into the porous cup by a rubber stopper. The top of the jar is covered by a cork which is nearly air tight. Surrounding the tube above the jar is a conical shield of paraffined cloth to keep out rain. In use the jar was sunk in the ground to within 2^{cm} of the top. In setting up

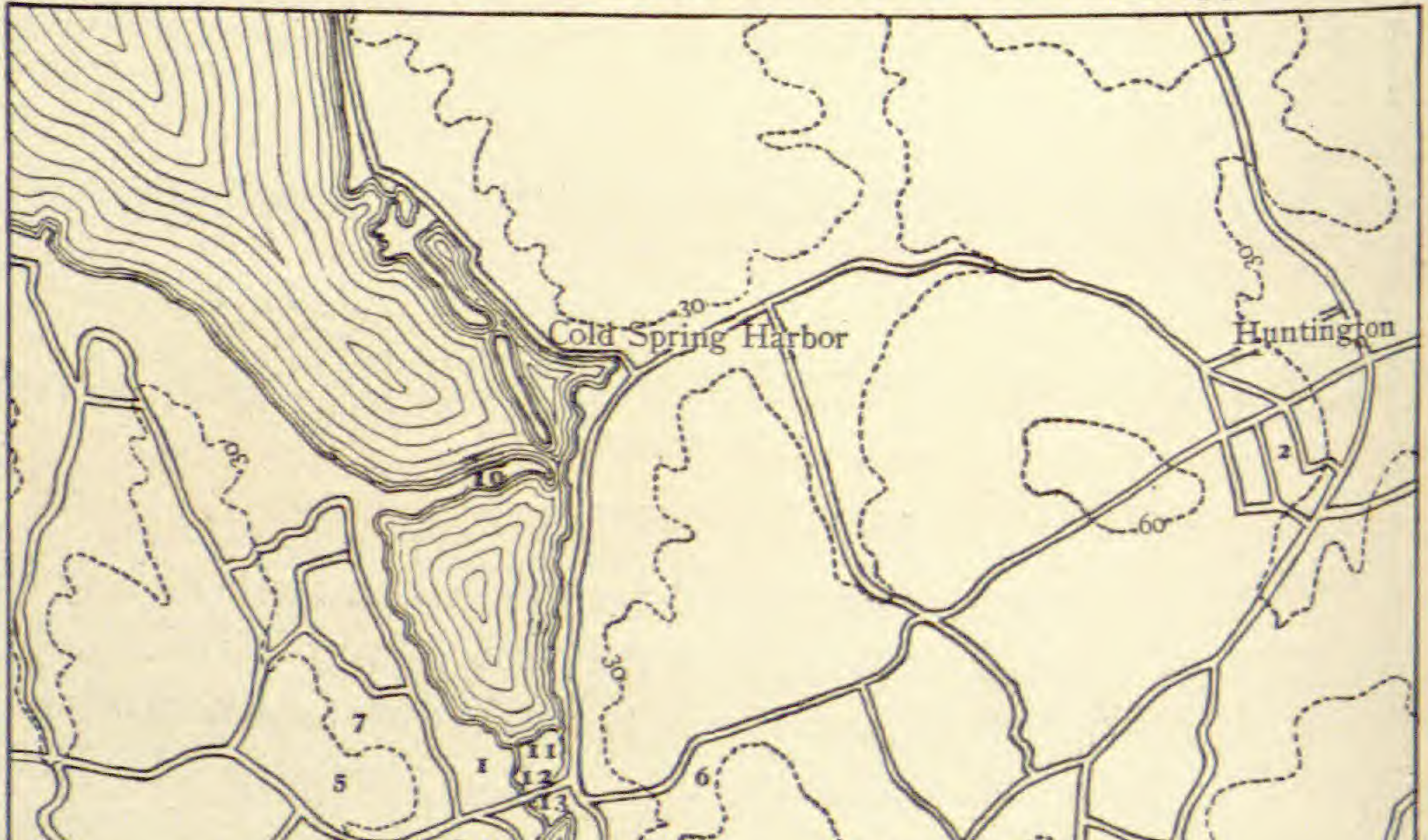

FIG. 1.—Sketch map showing positions of all stations except Station 9. Contour interval 30^{m}.

the instrument, the porous cup and tube were completely filled with water and the jar was filled to a mark near the top. At convenient intervals the loss of water by evaporation was determined by running in water from a burette and restoring the original water level. Because of the large water area in the jar the error in these readings is estimated to be $\pm 1^{cc}$.

Following are the vaporimeter readings for the several habitats, with their accompanying vegetation. The corresponding record for the standard instrument is given in each case, together with the comparative evaporation expressed in percentages of the standard.

Station 1 (standard).—Located in the east plot of the garden of the Station for Experimental Evolution; altitude above sea-level about 3^{m}. During the summer the area adjacent to the vaporimeter was occupied by plants of *Oenothera nanella*, set 1^{m} from the instrument and from each other. The small size of this variety of evening primrose left the instrument freely exposed to the sun and air. The soil is a dark-colored sandy loam (*figs. 2, 3*).

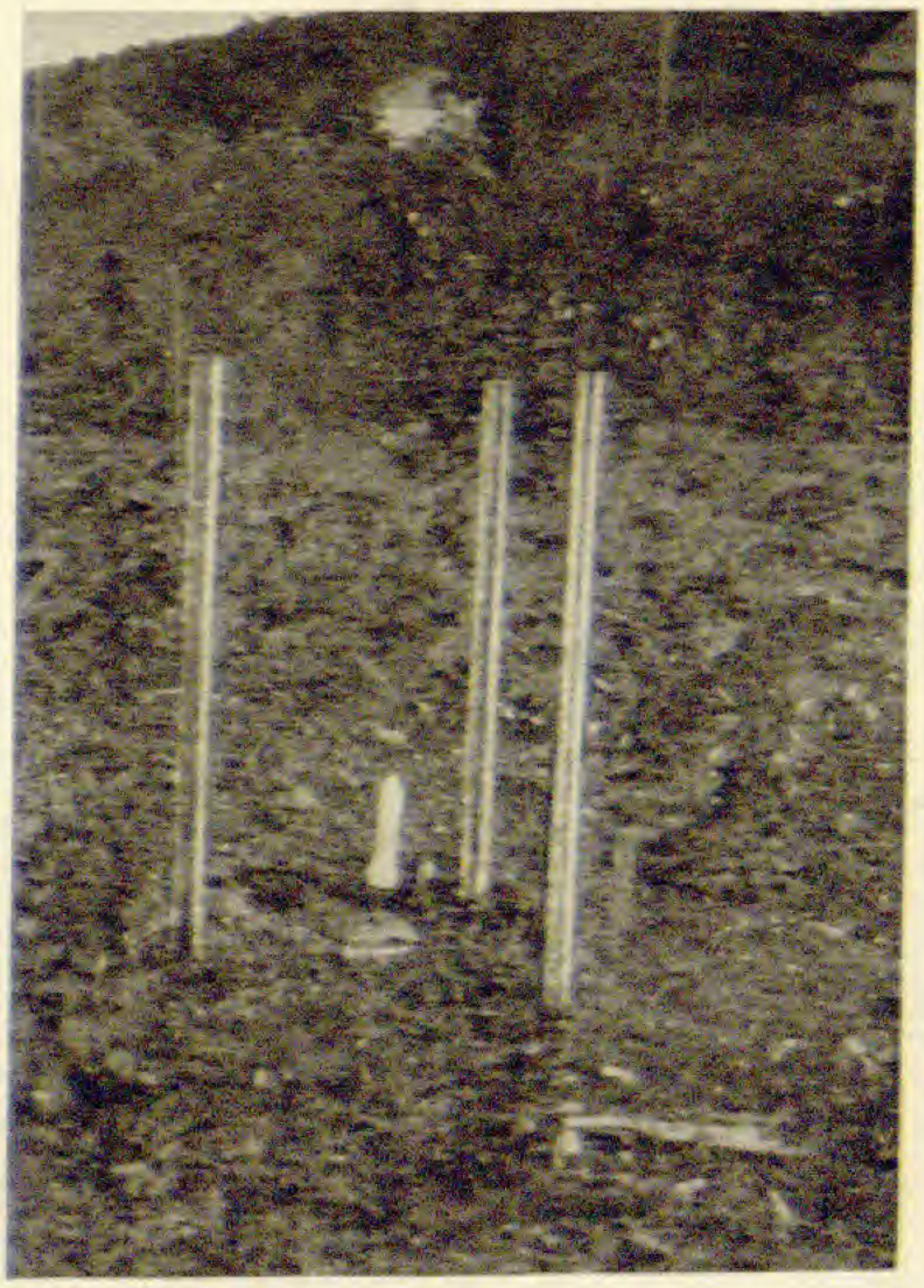

FIG. 2.—Standard instrument in position.

AMOUNT OF EVAPORATION BY WEEKS

Week ending May 27,	197^{cc}	Week ending July 8,	152^{cc}
" " June 3,	106	" " July 15,	152
" " June 10,	122	" " July 22,	114
" " June 17,	153	" " July 29,	160
" " June 24,	166	" " August 4,	102
" " July 1,	129	" " August 11,	104

Total for 12 weeks, 1657^{cc}

Station 2.—A garden plot between Hillside and Highland avenues, Huntington, about 3^{km} east of the standard. Conditions similar to those at the Carnegie garden, except the elevation, which is about

40^{m} above sea-level. Both this station and the standard were bare of vegetation at the time these comparative readings were made.

	June 5–10	June 10–19	June 19–24	Total (19 days)
Amt. evaporation, Station 2....	90.3^{cc}	145.5^{cc}	100.3^{cc}	336.1^{cc}
Amt. evaporation, standard....	102.2	207.3	112.6	422.1
Comparative evaporation..	88.3%	70.1%	89%	79.6%

This result indicates that for this nineteen-day period the increased elevation is correlated with decreased evaporation. This was quite unexpected, but on account of the small number of instruments at my disposal could not be tested further. The instrument used was subsequently tested and found to be perfectly standardized, so that there is no question about the correctness of this series of readings.

FIG. 3.—View from Station 1 across the Carnegie garden and salt marsh.

Station 3.—Gravel slide near St. John's Episcopal Church, about 0.25^{km} from the standard. The soil is a coarse sand and gravel; slopes eastward at an angle of 35°; altitude of station 25^{m}. The northern two-thirds of the gravel slide has been so recently disturbed by the removal of gravel, that it is free of vegetation except for some half-dead young trees which have fallen from above part way down the slope. There were none of these near the instrument. The southern third of the slide had been invaded by plants and had become more or less stable. The vaporimeter was placed on the border between these two areas.

Growing near the instrument were the following plants in very open association: *Polytrichum* sp.? *Androgopon scoparius*, *Deschampsia flexuosa*, *Comptonia peregrina*, *Ionactis linearifolia*, *Baptisia tinctoria*, *Epigaea repens*, and *Vaccinium pennsylvanicum*.

FIG. 4.—Station 4; partially invaded gravel slide.

	June 28–July 1	July 1–8	July 8–14	Total (16 days)
Amt. evaporation, Station 3....	34.0^{cc}	140.3^{cc}	145.6^{cc}	319.9^{cc}
Amt. evaporation, standard....	34.3	151.5	131.5	317.3
Comparative evaporation..	99.1%	92.6%	110.7%	100.8%

The bare gravel slide therefore shows an evaporation approximating that of the standard. As shown by synchronous temperature records, the surface on sunshiny days rises 20° above that of the garden, but the corresponding increase in evaporation seems to be largely compensated by the somewhat lower night rate. This gravel slide station represents the pioneer stage in the reforestation of a denuded area, and these percentages have a larger interest when compared with the stations representing later stages in this process.

FIG. 5.—Station 5; open forest on top of moraine.

Station 4.—Partly forested gravel slide (*fig. 4*) along the abandoned railroad grade midway between Wolfert and Tramp ponds. It is 2^{km} distant from Station 1; altitude 55^{m}; slopes south at an angle of about 30°. Scrub forest vegetation has invaded this slope and formed an open association. The principal trees (mostly under 7^m in height) are *Pinus rigida, Quercus coccinea, Q. marylandica, Q. prinus*, and *Castanea dentata*. The scattered low growth consists of *Pteridium aquilinum, Gaylussacia resinosa, Vaccinium pennsylvanicum, Myrica carolinensis*, and *Baptisia tinctoria*. The vaporimeter was placed in the partial shade of a pine tree, surrounded by a low clump of huckleberry. Readings were made during the same periods as at Station 3.

Fig. 6.—Typical mesophytic forest with abundant undergrowth, similar to Station 6.

	June 28–July 1	July 1–8	July 8–14	Total (16 days)
Amt. evaporation, Station 4....	14.3^{cc}	103.4^{cc}	72.3^{cc}	190.0^{cc}
Amt. evaporation, standard....	34.3	151.5	131.5	317.3
Comparative evaporation..	41.6%	68.2%	54.9%	59.5%

Since the slope is more toward the south than at Station 3, one might expect a greater evaporation rate, other conditions being equal. But the results indicate that in spite of direction of slope, the partial invasion of the gravel slide by vegetation has produced a 40 per cent. reduction in the evaporation.

Station 5.—Forest on top of moraine directly west of Station 1; altitude 43^m. This wood (*fig. 5*) is composed of *Quercus prinus, Q. velutina, Q. alba, Fagus americana, Castanea dentata, Betula lenta, Hicoria glabra*, and *Acer rubrum*. The trees attain a height of 20^m.

Nearly all of the undergrowth has been removed and the forest cover is not dense.

	July 15–23 (8 days)
Amt. evaporation, Station 5	71.6^{cc}
Amt. evaporation, standard	136.9
Comparative evaporation	52.2%

When this record is compared with that of Station 4, we see that the further development of the trees to true forest proportions reduces the evaporation near the soil by an additional 10 per cent.

Station 6.—Forest on the Joshua Jones farm along the "short road" to Huntington, 0.5^{km} east of the Carnegie garden. The land slopes gently to the northwest; altitude 50^{m}.

This is a fine example of the dominant forest of the region (*fig. 6*). The principal trees are *Quercus prinus*, *Q. velutina*, *Castanea dentata*, *Acer rubrum*, *Betula lenta*, and *Fagus americana*. The shrub layer is dominated by *Kalmia latifolia*, tree seedlings, *Sassafras sassafras*, *Azalea nudiflora*, *Viburnum acerifolium*, *Cornus alternifolius*, and *C. florida*. The herbaceous plants are *Chimaphila maculata*, *Dryopteris acrostichoides*, *Galium circaezans*, *Aster cordifolius*, *Leptorchis liliifolia*, *Peramium pubescens*, and *Phegopteris*.

	June 5–10	June 10–19	June 19–24	June 24–July 2	Total (27 days)
Amt. evaporation, Station 6..	49.3^{cc}	92.1^{cc}	37.6^{cc}	50.0^{cc}	229.0^{cc}
Amt. evaporation, standard..	102.2	207.3	112.6	148.8	570.9
Comparative evaporation	48.2%	44.4%	33.4%	33.6%	40.1%

The important difference between this station and the last is the increased undergrowth. When the record was started the trees were just beginning to leaf out. The effect of the increased shade and interference with air movements is well shown in the 15 per cent. reduction in evaporation between the first and last part of the record. When this record is compared with others, it must be on the basis of the last two weeks. It will be noted then that as reforestation proceeds in this region, the evaporation in the lower stratum of the forest decreases from 100 per cent. to about 33 per cent. when the climax forest type is reached.

Station 7.—Ravine forest just south of the "North lot" of the Carnegie Institution, about 0.5^{km} west of Station 1; altitude 10^{m};

soil surface slopes gradually to the northeast. This area is densely wooded and the herbaceous vegetation is very characteristic for the habitat. The trees are *Castanea dentata*, *Fraxinus americana*, *Prunus serotina*, *Betula lenta*, *Acer rubrum*, *Quercus prinus*, and *Tilia americana*. The shrub layer is made up of *Benzoin benzoin*, *Cornus florida*, *Rhus radicans*, *Viburnum acerifolium*, *Smilax rotundifolia*, and *S. herbacea*. The dominant herbs are *Washingtonia longistylis*, *Arisaema triphyllum*, *Trillium cernuum*, *Smilacina racemosa*, *Actaea alba*, *Impatiens biflora*, *Dryopteris acrostichoides*, and *Sericocarpus asteroides*.

FIG. 7.—Northern edge of swamp forest in which Station 8 was located.

	July 14–23 (9 days)
Amt. evaporation, Station 7	18.5^{cc}
Amt. evaporation, standard	141.9
Comparative evaporation	13.0%

Here we see a further marked reduction in the evaporation rate, when compared with the wooded hilltop and slopes. The presence of Benzoin, Trillium, and Arisaema, however, is in keeping with it.

Station 8.—Swamp forest at the head of St. John's pond, 0.5^{km} from the Carnegie garden (*fig. 7*); altitude 5^{m}. The soil is gravel and the water table is very near the surface. The forest cover is composed of *Quercus alba, Nyssa sylvatica, Liriodendron tulipifera, Fraxinus americana.* The dense shrub layer is dominated by *Hamamelis virginiana, Viburnum molle, Azalea viscosa, Clethra alnifolia, Alnus incana, Smilax rotundifolia, Rhus radicans, Benzoin benzoin,* and *Xolisma ligustrina.* The herbaceous layer contains *Osmunda cinnamomea, Spathyaema foetida, Viola cucullata, Trillium cernuum, Dryopteris noveboracensis, Arisaema triphyllum, Carex crinita, C. Asa-Grayi, Veratum viride,* etc.

	June 10–19	June 19–24	June 24–28	Total (18 days)
Amt. evaporation, Station 8....	20.2^{cc}	11.3^{cc}	9.5^{cc}	41.0^{cc}
Amt. evaporation, standard....	207.3	112.6	94.7	414.6
Comparative evaporation..	9.7%	10.0%	10.0%	9.9%

This record seemed very surprising at first, but as the records from the other habitats increased, it proved to be entirely in harmony with them. During the preceding summer I took some comparative temperature and relative humidity readings for this station and the Carnegie garden. Though the temperatures ran somewhat lower and the relative humidity higher, one would scarcely have predicted an evaporation difference of 90 per cent. for the lower stratum of the two stations. The failure to take into account the relative air movements in the two situations is the probable cause of the apparent discrepancy; but the comparison helps to emphasize the importance and efficiency of the porous-cup vaporimeter as a means of differentiating habitats.

Station 9.—Upper beach area, on "East Beach," 3^{km} north of Northport; altitude 4^{m}. This area is generally considered, on the basis of the vegetation, the most xerophytic along the Sound in the vicinity of Cold Spring Harbor. The direct distance from the Carnegie garden is 12^{km}. The vegetation is dominated by *Ammophila arundinacea, Rosa humilis, Panicum amarum, Lechea maritima, Artemisia caudata, Chrysopsis falcata, Polygonella articulata, Solidago sempervirens, Hudsonia tomentosa,* and *Opuntia opuntia.* The vaporimeter was placed among some shoots of *Rosa humilis,* just sufficiently screened so as not to attract attention. The coarse sandy substratum slopes gradually to the south at this point.

	June 27–July 19 (22 days)
Amt. evaporation, Station 9..........	332.1^{cc}
Amt. evaporation, standard...........	424.3
Comparative evaporation.........	78.2%

Station 10.—Upper beach on "Sand Spit" in Cold Spring Harbor, 1^{km} north of the Carnegie garden; altitude 3^{m}. The instrument was placed near the middle of the spit in the zone dominated by *Ammophila arundinacea.* Other near-by plants are *Solidago sempervirens, Cakile edentula, Asparagus officinalis, Euphorbia polygonifolia, Xanthium canadense, Cyperus Grayi, Polygonella articulata,* and *Verbascum thapsus.*

	July 2–8	July 8–15	Total (13 days)
Amt. evaporation, Station 10..........	112.9^{cc}	138.2^{cc}	251.1^{cc}
Amt. evaporation, standard...........	131.5	151.9	283.4
Comparative evaporation.........	85.8%	90.9%	88.6%

It will be noted that at both Stations 9 and 10 the evaporation is less than at the garden, in spite of the very xerophytic nature of the vegetation. I was unable to analyze this result further with the instruments and time at my disposal. It seems likely that the explanation lies in the low rate of evaporation during the night, due to low temperatures and increased humidity. They are more exposed to the temperature effects produced by the cooler water brought in at the flow of the tides and are more subject to fogs. The correctness of this suggestion can be tested only by an instrument which will record at hourly intervals. It is probable that the evaporation is higher on the beaches during periods of bright sunshine.

Station 11.—Salt marsh adjoining the garden of the Carnegie Institution. The vaporimeter was set up near the outer end of the middle line of the salt marsh (*fig. 8*). As this area is covered by water at extreme spring tides, the instrument was necessarily raised about 5^{dm} above the ground. The jar was protected from the sun by a heavy wrapping of felt paper. From the standpoint of the marsh vegetation the instrument stood on the tension line between the *Spartina polystachya* association and the zone dominated by *S. patens.* Other plants occurring near this point are *Atriplex hastata, A. arenaria, Tissa marina, Salicornia Bigelovii, Plantago maritima,* and *Limonium carolinianum.*

	June 14–23	Aug. 5–12	Total (14 days)
Amt. evaporation, Station 11..........	175.2^{cc}	171.2^{cc}	346.4^{cc}
Amt. evaporation, standard...........	141.9	129.7	271.6
Comparative evaporation.........	123.4%	131.9%	127.5%

It is here then that the greatest evaporation rate for this vicinity was recorded. This relation was not wholly unexpected, as high temperatures and low relative humidities had been found to occur here during the preceding summer; but the amount of the excess seemed large. Preceding the second attempt at measuring this rate, all of the instruments were brought to the garden and exposed side by side for four days. Their individual factors were recalculated and found to be practically the same as those calculated by LIVINGSTON. Further, a different instrument was used for the second period. Just how this increased evaporation over the salt marsh may be accounted for is not clear. The suggestion is offered that the deposition of salt on the vegetation is effective in reducing the vapor tension of the overlying stratum of air sufficiently to raise the evaporation rate.

FIG. 8.—View north from road across the salt marsh; Station 11 in the distance; Station 12 at the left.

Station 12.—Inner end of salt marsh, on the tension line between the *Spartina patens–Juncus Gerardi* association and the *Scirpus americana* association (*fig. 7*). Although the tide occasionally invades this area, the water proved by chemical test to be without a trace of chlorid at the next low tide. The vaporimeter was set up precisely as at Station 11.

	Aug. 5–13 (8 days)
Amt. evaporation, Station 12	112.8cc
Amt. evaporation, standard	129.7
Comparative evaporation	86.9%

It might be urged, in connection with the high percentage reading at Station 11, that the result was due to the elevation of the instrument. This cannot be offered, however, as an explanation of the great difference between Stations 11 and 12.

The other species of plants found near the station are *Eleocharis rostellata, Asclepias pulchra, Carex gracilis, Lysimachia terrestris, Eupatorium perfoliatum, Hibiscus moscheutos*, and *Dryopteris thelypteris.*

Station 13.—Fresh-water marsh—a continuation of the same marsh in which the two preceding stations were located. A road, elevated about 3^m above the marsh, separates this station from the last. I have no doubt but that this road interferes somewhat with the normal air drainage in its vicinity and may account in part for the low reading at Station 13. This station is sufficiently elevated to be beyond the reach of the highest tides. The vegetation about the instrument consisted of *Onoclea sensibilis, Lysimachia terrestris, Juncus effusus, Dryopteris thelypteris, Lycopus americana, Iris versicolor, Epilobium adenocaulon, Asclepias pulchra, Carex hystricina.* Near by grew *Acorus calamus, Eupatorium perfoliatum, Impatiens biflora, Ilysanthes attenuata, Rumex altissimus, Polygonum sagittatum, Scirpus americana, Myrica carolinensis*, etc.

	Aug. 5–13 (8 days)
Amt. evaporation, Station 13	57.0cc
Amt. evaporation, standard	129.7
Comparative evaporation	43.9%

When we compare this record with those obtained simultaneously at the last two stations, we see that there is a further decrease of 40 per cent. as we go farther away from the outer salt marsh. Even making allowances for the interference with air currents at this station, there would be a well-marked decrease in this direction.

Putting these three station records and the standard together we see that as we go west from the middle outer portion of the salt marsh into the garden, the rate decreases by a fourth. As we go south to the edge of the salt marsh the rate is decreased nearly a half, and when the middle of the fresh-water marsh is reached the rate has decreased to one-third.

GENERAL CONCLUSIONS

From the foregoing data, meager as they are, it is evident that we have in the porous-cup vaporimeter an instrument that will be of the greatest importance in the study of habitat conditions. Unlike so many of the recently exploited forms of instruments, it furnishes data that may be directly related to the plant. It has the great advantage over readings made from open water surfaces, in that the surface is constant and is continually exposed in the same way. Comparative readings may be obtained from different habitats by first standardizing the instruments.

The principal objection to the porous cup is its inability to withstand frost; this makes it useless in winter and early spring; it also limits its use at high elevations. Its usefulness might be greatly increased by making it self-recording, so that the diurnal variations could be accurately obtained.

The data which my observations furnish are of course characteristic only of the stratum within a meter of the soil surface. In the forest as we go from soil to tree-top this relative evaporation must increase; but it is in the lowest stratum that the seedlings, which are to determine the future of the area, have their struggle with the environment. With these data in hand it is not difficult to see why seedlings of Trillium, Arisaema, and Veratrum are successful in the swamp forest with its 10 per cent. relative evaporation; why they fail in the open hillside forest with its 50 per cent. rate; and why they are never seen on the near-by gravel slide with its relative rate of 100 per cent. in addition to its unstable character.

The following diagram (*fig. 9*) shows in a more graphic way the comparative rates of evaporation in the lowest stratum of the common habitats about Cold Spring Harbor.

In the reforestation of the gravel slides in this locality it will be seen that the greatest decrease in the demands for transpiration on the part of seedlings takes place during the first stages. This greatly aids in accounting for the well-known fact that the development toward a closed association proceeds with such increasing rapidity when once a few plants gain a foothold. Attention has been frequently called to the importance of pioneers as shade-producers, while their effectiveness in reducing transpiration has been underestimated.

The fact that the weekly evaporation rate for a beach covered with xerophytes should be less than that for a garden may seem anomalous. But when the diminished ground-water supply, the sterility of the soil, and the probable high rate of evaporation during short periods are taken into account, the elimination of more mesophytic species is not surprising.

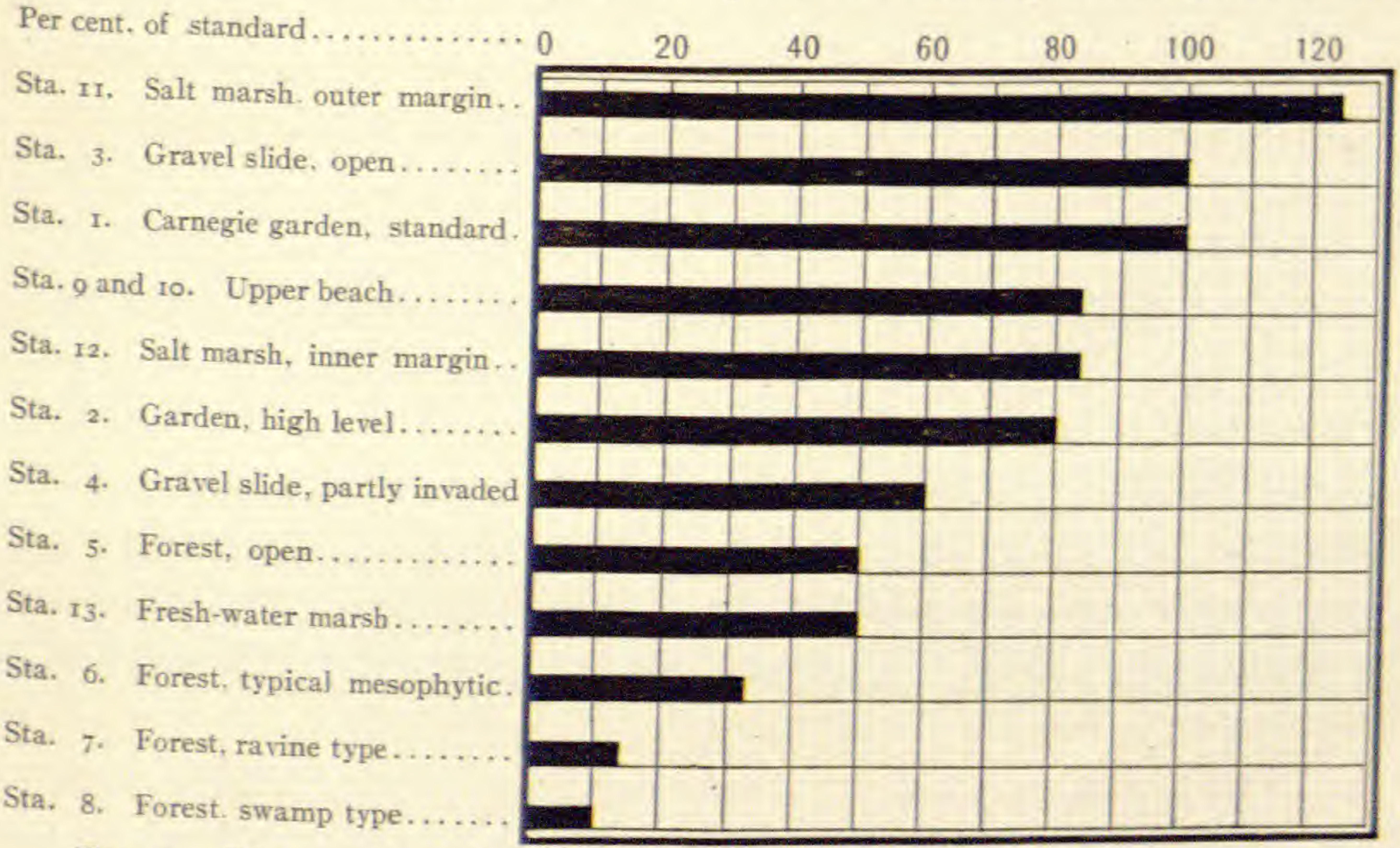

FIG. 9.—Diagram showing comparative evaporation rate in the lowest stratum of the several plant societies.

In the case of the high-level and low-level gardens further observations may alter these relative figures.

The high rate of evaporation in the salt marsh helps in the understanding of the structure of these plants.

Further work upon the rate of evaporation at different levels within the forest will aid greatly in estimating the factors involved in competition between trees. For a complete understanding of habitat conditions in general it seems essential that an instrument which will record the losses for smaller time intervals be devised.

CHARLESTON, ILL.

FURTHER STUDIES OF SOLUTION TENSION AND TOXICITY IN LIPOLYSIS[1]

RAYMOND H. POND

This paper is really a continuation of an earlier one[2] which supplied data showing that the toxicity of certain salts in lipolysis does not vary inversely as the decomposition tension of those salts. As my conclusion disagrees with that of MATHEWS,[3] MCGUIGAN,[4] and CALDWELL,[5] and as BERG and GIES[6] found that the nature of the zymolyte is an important factor in peptolysis and tryptolysis, it seemed desirable to determine whether the same general relation found by me for the toxicity of several nitrates in the lipolysis of ethyl butyrate would also hold in the case of some other ester. Ethyl acetate was selected as the zymolyte because its saponification is a monomolecular reaction, because acetic acid is a close homologue of butyric acid, and because LOEVENHART and PEIRCE[7] found that the hydrolysis of ethyl acetate is inhibited to a greater extent by sodium fluorid than that of ethyl butyrate. They also expressed the view that the inhibiting effect of sodium fluorid is dependent upon the acid radical from which the ester is derived, while the alcohol arising in the saponification is a comparatively indifferent factor.

Since the same general procedure has been followed here as in the preceding investigation, many of the details given before will be omitted

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[1] From the laboratories of the New York Botanical Garden.

[2] POND, RAYMOND H., Solution tension and toxicity in lipolysis. Amer. Jour. Physiology 19:258–283. 1907. Contributions from the New York Botanical Garden 94.

[3] MATHEWS, A. P., The relation between solution tension, atomic volume, and the physiological action of the elements. Amer. Jour. Physiology 10:290–323. 1904.

[4] MCGUIGAN, HUGH M., The relation between the decomposition tension of salts and their antifermentative properties. Amer. Jour. Physiology 10:444–451. 1904.

[5] CALDWELL, J. S., The effects of toxic agents upon the action of bromelin. BOT. GAZETTE 39:409–419. 1905.

[6] BERG, W. N., and GIES, WM. J., Studies of the effects of ions on catalysis, with particular reference to peptolysis and tryptolysis. Jour. Biol. Chem. 2:489–546. 1907.

[7] LOEVENHART, A. S., and PEIRCE, GEO., The inhibiting effect of sodium fluorid on the action of lipase. Jour. Biol. Chem. 2:379–413. 1907.

here. Most of the solutions used were made up from the same stock solutions and all were upon the same basis of molecular weight. The lipase used was taken from the same sample. Professor GIES has been my counselor in this work as in the other, and the same acknowledgments are due the New York Botanical Garden for the exceptionally favorable facilities afforded.

METHOD

When ethyl acetate is decomposed by the action of lipase, the products are supposed to be the same as in any case of its saponification, namely, acetic acid and ethyl alcohol. The amount of acid liberated from initially neutral acetate under given conditions is an index of the amount of enzyme activity under those conditions, and can be expressed in cubic centimeters of KOH solution required for neutralization. Since boiling destroys the activity of the enzyme, and since the acetate does not become appreciably acid in such a boiled solution of the enzyme during an incubation of 5 hours at 40° C., a boiled solution of the enzyme can be used as a control. The difference then in acidity of a boiled and of an unboiled solution of the enzyme of equal concentration, to which the same amount of ester has been added, expresses the amount of enzyme activity under the conditions imposed. Six vials were used for each concentration of the toxic salt. To each vial were added 2^{cc} of the toxic salt solution of a certain concentration, say $m/32$. Then to each of three of the vials were added 2^{cc} of the unboiled, filtered enzyme solution having a given concentration, say 0.10 per cent. To each of the other three vials were then added 2^{cc} of a portion of the same enzyme solution which had been boiled. Then to each of the six vials was added 0.10^{cc} of neutral ethyl acetate free from alcohol. The stoppered vials were placed in an incubator for 5 hours at 40°C. After incubation each vial was titrated with $m/40$ KOH, using phenolphthalein as an indicator. The difference between the boiled and unboiled solutions showed the amount of enzyme activity for $m/64$ of the salt used with a concentration of the enzyme of about 0.05 per cent. Filtering the enzyme solution makes the exact concentration uncertain. All of the salts used were nitrates except that of mercury, which was the bichlorid.

Since the acetate has the lower boiling-point and decomposes more rapidly in the presence of water, one would expect that it would be more readily saponified by the lipase. A 0.0125 per cent. solution of the enzyme yielded from 0.10^{cc} of butyrate enough acid to require 0.20^{cc} of $m/20$ KOH for neutralization. The same concentration of enzyme acting for one hour longer at the same temperature on the same amount of acetate gave the figure 0.06^{cc} of $m/40$ KOH. A stronger solution of the enzyme (0.0250 per cent.) acting on the acetate gave 0.08^{cc} of $m/40$ KOH. From this one might think that the butyrate is the less stable in the presence of the enzyme, but it may be that the products of saponification are more inhibiting in the one case than in the other. The concentration of the enzyme in most of

TABLE I: RELATIVE TOXICITY WITH SAME CONCENTRATION OF ENZYME

Potassium and sodium in contemporaneous test

POTASSIUM

m	Control[8]	Enzyme[8]	Increase[8]
Water[8]........	0.07	0.25	0.18
32............	0.06	0.20	0.14
16............	0.06	0.19	0.13
8............	0.06	0.16	0.10
4............	0.06	0.13	0.07
2............	0.06	0.13	0.07
1............	0.06	0.10	0.04

SODIUM

m	Control	Enzyme	Increase
32............	0.07	0.20	0.13
16............	0.07	0.17	0.10
8............	0.07	0.15	0.08
4............	0.07	0.13	0.06
2............	0.07	0.10	0.03
1............	0.07	0.10	0.03

Enzyme 0.05 per cent. Incubation 5 hours at 40 C.

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[8] In the column headed "*m*" are given the various concentrations of the toxic salt in fractions of molecular strength. In this column the word "Water" means that 2^{cc} of water were used instead of the toxic solution in order to ascertain the uninhibited activity of the enzyme in 0.05 per cent. strength. In the column headed "Control" are given the figures for the final acidity (cc. $m/40$ KOH) of the boiled preparations containing the toxic salt in the concentration indicated by the corresponding figure in the column headed "*m*." The column headed "Enzyme" shows the final acidity of the unboiled preparations corresponding to the controls. The column headed "Increase" shows the difference in acidity between the controls and the unboiled preparations and expresses the amount of saponification allowed by the concentration of the toxic solution indicated by the corresponding figures in the column headed "*m*."

the tests was 0.05 per cent., that is, the filtrate obtained from the solution of 0.20^{gm} of the enzyme powder in 200^{cc} of water, filtered and then diluted one-half by the addition of an equal volume of the toxic solution. During 5 hours' incubation at 40° C., 0.1^{cc} of neutral acetate in 4^{cc} of water remains neutral.

In this test, as in several that follow, it was observed that while there was sediment in each of the vials containing boiled enzyme, the vials containing the unboiled solutions were at the close of the incubation entirely clear and free from sediment. There is no doubt therefore that partial inhibition of the enzyme is possible and actually occurs in the absence of any precipitation whatever. In this table we note that the uninhibited activity of the enzyme is expressed by 0.18^{cc}. The most concentrated solutions of the salts reduce this to 0.04 for potassium and to 0.03 for sodium. The amount of inhibition is therefore very considerable, and yet there was no precipitation even after an incubation period of 5 hours at 40° C. In this test the nitrates of sodium and of potassium are certainly to be regarded as equitoxic.

TABLE II: *Potassium, sodium, and lithium in contemporaneous test*

POTASSIUM

m	Control	Enzyme	Increase
Water	0.10	0.25	0.15
8	0.10	0.15	0.05
4	0.10	0.13	0.03
1	0.10	0.13	0.03

SODIUM

m	Control	Enzyme	Increase
8	0.10	0.20	0.10
4	0.08	0.16	0.08
2	0.07	0.15	0.08
1	0.07	0.12	0.05

LITHIUM

m	Control	Enzyme	Increase
8	0.08	0.17	0.09
4	0.09	0.15	0.06
2	0.09	0.13	0.04

Enzyme 0.05 per cent. Incubation 4 hours at 45° C.

So far as this particular test is concerned, it can hardly be said that the salts are equitoxic. On the other hand, it cannot be positively concluded which of the three is the most toxic. Comparison of the individuals of the series leads to nothing decisive. The next table shows that fluctuating differences occur.

TABLE III

POTASSIUM

m	Control	Enzyme	Increase
Water	0.09	0.23	0.14
8	0.10	0.20	0.10
4	0.09	0.18	0.09
2	0.09	0.16	0.07
1	0.09	0.12	0.03
SODIUM			
8	0.07	0.20	0.13
4	0.08	0.18	0.10
2	0.08	0.15	0.07
1	0.08	0.13	0.05
LITHIUM			
8	0.10	0.17	0.07
4	0.09	0.16	0.07
2	0.09	0.13	0.04

Enzyme 0.05 per cent. Incubation 5 hours at 45 C.

There seems to be some indication that lithium is more toxic than sodium or potassium, but the difference is too insignificant for a conclusion. In all the vials the unboiled solutions were free from sediment. Since the salt solutions themselves are neutral in all the concentrations tried, the column showing acidity of the reagent has been omitted. The titration with the *m*/40 KOH instead of the *m*/20 KOH used in the previous investigation with ethyl butyrate perhaps brings out greater variations. The acidity of the control is usually with each of these salts about the same in all concentrations and just about what the plain enzyme solution itself is. This confirms the observation made in the work with ethyl butyrate that there is no positive sign of any chemical action between the substance of the enzyme solution and that of the toxic solution.

In this test the two salts are neutral in all concentrations tried. One drop of the *m*/40 KOH gives a deep purple with 4^{cc} of the salt solution in any of the concentrations used in the test. The unboiled preparations were all free from sediment after incubation. Total inhi-

TABLE IV: *Barium and magnesium in contemporaneous test*

BARIUM

m	Control	Enzyme	Increase
Water	0.10	0.22	0.12
128	0.10	0.22	0.12
64	0.10	0.20	0.10
32	0.10	0.18	0.08
16	0.10	0.17	0.07
8	0.10	0.16	0.06
MAGNESIUM			
128	0.10	0.22	0.12
64	0.10	0.20	0.10
32	0.10	0.18	0.08
16	0.10	0.17	0.07
8	0.10	0.16	0.06

Enzyme 0.05 per cent. Incubation 4.5 hours at 40 C.

bition seems to be impossible for this concentration of the enzyme as *m*/4 of the salts is almost saturation at room temperature. In this test the two salts are certainly equitoxic.

TABLE V: *Barium and strontium in contemporaneous test*

BARIUM

m	Control	Enzyme	Increase
Water	0.13	0.27	0.14
128	0.13	0.27	0.14
64	0.13	0.24	0.11
32	0.13	0.22	0.09
16	0.13	0.20	0.07
8	0.13	0.16	0.03
STRONTIUM			
128	0.13	0.27	0.14
64	0.13	0.24	0.11
32	0.13	0.22	0.09
16	0.13	0.20	0.07
8	0.13	0.16	0.07

Enzyme 0.05 per cent. Incubation 5 hours at 40 C.

In this test the two salts are certainly equitoxic. Comparison with Table IV however shows inconstancy. Thus, *m*/8 of barium in Table IV allows 0.06 as compared with 0.03 in Table V. On the other hand, in both cases, *m*/128 of barium allows uninhibited activity. Again, the figure indicating full activity in Table IV is less than that in Table V, and yet *m*/8 barium inhibits more in the latter than in the former. It is of course simply a matter of judgment how such results are to be interpreted. Since barium and strontium are equitoxic in contemporaneous tests and since the same is true of barium and magnesium, it is reasonable to conclude that the three salts are equitoxic. This same relation was found in the case of ethyl butyrate. In this table (V) the control figure is higher than that in Table IV. There is, however, a corresponding increase in the figures in the enzyme column. Just what causes such fluctuation from day to day is difficult to tell, but there seems to be sufficient uniformity in the test as a whole. For the sake of assurance another test was made, and this gave practically identical figures with those in Table IV. The figure for *m*/8 barium shows some irregularity in this next table.

TABLE VI: *Barium and magnesium in contemporaneous test*

BARIUM

m	Control	Enzyme	Increase
Water	0.10	0.23	0.13
128	0.10	0.22	0.12
64	0.10	0.20	0.10
32	0.10	0.17	0.07
16	0.10	0.16	0.06
8	0.10	0.13	0.03

MAGNESIUM

m	Control	Enzyme	Increase
128	0.10	0.22	0.12
64	0.10	0.20	0.10
32	0.10	0.18	0.08
16	0.10	0.16	0.06
8	0.10	0.16	0.06

Enzyme 0.05 per cent. Incubation 5 hours at 40 C.

In the work with ethyl butyrate I found barium, strontium, and magnesium to be equitoxic with each other, but all were more toxic than either sodium, potassium, or lithium, which latter were equitoxic with each other. In the foregoing tables there are some indications that barium, strontium, and magnesium are more toxic than the sodium, potassium, lithium group. On the other hand, the differences between lithium and magnesium are not so great. Thus, *m*/8 of lithium in Table II allows 0.09, while *m*/16 of magnesium in Table IV allows 0.07. Again, *m*/8 of lithium in Table III allows 0.07. From the foregoing tables it would be impossible to say that the barium group is more toxic than the sodium group on the equinormal basis.

It may be that the zymolyte ethyl acetate tends to minimize the difference in relative toxicity as compared with the zymolyte ethyl butyrate. While this is a plausible explanation, it has little value in the absence of convincing evidence, and we cannot select the determining factor. Why the variations in the different tests should be relatively so wide is not understood, especially in view of the precautions to avoid error.[9] The following test shows that barium is more toxic than lithium under the same conditions as imposed in a contemporaneous test. This emphasizes the apparent tendency of the barium group to exceed the sodium group in toxicity, and such a conclusion is perhaps permissible in view of all the results of this work and that on ethyl butyrate, but it would certainly be difficult to say how much more toxic.

TABLE VII: *Barium and lithium in contemporaneous test*

BARIUM

m	Control	Enzyme	Increase
Water	0.10	0.20	0.10
256	0.09	0.16	0.07
128	0.09	0.15	0.06
64	0.09	0.15	0.06
32	0.10	0.13	0.03
16	0.10	0.12	0.02
8	0.10	0.10	0.00

LITHIUM

m	Control	Enzyme	Increase
64	0.10	0.20	0.10
32	0.10	0.18	0.08
16	0.10	0.17	0.07
8	0.10	0.12	0.02
4	0.10	0.11	0.01
2	0.10	0.10	0.00

Enzyme 0.05 per cent. Incubation 5 hours at 40° C.

In the preceding tests it will be remembered that the salts themselves are neutral in all the concentrations used, and that the acidity of the reagent is quite uniformly the same for all the concentrations of a given salt, and that the acidity of the control is the acidity of the enzyme solution itself, as is indicated by the figure for "Water" in the control column. It is apparent that the highest saturation is required

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[9] Possibly the enzyme solutions differed sufficiently to account for the discrepancies. Then too, the insolubility of the zymolyte may have been responsible to some extent.

for marked inhibition, and there is no evidence of chemical action between the enzyme substance and the toxic salt. The cause of the inhibition may be simply osmotic. In all of the remaining tests it will be noted that the toxic salt solution has more or less acidity of its own (due to hydrolytic dissociation) according to the dilution, and in several cases the control acidity is greater than the sum of the enzyme acidity and of the reagent acidity. It seems, in some cases at least, that there is chemical action between the substance of the enzyme solution and the toxic salt which results in the liberation of acid. In the higher dilutions of the toxic salt the acidity of the salt itself approaches zero, and the acidity of the control tends to be identical with that of the enzyme solution itself. Then again, at the point of total inhibition the reagent acidity and the control acidity are often equal.

TABLE VIII: *Cadmium and cobalt in contemporaneous test*

CADMIUM

m	Reagent[10]	Control	Enzyme	Increase
Water.........	....	0.10	0.22	0.12
1024.........	0.02	0.20	0.33	0.13
512.........	0.04	0.30	0.40	0.10
256.........	0.06	0.40	0.50	0.10
128.........	0.10	0.55	0.60	0.05
64.........	0.20	0.60	0.63	0.03
32.........	0.30	0.90	0.90	0.00

COBALT

m	Reagent[10]	Control	Enzyme	Increase
1024.........	0.03	0.20	0.33	0.13
512.........	0.06	0.33	0.43	0.10
256.........	0.08	0.43	0.52	0.09
128.........	0.10	0.70	0.77	0.07
64.........	0.15	0.90	0.92	0.02
32.........	0.30	1.00	1.00	0.00

Enzyme 0.05 per cent. Incubation 5 hours at 40° C.

All of the vials contained sediment at the close of incubation. In the unboiled *m*/1024 there was very little precipitate. Before incubation the unboiled solutions remained water clear after the mixture of enzyme and toxic salt until placed in the oven, so that the precipitation was slow.

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[10] This column gives the acidity of 4^{cc} of the toxic salt solution of the concentration indicated by the corresponding figure in the column headed "*m*."

In comparison with the preceding tables there are some points of difference here. The reagent itself is acid and the unboiled preparations show a deposit of substance which tends to be zero in quantity as the dilution of the reagent increases and the acidity consequently decreases. The point of uninhibited activity lies close to that of the lowest concentration of the toxic solution having zero acidity. The acidity of the control is greater than the sum of the reagent acidity and the acidity of the enzyme solution. In this table (VIII) it will be noted that the reagent acidity for $m/32$ cadmium is 0.30, while the corresponding control figure is 0.90. A similar relation holds for cobalt. The same relation was observed for these salts in the work on ethyl butyrate. That cadmium and cobalt are more toxic than sodium, potassium, lithium, barium, strontium, and magnesium is certain. Thus it appears that the first inhibitory effect in our series, which seems more likely to be due to chemical action than to osmotic surface tension or other physical influences, is associated with substances having a natural acidity.

TABLE IX: *Copper and zinc in contemporaneous test*

COPPER

m	Reagent	Control	Enzyme	Increase
Water........	....	0.07	0.19	0.12
16384........	0.00	0.10	0.19	0.09
8192........	0.05	0.13	0.19	0.06
4096........	0.10	0.17	0.22	0.05
2048........	0.20	0.23	0.27	0.04
1024........	0.40	0.40	0.40	0.00
ZINC				
16384........	0.00	0.10	0.19	0.09
8192........	0.05	0.13	0.19	0.06
4096........	0.10	0.17	0.22	0.05
2048........	0.20	0.25	0.30	0.05
1024........	0.40	0.40	0.40	0.00

Enzyme 0.05 per cent. Incubation 5 hours at 40° C.

In this test copper and zinc are certainly equitoxic. Copper is much more active in the precipitation of substance from the solution. All the unboiled solutions with zinc were free from sediment after incubation, showing that inhibition is not necessarily associated with precipitation. In the copper solutions sediment was present in all concentrations except *m*/16384. The amount of the precipitate varies directly with the concentration of the reagent.

TABLE X: *Copper and lead in contemporaneous test*

m	Reagent	Control	Enzyme	Increase
COPPER				
Water........	....	0.10	0.20	0.10
16384........	0.00	0.10	0.20	0.10
8192........	0.05	0.15	0.20	0.05
4096........	0.10	0.18	0.22	0.04
2048........	0.20	0.23	0.26	0.03
1024........	0.40	0.40	0.40	0.00
LEAD				
16384........	0.00	0.10	0.20	0.10
8192........	0.00	0.10	0.20	0.10
4096........	0.03	0.15	0.25	0.10
2048........	0.07	0.20	0.25	0.05
1024........	0.16	0.35	0.40	0.05

Enzyme 0.05 per cent. Incubation 5 hours at 40° C.

All the vials except the *m*/16384 contained sediment at the close of incubation. The figures for copper are practically a duplicate of those in Table IX. Lead seems to be not quite so toxic as copper in

TABLE XI: *Copper, lead, and zinc in contemporaneous test*

m	Reagent	Control	Enzyme	Increase
COPPER				
4096.........	0.10	0.17	0.22	0.05
2048.........	0.20	0.23	0.26	0.03
1024.........	0.40	0.40	0.40	0.00
512.........	0.70	0.70	0.70	0.00
LEAD				
4096.........	0.03	0.16	0.22	0.06
2048.........	0.07	0.24	0.28	0.04
1024.........	0.16	0.40	0.40	0.00
512.........	0.35	0.50	0.50	0.00
ZINC				
4096.........	0.10	0.18	0.23	0.05
2048.........	0.20	0.26	0.30	0.04
1024.........	0.40	0.50	0.50	0.00
512.........	0.85	0.85	0.85	0.00

Enzyme 0.05 per cent. Incubation 5 hours at 40° C.

this test. The reagent acidity of lead is correspondingly less than that of copper, although the control figures agree fairly well with those of copper. The point of total inhibition for lead is not determined in this test, so it is not apparent whether the acidity of the reagent and control is identical at that point as was found to be the case with copper and zinc.

In this test lead proves to be equitoxic with copper and zinc. The figures for copper agree closely with those preceding. The figures for zinc agree fairly well, the important difference being that at the point of total inhibition the reagent acidity and control acidity are not identical for the *m*/1024 concentration, although at *m*/512 the coincidence may be noted. The following test is sufficient confirmation of the relation here found.

TABLE XII

m	Control	Enzyme	Increase
COPPER			
4096	0.13	0.20	0.07
2048	0.20	0.23	0.03
1024	0.40	0.40	0.00
512	0.70	0.70	0.00
LEAD			
4096	0.15	0.19	0.04
2048	0.20	0.23	0.03
1024	0.30	0.30	0.00
512	0.50	0.50	0.00
ZINC			
4096	0.15	0.20	0.05
2048	0.23	0.27	0.04
1024	0.45	0.45	0.00
512	0.85	0.85	0.00

Enzyme 0.05 per cent. Incubation 5 hours at 40° C.

All the unboiled zinc solutions were free from sediment. The copper and the lead solutions all contained sediment, though the *m*/4096 of each contained only the smallest traces. While total inhibition may apparently occur in the absence of any precipitation, there is always (with the exception of cadmium and cobalt in Table VIII) some inhibition accompanying precipitation.

TABLE XIII: *Mercury and silver in contemporaneous test*

MERCURY

m	Reagent	Control	Enzyme	Increase
Water........	....	0.10	0.18	0.08
65536........	0.00	0.10	0.15	0.05
32768........	0.00	0.10	0.15	0.05
16384........	0.00	0.10	0.12	0.02
8192........	0.05	0.10	0.10	0.00
4096........	0.10	0.13	0.13	0.00

SILVER

m	Reagent	Control	Enzyme	Increase
32768........	0.00	0.10	0.15	0.05
16384........	0.00	0.10	0.13	0.03
8192........	0.00	0.10	0.13	0.03
4096........	0.00	0.10	0.12	0.02
2048........	0.00	0.10	0.10	0.00

Enzyme 0.05 per cent. Incubation 5 hours at 40 C.

In this test mercury is more toxic than silver, even on the equinormal basis, though the difference here is not nearly so great as was found with ethyl butyrate. Of course the greater concentration of the enzyme used here introduces a factor in addition to that of the different zymolyte. Mercury in comparison with silver is like copper in comparison with zinc in regard to precipitating power. The point of total inhibition is close to that of the first appearance of a precipitate,

TABLE XIV

MERCURY

m	Control	Enzyme	Increase
131072........	0.10	0.15	0.05
65536........	0.10	0.15	0.05
32768........	0.10	0.13	0.03
16384........	0.10	0.10	0.00
8192........	0.10	0.10	0.00
4096........	0.13	0.13	0.00

SILVER

m	Control	Enzyme	Increase
131072........	0.10	0.13	0.03
65536........	0.10	0.13	0.03
32768........	0.10	0.13	0.03
16384........	0.10	0.12	0.02
8192........	0.10	0.12	0.02
4096........	0.10	0.10	0.00

Enzyme 0.05 per cent. Incubation 5 hours at 40° C.

but with silver total inhibition may occur in the absence of any precipitation. The following test reinforces the result of this one.

In Table XIV the reagent acidity is omitted, as it may easily be seen in Table XIII and most of the values are zero anyhow. The relative toxicity is the same, athough the exact point of total inhibition is different. In the case of silver there was no deposit in any of the unboiled solutions, while with mercury there was deposit in *m*/16384 and in higher concentrations, while greater dilutions were free from deposit. To determine at what point precipitation first appears in the case of silver the following test was made.

TABLE XV: *Silver in solitary test*

SILVER

m	Control	Enzyme	Increase
Water.........	0.10	0.15	0.05
262144........	0.10	0.15	0.05
131072........	0.10	0.15	0.05
65536........	0.10	0.13	0.03
32768........	0.10	0.13	0.03
16384........	0.10	0.12	0.02
8192........	0.10	0.12	0.02
4096........	0.10	0.10	0.00
2048........	0.10	0.10	0.00
1024........	0.15	0.15	0.00

Enzyme 0.05 per cent. Incubation 5 hours at 40 C.

The lowest concentration of the reagent to show any natural acidity was the *m*/1024, and greater dilutions were neutral to the indicator used. The only vials of the unboiled series to show a deposit after incubation were the *m*/1024.

A comparison of the results of this work on the acetate with those obtained in the work on ethyl butyrate shows that the same general relation holds for both. The metals fall into groups according to toxicity. The groups are not so clearly separated in the case of the acetate, but still sufficiently distinct to confirm the conclusion drawn in regard to the lipolysis of ethyl butyrate.

In the work on the ethyl butyrate it was found that the relative toxicity changes according to the concentration of the enzyme—a result not in accord with the conclusion of CALDWELL (*l. c.*). The following tests show that concentration of the enzyme is a factor in relative toxicity, and adds emphasis to my result with ethyl butyrate.

TABLE XVI

RELATIVE TOXICITY WITH VARYING CONCENTRATIONS OF THE ENZYME

Copper and zinc in contemporaneous test

COPPER

m	Control	Enzyme	Increase
2048	0.27	0.32	0.05
1024	0.42	0.46	0.04
512	0.77	0.80	0.03
256	1.50	1.50	0.00
128	2.90	2.90	0.00
ZINC			
2048	0.30	0.38	0.08
1024	0.47	0.53	0.06
512	0.90	0.96	0.06
256	1.73	1.77	0.04
128	3.30	3.30	0.00

Enzyme 0.10 per cent. Incubation 5 hours at 40 C.

In Tables IX, XI, and XII, copper and zinc have the same point of total inhibition, namely *m*/1024. In those tests the concentration of the enzyme was 0.05 per cent. Here when the concentration of the enzyme is doubled the relative toxicity is changed. The point of total inhibition for copper is *m*/256 and for zinc *m*/128. In Table XVII where the concentration of the enzyme is still higher, the difference in toxicity is more pronounced.

TABLE XVII

COPPER

m	Control	Enzyme	Increase
2048	0.33	0.47	0.14
1024	0.50	0.55	0.05
512	0.80	0.85	0.05
256	1950	1.50	0.00
128	2.90	2.90	0.00
ZINC			
2048	0.32	0.47	0.15
1024	0.50	0.65	0.15
512	0.87	1.02	0.15
256	1.70	1.80	0.10
128	3.40	3.50	0.10

Enzyme 0.15 per cent. Incubation 5 hours at 40 C.

In the work on ethyl butyrate, cadmium, cobalt, and zinc were found to become equitoxic with increasing concentration of the enzyme. Here the relative toxicity of zinc as compared with that of copper decreases with increasing concentration of the enzyme. In both cases the tendency of zinc to lose in relative toxicity with increasing concentration of the enzyme is apparent.

TABLE XVIII: *Mercury and silver in contemporaneous test*

MERCURY

m	Control	Enzyme	Increase
8192	0.20	0.25	0.05
4096	0.25	0.25	0.00
2048	0.33	0.33	0.00
1024	0.35	0.35	0.00
512	0.53	0.53	0.00

SILVER

m	Control	Enzyme	Increase
8192	0.20	0.25	0.05
4096	0.20	0.24	0.04
2048	0.20	0.23	0.03
1024	0.26	0.26	0.00
512	0.33	0.33	0.00

Enzyme 0.10 per cent. Incubation 5 hours at 40 C.

Here the point of total inhibition for copper is the same as with the lower concentration of the enzyme in the preceding table, but the zinc fails to inhibit totally and shows plainly that it loses toxicity faster than copper as the concentration of the enzyme increases.

TABLE XIX

MERCURY

m	Control	Enzyme	Increase
8192	0.25	0.40	0.15
4096	0.25	0.35	0.10
2048	0.35	0.40	0.05
1024	0.50	0.55	0.05
512	0.85	0.88	0.03

SILVER

m	Control	Enzyme	Increase
8192	0.25	0.35	0.10
4096	0.25	0.35	0.10
2048	0.25	0.32	0.07
1024	0.28	0.33	0.05
512	0.35	0.40	0.05

Enzyme 0.15 per cent. Incubation 5 hours at 40° C.

In Tables XIII and XIV mercury is four times more toxic than silver on the equimolecular basis. The same relative toxicity is maintained here as in those tables, although greater concentration of both salts is required for total inhibition.

Here the two salts are practically equitoxic, so that in some cases, with increasing concentration of the enzyme, salts, which are equitoxic become unequal in toxicity, while others that are not equitoxic under certain conditions become so under other conditions. Perhaps the only conclusion that can be safely drawn from this group of tests is that the concentration of the enzyme influences relative toxicity, and that a relation which holds for one concentration of the enzyme may not hold for another four or five times greater.

RELATIVE TOXICITY OF EQUICATIONIC SOLUTIONS

By calculation[11] it may be determined that copper and lead of *m*/2048 concentration are equicationic, and by examination of my results it is seen that those solutions are about equitoxic. On the other hand, *m*/1971 of barium is equicationic with copper *m*/2048,

TABLE XX: ETHYL BUTYRATE

Barium, lead, copper, and potassium in contemporaneous test

m	Control	Enzyme	Increase
BARIUM			
Water	0.00	0.20	0.20
2048	0.00	0.20	0.20
1024	0.00	0.20	0.20
POTASSIUM			
2048	0.00	0.20	0.20
1024	0.00	0.20	0.20
LEAD			
2048	0.10	0.30	0.20
1024	0.20	0.30	0.10
COPPER			
2048	0.15	0.30	0.15
1024	0.20	0.30	0.10

Enzyme .0125 per cent. Incubation 4 hours at 35° C.

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[11] All of the equicationic calculations were made for me by Dr. WM. N. BERG of the Laboratory of Biological Chemistry of Columbia University.

but reference to the tables shows that such a concentration of barium is entirely without inhibitory effect upon the concentration of the enzyme tried. So by calculation it is seen that *m*/2030 of potassium is equicationic with copper *m*/2048, but reference to the table shows that potassium of such concentration is not toxic. In order to leave no doubt that cation concentration is not a determining factor in toxicity to the extent that equicationic solutions are equitoxic, the following tests were made.

According to calculation, the following solutions are equicationic: copper and lead *m*/2048, potassium *m*/2030, barium *m*/1971. Instead of making the exact concentrations for barium and potassium, it was assumed that if *m*/1024 and *m*/2048 do not inhibit, intermediate concentrations would not. Examination of the results shows that there is no basis for regarding equicationic solutions as even probably equitoxic. Neither of the concentrations of barium tried nor of potassium are inhibiting, while both of copper and one of lead are. By calculation it is easy to see that there is no fixed relation apparent between equitoxicity and equicationic concentration. The exceptions may be multiplied. Thus in the butyrate tests in my other paper, copper *m*/512 and lead *m*/512 are equicationic, equidissociated, and equitoxic. On the other hand, barium *m*/8 and copper *m*/512 are equitoxic, but the relative number of cations per unit volume of those elements in those concentrations is 38 to 1. That is, in unit volume of barium *m*/8 there are 38 cations as compared with 1 cation in unit volume of copper *m*/512. The test shown in Table XXI with ethyl acetate shows that equicationic solutions may totally inhibit or may not inhibit at all according to the salts compared.

According to the calculation from the conductivity tables, the following nitrates have a cation concentration of *m*/80.7: barium *m*/64, copper *m*/70, lead *m*/64, and potassium *m*/76. Reference to the above test shows that all of those concentrations are included within the limits of the test, and yet copper and lead totally inhibit, while barium and potassium do not inhibit at all.

DISCUSSION

The attempt to correlate physiological action with certain physical or chemical properties either of the ions or of the atoms has thus far been unsuccessful.[12] In view of the really large attention that has been devoted to this subject, it seems surprising that no generalization has been satisfactorily established (BERG and GIES, *l. c.*). The affinity of ions for their charges is such a fundamental property that we can hardly conceive of a chemical reaction involving solution in which this affinity or solution-tension, as some have called it, does not play a part. On the other hand, there is abundant evidence offered by my experiments to show that there is no fixed relation between toxicity and solution-tension to the extent that the toxicity of substances can be exactly estimated from their solution-tension (POND, *l. c.* 274). There is difficulty even in agreeing upon a standard of toxicity. The minimum lethal dose measures the fatal toxicity, but is it a standard of toxicity? Does total inhibition measure the toxicity in my own experiments? In Table XIII we see that $m/16384$ and $m/32768$ of both mercury and silver are inhibiting and equally inhibiting, but for

TABLE XXI: ETHYL ACETATE

Barium, lead, copper, and potassium in contemporaneous test

m	Control	Enzyme	Increase
COPPER			
Water	0.12	0.27	0.15
128	2.90	2.90	0.00
64	5.70	5.70	0.00
LEAD			
128	2.10	2.10	0.00
64	4.20	4.20	0.00
BARIUM			
128	0.12	0.27	0.15
64	0.12	0.27	0.15
POTASSIUM			
128	0.12	0.27	0.15
64	0.12	0.27	0.15

Enzyme 0.10 per cent. Incubation 5 hours at 40 C.

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[12] The following paper offers a recent and thorough digest of the more important literature of this subject: BERG, WM. N., The relation between the physiological action of ions and their physico-chemical properties. New York Medical Journal for July 20 and July 27, 1907. pp. 42.

total inhibition four times greater equimolecular concentration of silver is required than of mercury. (See also POND, *l. c.* 268.)

A critical examination of my own results and those of others fails to reveal any general relation without exceptions, or any property or factor that can be regarded as exclusively fundamental and as solely determining the relative toxicity of a wide series of salts.

Each of the salts I have tested is a nitrate except one, the chlorid of mercury. The members of the series fall into two groups, the neutral salts which do not hydrolytically dissociate in aqueous solution, and the hydrolytes which do so dissociate and whose solution is therefore acid or alkaline according to whether the H or OH ion is predominant. All of the hydrolytes of my series are acid, but as the dilution increases the acidity decreases to zero as here tested.

This difference between the neutral salts and the hydrolytes shows very clearly in the acidity of the controls. The controls of the neutral salts have practically the same acidity as the enzyme solution itself, that is, there is no evidence of any alteration of the acidity of the boiled enzyme solution as a consequence of the mixture with the neutral salt solution. Thus in Tables I–VII inclusive the acidity of the control is about the same for each concentration of the salt. This acidity is close to if not identical with the acidity of the plain boiled enzyme solution as indicated by the figures corresponding to "Water."

In the other tables, however, where the salts have an acid reaction in consequence of their hydrolytic dissociation, the acidity of the control and of the reagent varies considerably (POND, *l. c.* 283). Thus in Table VIII the acidity of the plain control without salt present is 0.10. The acidity of $m/1024$ cadmium is 0.02, but when the control for $m/1024$ is observed, the figure is 0.20 or considerably more than the sum of reagent and enzyme. In regard to this behavior there is much variation among the hydrolytes. Thus at the point of total inhibition the reagent acidity and control acidity may be identical, as with copper and zinc in Table IX, or considerably different, as in Table VIII with cadmium. It is difficult to interpret this with assurance.

It is notable that the neutral salts are not of equal toxicity either in the saponification of the acetate or of the butyrate (POND, *l. c.* 265, 266). Thus in Table VII barium is more toxic than lithium, so that we cannot correlate the degree of toxicity with the degree of hydrolytic dissociation in any close way, though it is true that all of the hydrolytes in the tests I have made are more toxic than any of the neutral salts.

The fact that the hydrolytes themselves are potent in saponification has long been known, in fact the degree of hydrolysis has been measured by the rate of the saponification of esters.[13] Such salts have also been found to be potent in the inversion of sugars.[14] It was supposed that the inverting or saponifying power is associated with hydrolytic dissociation, the H or the OH ions being the active agents. LEY[15] (p. 214), however, found that KCl can invert sugar, so that other ions than those resulting from hydrolytic dissociation are potent. Thus it seems that exceptions arise to intercept the formulation of any generalization.

Significant also is the fact that dilutions of silver and mercury so great that the reaction is neutral as tested are capable of causing total inhibition. On the other hand, solutions of the neutral salts which are comparatively very concentrated are not inhibiting. Thus in Table XIII, $m/2048$ of silver is totally inhibiting but not measurably acid. In some cases we find that solutions of equal acidity are also equitoxic (Tables VIII and IX especially). This relation, however, fails in some cases (Tables X and XI). Moreover, partial or total inhibition may occur with the hydrolytes at dilutions too great to show acidity as here tested. Thus copper and zinc in Table IX show partial inhibition at $m/16384$, which is neutral, and mercury at $m/16384$ in Table XIII shows almost total inhibition without acidity, while silver in Table XIII shows total inhibition in a neutral concentration of the salt.

The relative toxicity changes as the concentration of the enzyme changes, provided the difference is enough. Thus in Tables IX, XI, and XII copper and zinc are equitoxic, but in Table XVI they are less equal, and in Table XVII still less so with the increasing concentration of the enzyme. Similar variations were found in the tests with ethyl butyrate (POND, *l. c.* 276).

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[13] SHIELDS, JOHN, Ueber Hydrolysis in wässerigen Salzlösungen. Zeitsch. physikalische Chem. **12**:167–187. 1893.

[14] LONG, J. H., On the inversion of sugar by salts. Jour. Amer. Chem. Soc. **18**:120–130. 1896.

[15] LEY, H., Studien über die hydrolytische Dissociation der Salzlösungen. Zeitsch. physikalische Chem. **30**:193–257. 1899.

Just to what extent precipitation during incubation is a factor in inhibition is difficult to say. The results show that precipitation occurs in concentrations which cause total and often only partial inhibition. However, with zinc (Tables IX and XII) and silver (Tables XIV and XV) we find total inhibition without precipitation.

The enzyme itself, that is, the substance exhibiting zymolytic power, is probably a reversible colloid, and since it was not precipitated by the neutral salts used, it may be assumed to have gone into colloidal solution rather than into suspension. Perhaps it also is a hydrolyte liberating both H and OH ions. If this is the case, its saponifying power may be associated with its hydrolytic dissociation, just as has been that of the salt hydrolytes. The toxicity of the salt might even be referred to the possibility that the excess of H or of OH ions resulting from the hydrolysis of the salt prevented the hydrolytic dissociation of the enzyme substance and therefore caused the inhibition[16] (p. 214). If so, to what is the toxicity of the neutral salts to be referred? Also, why are the very highly diluted and therefore neutral solutions of silver and mercury capable of causing total inhibition? May not the chemical nature of the atom be a factor? Since convincing evidence is not available for a negative answer, and since theoretical argument based upon the negative assumption leads to embarrassing conclusions,[17] it is obvious that in any effort to discover final causes this possibility should not be ignored.

The tests with equicationic solutions show the futility of any attempt at generalization in that direction and at every hand exceptions seem to arise.

CONCLUSION

In the zymolytic saponification of ethyl acetate as in that of ethyl butyrate, the toxicity of the salts tested does not under the conditions specified vary inversely with the decomposition tension of those salts.

The concentration of the enzyme is a factor in relative toxicity in some cases.

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[16] Bredig, G., Beiträge zur Stoichiometrie der Ionenbeweglichkeit. Zeitsch. physikalische Chem. 13:191–288. 1894.

[17] Berg, *l. c.* pp. 15–20.

Note.—In referring to Pond (*l. c.*), note the following errata: p. 274, bottom line, "Zinc and cadmium" should read Zinc and barium; p. 283, line 13 from top, "Table XIII" should read Table XIV.

A QUANTITATIVE STUDY OF TRANSPIRATION

GRACE LUCRETIA CLAPP

(WITH TWO FIGURES AND THIRTY GRAPHS)

In the Laboratory of Plant Physiology of Smith College a systematic series of studies is being made to determine which of the plants available to American teachers are best for the demonstration, or investigation, of each of the principal physiological processes, and how much may be expected of them. Some of these studies in physiological constants have already been completed and others are well under way. I have undertaken to determine these facts for the process of transpiration. This work has been done under the direction of Professor W. F. GANONG, whose advice and supervision I gratefully acknowledge.

Transpiration has been extensively investigated from several points of view, and the results up to 1904 are all summarized in BURGERSTEIN'S admirable monograph.[1] It shows that investigations have been directed mainly to explain the amounts of transpiration as depending either upon structural features or upon physiological processes, or as controlled by physical changes. Special work now being done by LIVINGSTON, LLOYD, and others at the Carnegie Desert Laboratory in Arizona is likely greatly to extend our knowledge of transpiration as an ecological factor in the plant life of the desert. But nothing has as yet been done in the direction of the present study. I have not been concerned with the explanation of transpiration upon either a physical or a physiological basis, nor yet with the relations between absolute and relative transpiration, but simply with the fact that plants lose appreciable amounts of water. I have sought to determine with precision the actual amounts of water lost by plants, growing under the ordinary conditions arising in any greenhouse, and simultaneously have determined the transpiration under conditions which admit of control and repetition.

My procedure was as follows. In each species studied, two well-grown plants, of as nearly the same size as could be found, were chosen at maturity, when increase of leaf surface is at a minimum.

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[1] BURGERSTEIN, ALFRED, Die Transpiration der Pflanzen. Jena. 1904.

Two or three days before use, these were brought into the experimental greenhouse, where conditions of heat, moisture, and light were practically the same as in the greenhouse proper. Any plant likely to be pot-bound was repotted in the soil mixture for common plants two or three weeks before the test, in order that it might become properly adjusted.

The actual transpiration was determined throughout by the most accurate known method, that of weighing. To prevent evaporation from the pot and soil, each pot was covered by an aluminum or zinc shell of proper size, roofed over by rubber sheeting. This was tightly clasped to the plant, cemented along the seam, and fastened to the shell by a metal band, removable to permit the plant to be watered.

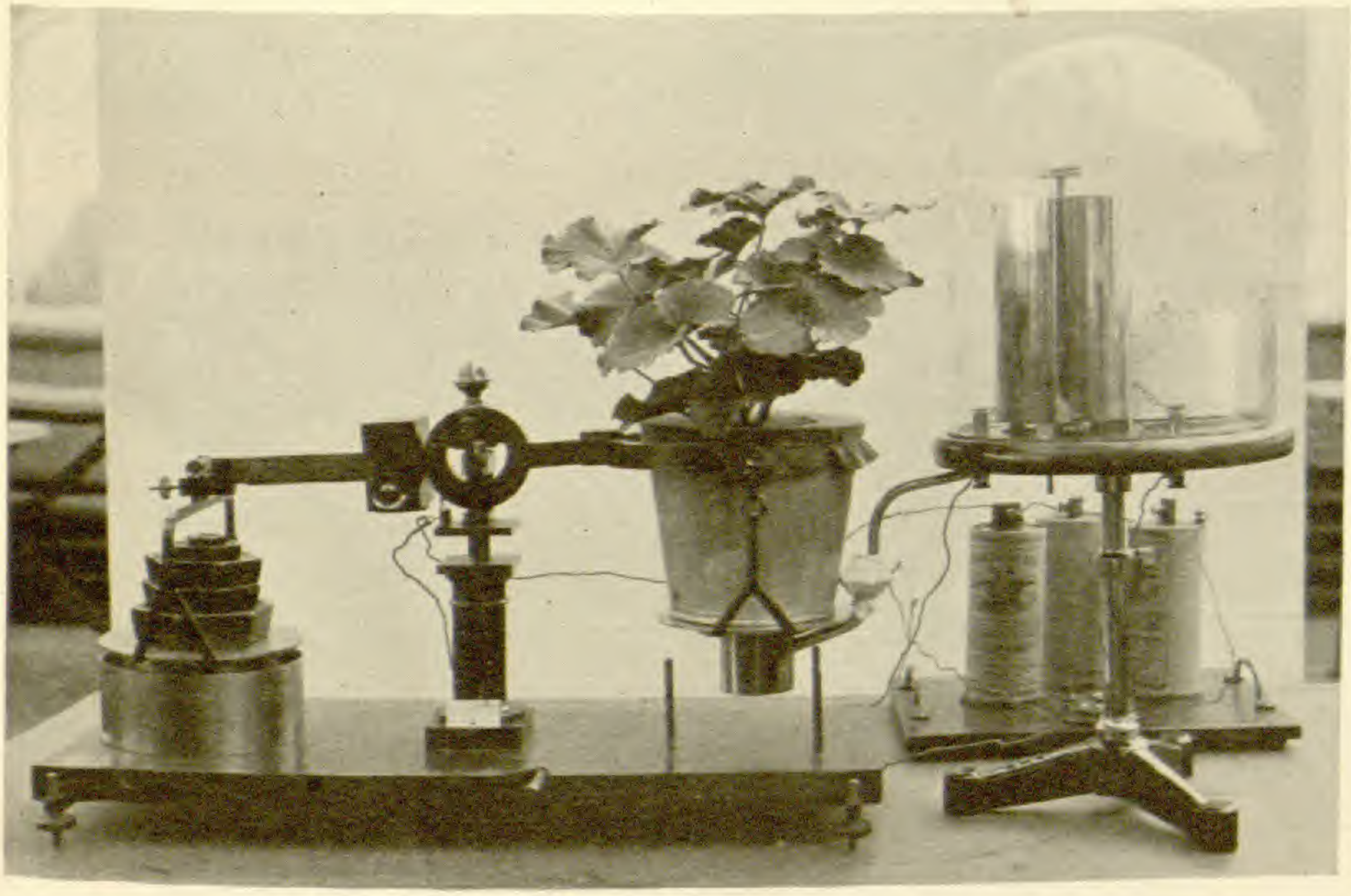

FIG. 1

The plant to be kept under greenhouse conditions was then placed on a specially constructed balance which was brought into electrical connection with the autographic transpirometer invented by Professor GANONG and described by him in this journal.[2] The entire arrangement, slightly modified from the original form, is well shown

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[2] GANONG, W. F., New precision appliances for use in plant physiology. BOT. GAZETTE 39:145. 1905.

in the accompanying *fig. 1*. By this apparatus the time and amount of transpiration are recorded continuously.

Each plant was under observation for six consecutive days (Monday morning to Saturday afternoon): climatic changes were recorded in the near vicinity by the thermograph, hygrograph, and barograph, but as changes of pressure have no appreciable effect on the transpiration the records are not given. There is no satisfactory instrument recording the varying intensities of light and darkness in a form permitting the construction of a graph. Therefore the changes of light were recorded from observation and were afterward reduced to a graph form by the following method.[3] CLEMENTS' table[4] of light intensities for the different sun altitudes (the angle of the sun's rays with a horizontal surface) for Lincoln, Neb. (41° N. lat.), was taken as a basis and changed to suit the latitude of Northampton (42° 19″ N.). Then, since the intensities for any hour of the day obtained from measurements on the celestial globe and graphically represented for a brilliant cloudless day form a parabola with greatest value at sun noon, it was possible to construct graphs showing full sunlight for each day of the year. For cloudy days the percentage of intensities was determined from the following arbitrary basis, the percentages being those of full sunlight for the given time.

Slight fleecy clouds and haze	90 per cent.
Heavy clouds alternating with blue sky	85 per cent.
Light open clouds, shadows cast	80 per cent.
Overcast, dark clouds, not rainy	60 per cent.
Overcast, with dark clouds and heavy rain	50 per cent.
Overcast, with dark clouds and snow	40 per cent.

The second plant was intended partly as a check upon results obtained from the first, but especially as a standard exposed to conditions which admit of comparison of one kind with another, and which can be repeated by other students. This plant was placed in a large glass case (*fig. 2*) of some 652 liters capacity where conditions were partly controlled, and were registered by the thermograph and hygrograph. The temperature was kept within the range of 19–21° C. and the moisture between 45 and 55 per cent. of saturation. The tempera-

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[3] For aid in the calculation of the light values I wish to thank Dr. HARRIET BIGELOW of the Astronomy Department.

[4] CLEMENTS, F. E., Research methods in ecology 57. 1905.

ture was raised by an electric heater in the bottom of the case, and lowered by a metal coil, cooled by water from the street pipes, in the top of the case. To promote prompt and even warming or cooling, electric fans of small size were made to blow air over the heating or cooling arrangements. Moisture was decreased by uncovering dishes of calcium chlorid and increased by exposing wet sponges and cloths. The case was always brought to the standard conditions, the plant was weighed and left in *still air* for as long a time as the conditions remained

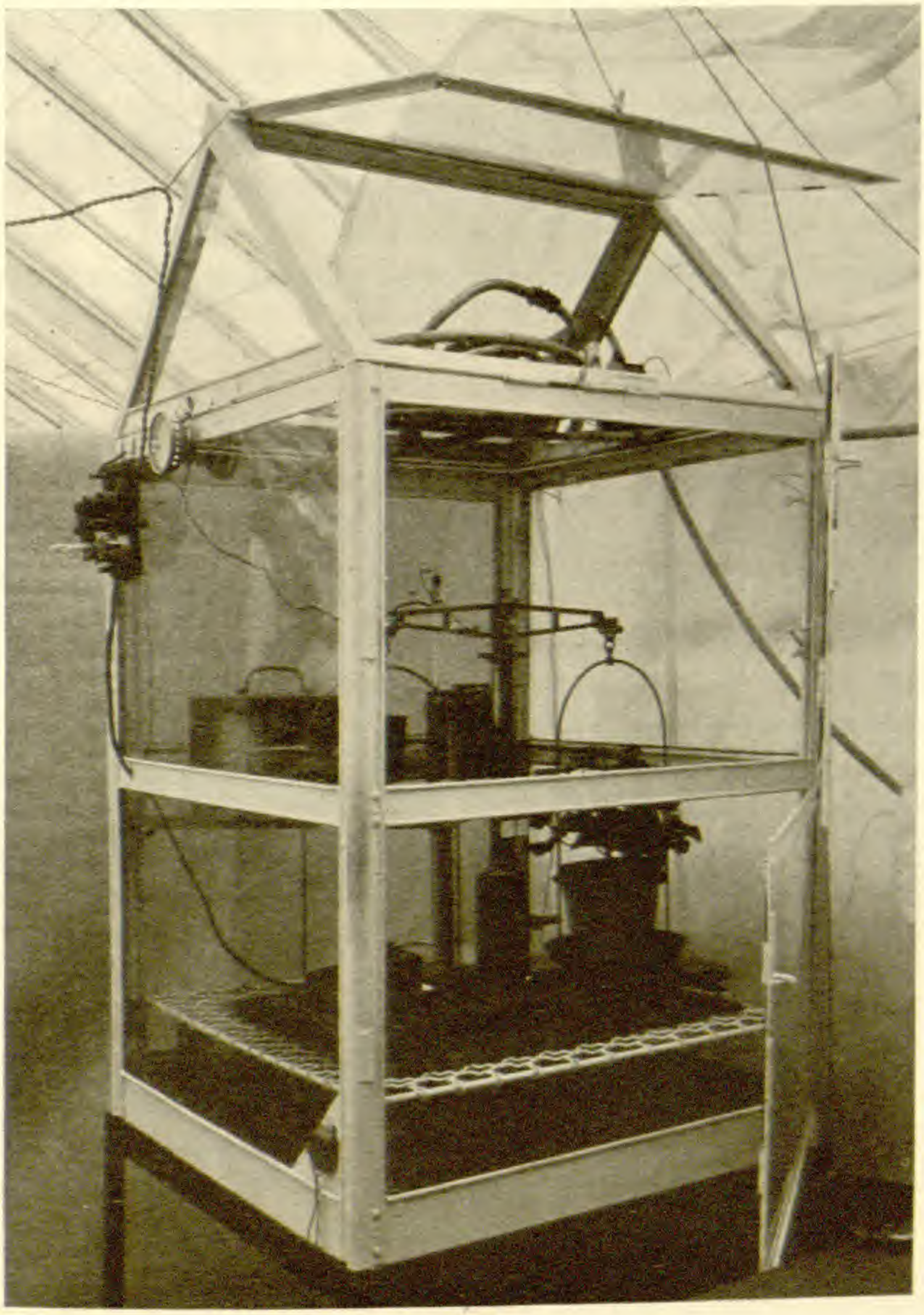

FIG. 2

within the prescribed limits; then it was weighed again, the results being reduced to an hour basis. The light was kept bright and diffuse by manipulation of a white curtain. The value of the light was considered about 90 per cent. of that received by the plant under ordinary conditions. At times when the sun did not cast a shadow both plants were exposed to the same intensity. To keep the plant under conditions of aeration as nearly normal as possible, the case was opened every morning (when the plant was watered) and the air blown out by a large electric fan.

To allow the transpiration of these plants to be compared with one another, and especially to permit the transpiration of different sizes and kinds to be compared, it was necessary to reduce their transpiration values to a common standard. For this I adopted the unit system of grams per hour per square meter of leaf surface, which for convenience may be designated the M^2HG standard. Leaf area was taken for one side only and petioles were ignored unless the blade extended along the edge, when an approximate value of half the petiole was added. This system has great advantages in convenience over one in which both leaf surfaces are estimated, or in which the stems and petioles are taken into account, and since in general in any given kind of plant the amount of petiole and stem surface must be approximately proportional to the leaf surface, no error is involved in a comparative study. In certain studies, involving structural relations, etc., the stem and petiole areas would have to be considered. The surface area was computed by tracing the leaves on homogeneous paper, and weighing them after they were cut out; this value was then reduced to a square meter of surface, by the familiar method, viz., from the determination of the weight of a known unit area of the paper. Photographs of the plants kept under ordinary greenhouse conditions were made, all upon the same scale and against the same background (ruled in square centimeters). These are reproduced with their respective curves below, with the exception of three, of which the plates were accidentally destroyed.

The results of the study I have endeavored to express in the ways which will render them most useful. First in importance are the exact figures, which are contained in the following table and are reduced to average per hour. In the plant under greenhouse condi-

NAME OF PLANT	GREENHOUSE CONDITIONS									STANDARD CONDITIONS						
	Actual loss per hour				Area of plant (m^2)	M^{2}HG				Actual loss per hour			Area of plant (m^2)	M^{2}HG		
	Min. (g)	Average		Max. (g)		Min.	Average		Max.	Min. (g)	Average Day (g)	Max. (g)		Min.	Average Day	Max.
		Day (g)	Night (g)				Day	Night								
Abutilon striatum	.123	1.731	.210	3.5	.05463	2.25	31.680	3.839	64.06	.36	1.334	2.4	.05002	7.19	26.669	47.98
Begonia argentea guttata	.123	1.861	.356	3.5	.07465	1.648	24.947	4.776	47.05	1.08	1.375	1.58	.07216	14.979	19.070	21.914
Cestrum elegans	.22	1.524	.565	3.09	.06383	3.445	23.864	8.846	48.387	1.	1.187	1.15	.07043	14.198	16.860	16.328
Chrysanthemum frutescens	.91	9.903	2.069	18.5	.12849	7.082	77.069	16.099	143.98	6.6	8.830	12.05	.12955	50.945	68.159	93.014
Cineraria stellata	.5	4.084	1.233	8.5	.15866	3.15	25.740	7.770	53.57	2.4	3.045	3.35	.11274	21.287	27.005	29.714
Coleus Blumei (var.)	.43	2.470	.819	5.09	.12164	3.53	20.305	6.732	41.84	1.33	2.335	3.05	.11068	12.197	21.096	27.586
Cucurbita Pepo	.19	6.437	.774	13.	.12420	1.53	51.832	6.229	104.66	1.33	3.112	4.48	.13860	9.595	22.455	32.32
Euphorbia pulcherrima	.34	3.442	1.155	6.	.07372	4.61	46.680	15.674	81.38	2.58	4.207	5.1	.07385	34.935	56.960	69.058
Ficus elastica	.153	2.882	.502	4.33	.07070	2.16	40.760	7.100	61.24	.7	2.121	3.5	.08070	8.67	26.280	43.37
Fuchsia speciosa	.45	5.526	1.128	11.	.09976	4.51	55.390	11.305	110.26	2.8	4.517	6.7	.12323	22.72	36.650	54.36
Hedera Helix	.08	1.656	.213	3.09	.07090	1.128	23.356	3.007	43.58	.3	1.077	2.25	.06308	4.755	17.073	35.669
Helianthus annuus	.386	6.670	.974	13.	.05056	7.634	131.920	19.264	257.12	2.6	3.136	5.08	.06275	41.43	49.976	80.956
Heliotropium peruvianum	.83	4.355	1.441	9.	.13009	6.38	33.470	11.070	69.18	1.6	3.758	9.2	.14274	11.209	26.327	64.45
Impatiens Holstii	.21	.946	.280	5.	.02383	4.74	27.708	9.212	81.75	1.78	2.168	2.12	.06618	26.89	31.530	32.03
Ipomoea purpurea	.084	4.783	.434	8.5	.06665	1.26	71.755	6.508	127.53	.9	1.76	1.22	.07295	12.337	24.127	16.72
Lupinus albus	.119	2.855	.344	5.	.03615	3.29	78.970	9.515	138.31	.81	2.399	3.58	.04033	20.084	59.484	88.83
Lycopersicum esculentum	.18	8.085	.803	15.5	.10673	1.686	75.750	7.52	145.23	1.8	3.284	4.07	.11415	15.768	28.769	35.65
Pelargonium domesticum	.116	5.156	.497	12.5	.08657	1.339	59.558	5.738	144.39	1.05	3.380	5.4	.06856	15.315	49.299	78.76
Pelargonium peltatum	.169	3.600	.446	7.	.07065	2.392	50.919	6.312	99.08	1.9	2.640	3.65	.09415	20.18	28.104	38.76
Pelargonium zonale	.44	6.940	1.135	17.	.14794	2.97	46.910	7.672	114.91	1.58	4.17	7.95	.14669	10.77	28.427	54.145
Phaseolus vulgaris	.3	2.348	.623	6.5	.06878	4.36	34.133	9.059	94.58	.51	.816	1.05	.06956	7.33	14.74	15.094
Primula sinensis	.155	1.242	.375	2.77	.07452	2.799	16.670	5.029	37.17	1.4	2.408	3.8	.09895	14.148	24.33	38.40
Ricinus communis	.29	1.694	.563	5.	.06115	4.74	27.708	9.212	81.75	1.78	2.168	2.12	.06618	26.89	32.754	32.03
Salvia involucrata	.315	8.313	1.123	12.5	.13382	2.35	62.121	8.390	93.404	2.9	4.350	6.15	.12892	22.49	33.750	47.79
Senecio mikanioides	.21	3.126	.737	8.	.06309	3.328	49.540	11.680	126.78	1.05	2.297	3.64	.06689	15.69	34.340	54.40
Senecio Petasitis	.57	9.708	2.492	19.	.14581	3.909	66.579	17.093	130.306	4.35	5.363	6.65	.18227	23.865	29.420	36.484
Tradescantia zebrina	.105	.819	.166	1.45	.02610	4.023	31.402	6.341	55.56	.19	.581	.93	.02840	6.69	20.471	32.746
Tropaeolum majus	.1	9.668	1.813	15.	.12801	7.81	75.520	14.159	117.17	4.45	7.880	11.07	.15056	9.423	52.335	73.505
Vicia Faba	.129	2.296	.258	4.67	.04117	3.13	55.760	6.26	113.43	1.3	3.440	4.95	.05030	25.785	68.389	98.409
Zea Mays	.195	5.560	.703	12.	.12655	1.541	43.931	5.553	94.824	.7	1.990	3.08	.10464	6.689	19.017	29.434

tions I have given this average loss for the day and night separately, and have added the extremes observed in order to show how widely the transpiration may vary in the same plant. In the plant under standard conditions similar results are given, though for day only.

A comparison of the M^2HG figures for the ordinary and standard conditions of the same plant will reveal some remarkable discrepancies. Thus, Helianthus under the ordinary conditions stands far ahead of any other plants studied, but under the standard conditions it is surpassed by several. The explanation of such differences is evident. The week in which the experiment was tried may have been one of exceptional dryness and brightness, or one in which air currents from ventilation, or perhaps greater heat, increased the transpiration of the plant under ordinary conditions but could not materially affect that under standard conditions. It is evident therefore that for comparison of the transpiration of one plant with another the columns under ordinary conditions have little value, and for this the column under standard conditions should be used. It is in order to exhibit clearly the effects of such external conditions upon transpiration that I have plotted for the same plants the transpiration in graphs, along with the graphs of external conditions, in the series of diagrams which follow below. Another peculiarity of the tables is found in the great range in the same plant under standard conditions. This comes in part from the difficulty of keeping the conditions constant, in part from the great sensitiveness of the process to even slight alterations of external conditions, but chiefly from the variations in light. Absolute proof of this could only be obtained from a series of careful experiments where all factors would be under control and only one varied at a time. It can reasonably be inferred, however, on this ground: on bright sunny days there was a gradual increase and decrease proportional to the light intensity. On cloudy days or when the roof was covered with snow, with the curtain aside, the loss per hour varied within much narrower limits. Comparison of the losses at the same hours on different days also justifies this conclusion.

It will occur to the reader at this point that not enough plants of each species were used to give results which are fair tests of the transpiration of a given species. This criticism is only in part just. It is obviously impracticable to provide either the excessive time or the numerous instruments requisite for obtaining averages from several plants. Aside from this, however, the results are more representative than may appear at first sight. On the one hand the plants were carefully chosen from a considerable number as typical specimens of their kinds, and on the other, according to QUÉTELET'S law, any single plant taken at random is more likely to fall close to the mean than far from it. Hence, taking our thirty plants collectively, while some of them may deviate considerably from the mean of their kind, the great majority must lie more or less close to it.

Although the table of figures expressing the total quantities of transpiration has its value, it cannot be made to throw light upon the effect of external conditions upon transpiration. In consequence graphs made of each plant under *greenhouse conditions* were plotted from the records of the autographic instruments. In all cases they are for six days, the day and night being indicated on the base line. Each abscissa space represents 2½ hours of time; each ordinate space in the transpiration curve equals either 1, ½, or 2 grams of water lost, as indicated in each case, the difference being necessary to bring the curves of the plants within practical ranges; one vertical space in the temperature curve represents 5°; in the curve of moisture, 1 per cent. of saturation; and in that of light, 5, 2.5, or 10 per cent. of intensity. The curve of moisture has been inverted into one of dryness so that all physical conditions tending to increase transpiration have upward turns, and *vice versa*. The curve of light shows the intensities available to the plant, not what the plant actually used; this varies with the structure and the internal physiological processes.

As indicated above, these graphs have two values. The first is to show under what external conditions the figures for ordinary conditions given in the table were obtained. It is obvious that these values are profoundly affected by the conditions prevailing when the experiment was in progress. The second value is to present as clearly and graphically as possible the relations of the rate of transpiration to external conditions. It is not supposed that these graphs can bring out any knowledge of transpiration new in the abstract, but they do show this relation with gratifying clearness for our most familiar plants, and in a form which, it is hoped, will make them valuable in educational work.


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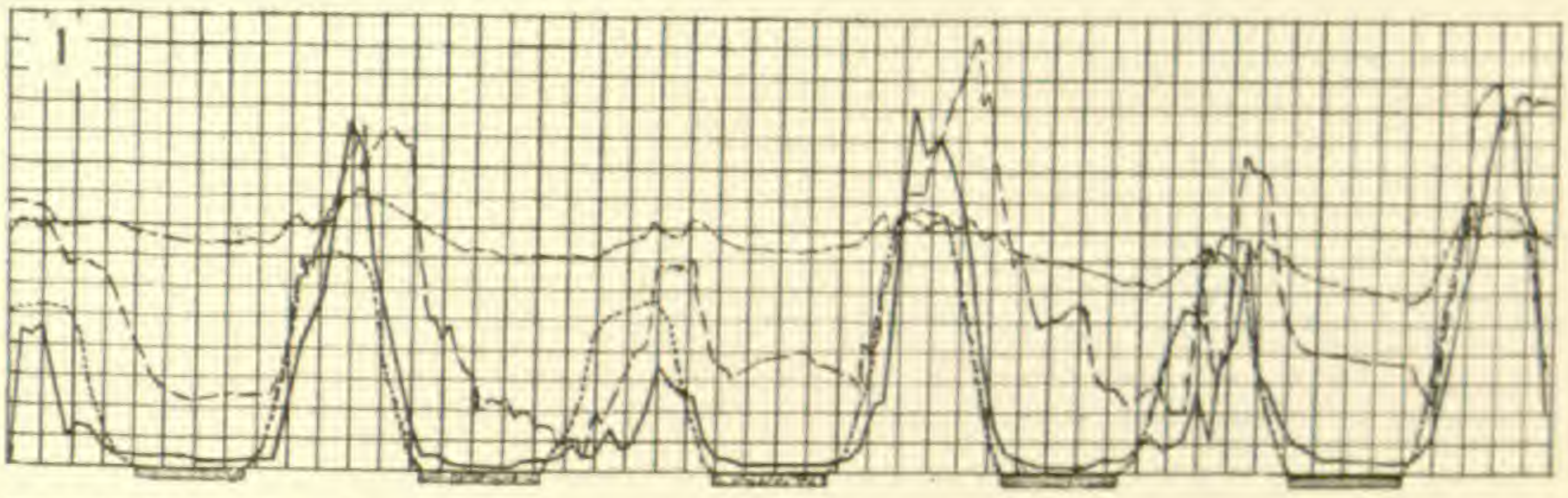
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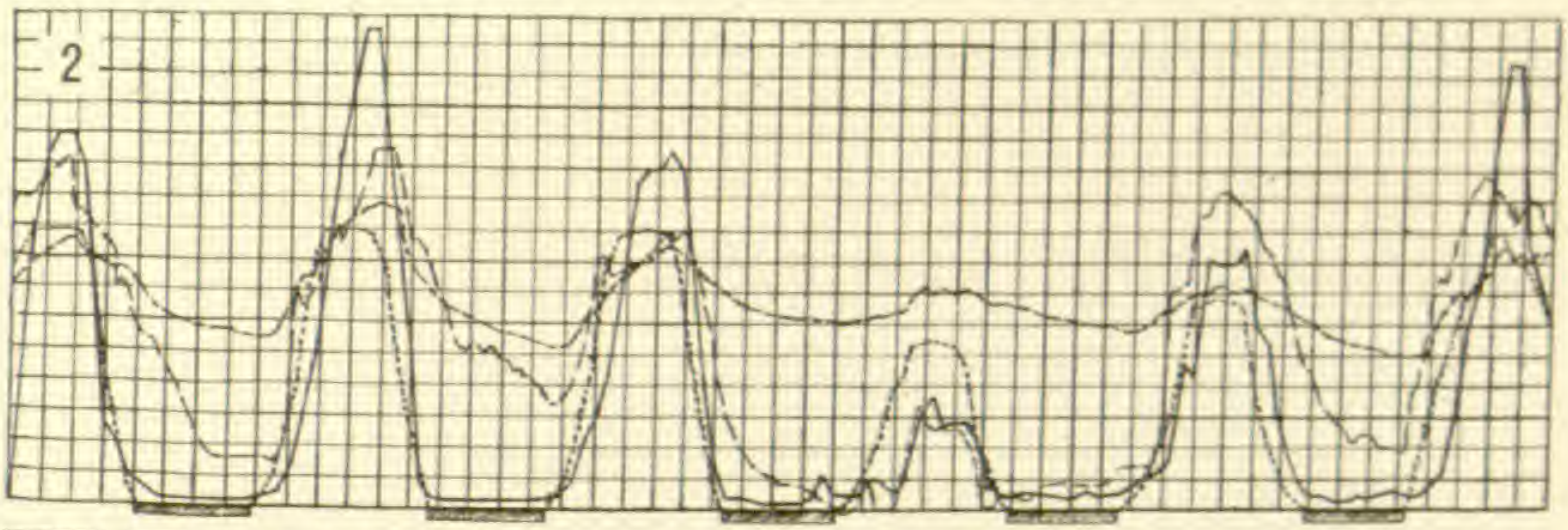
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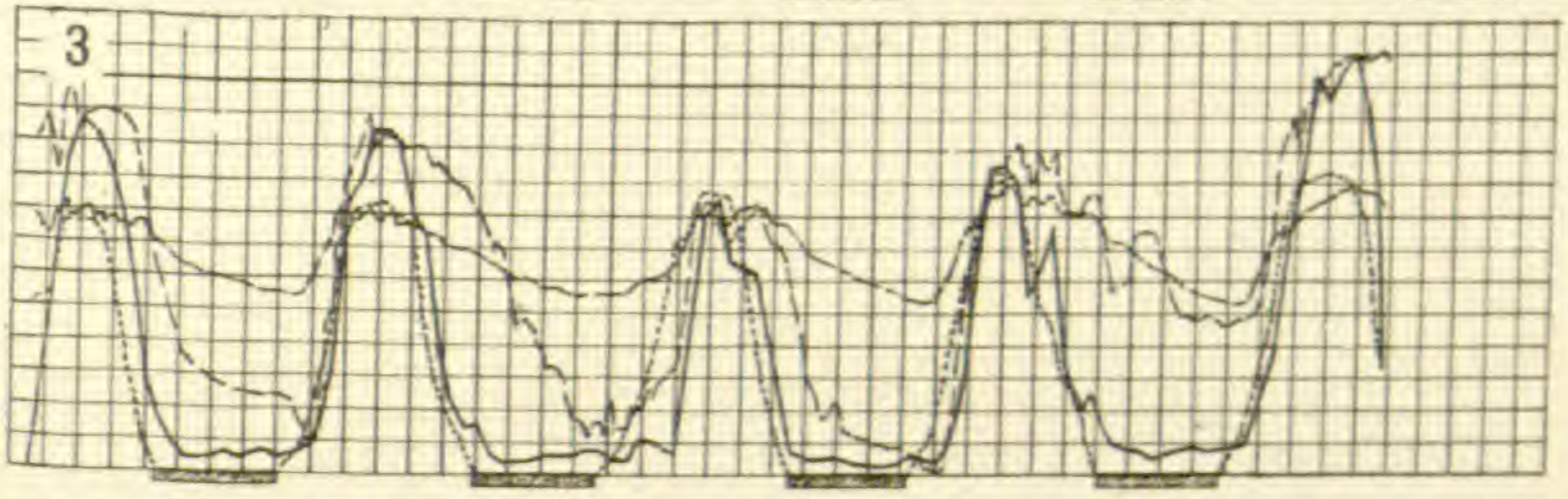
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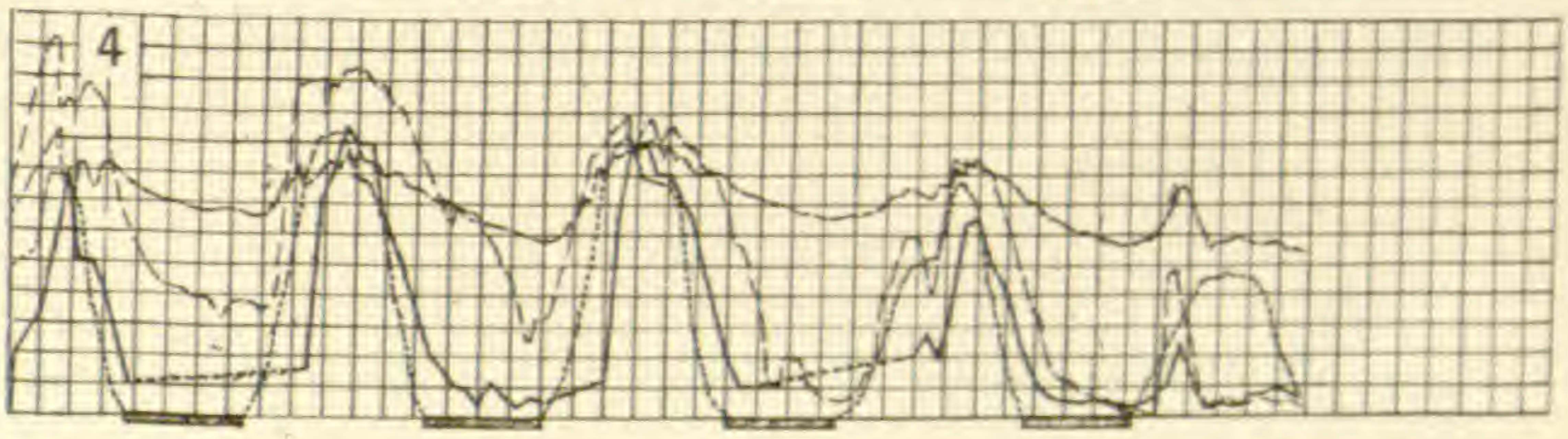
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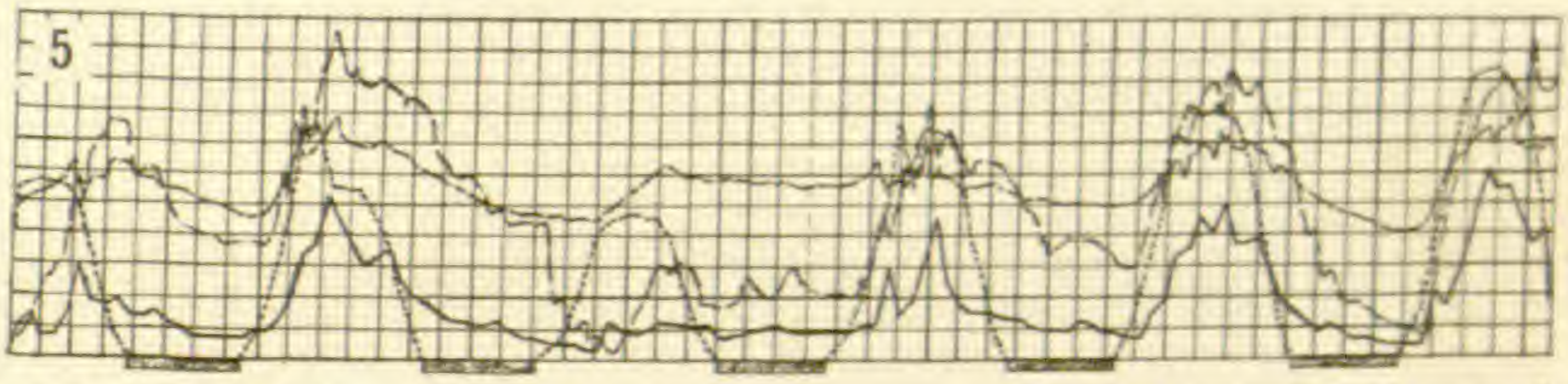
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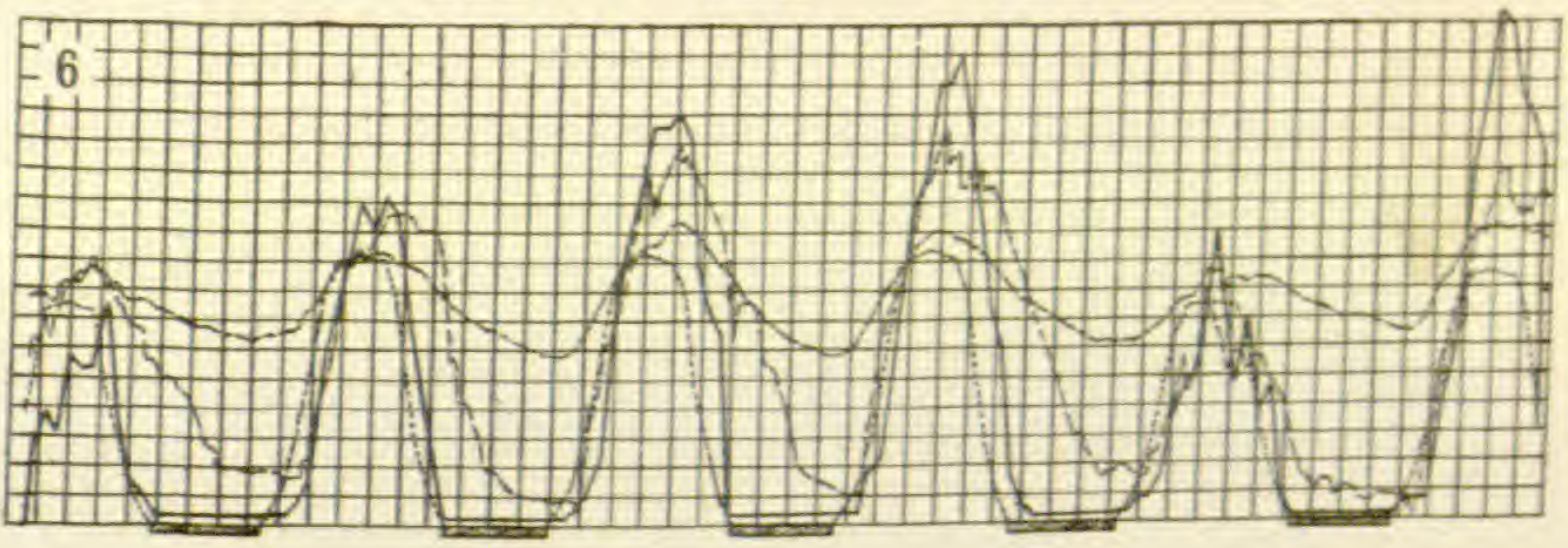
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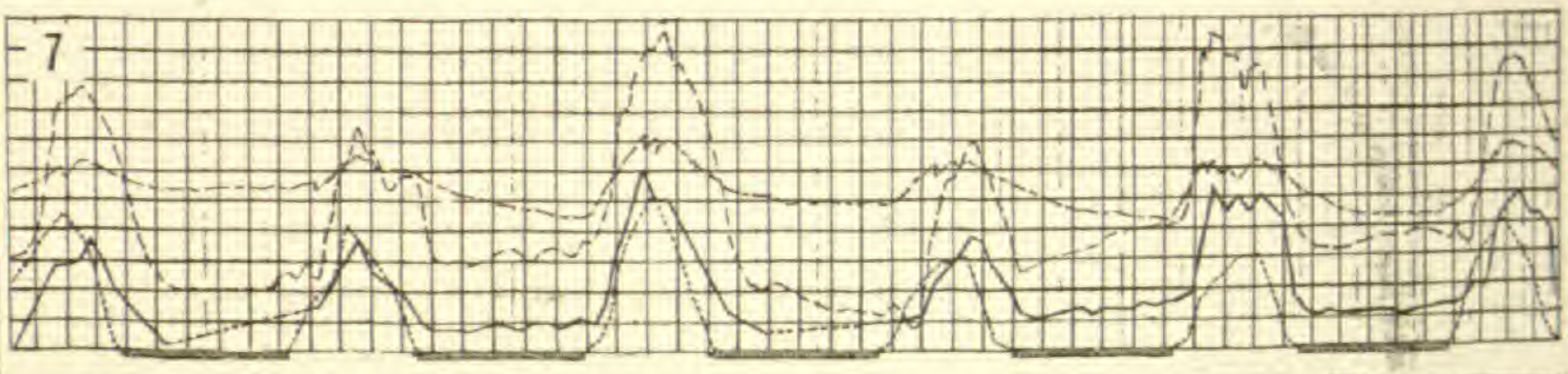


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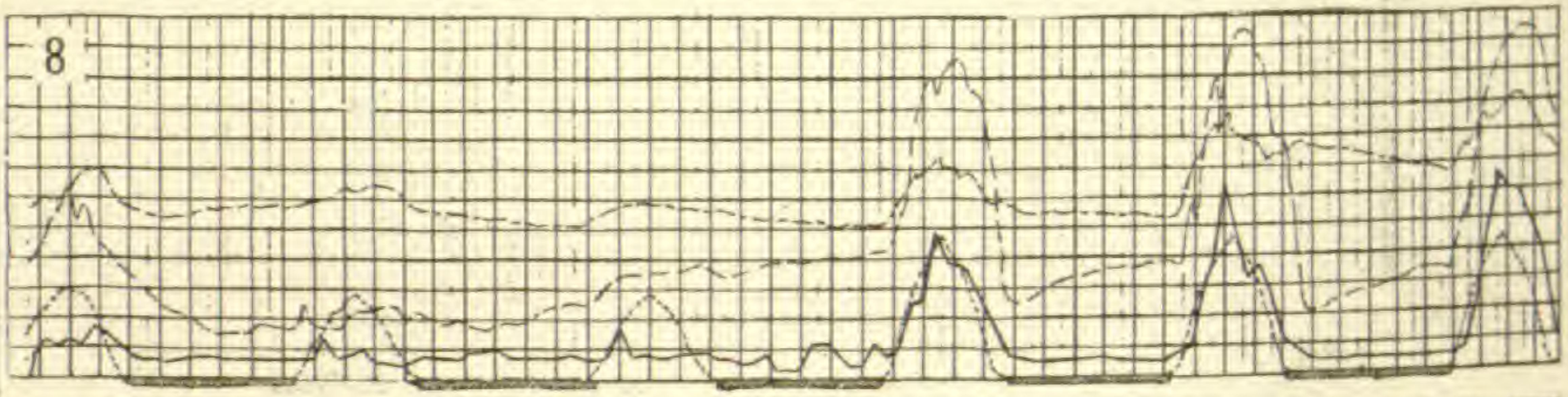


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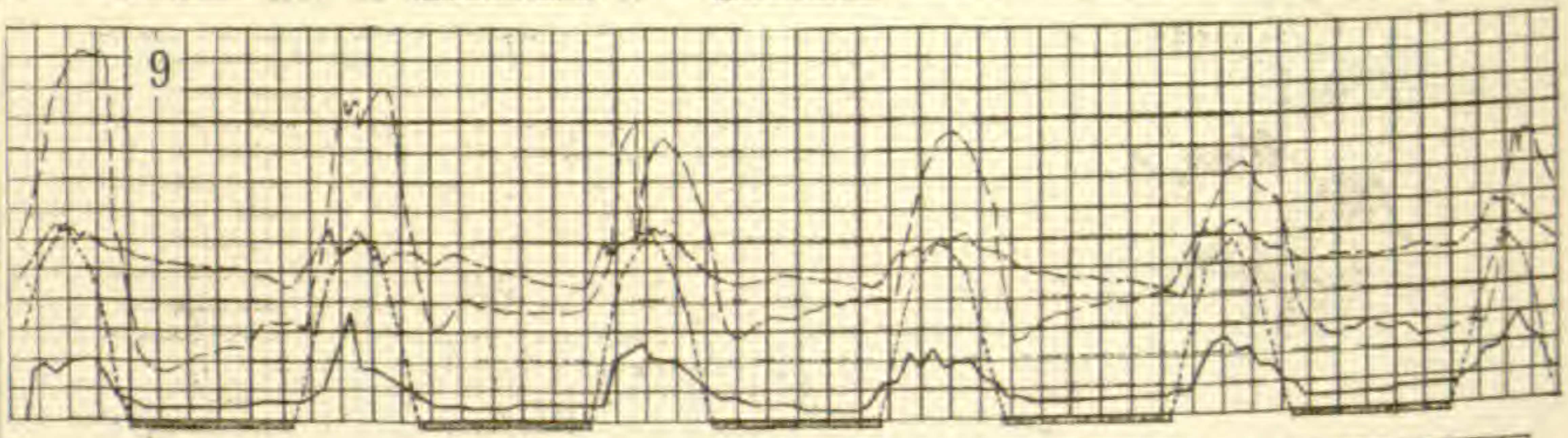


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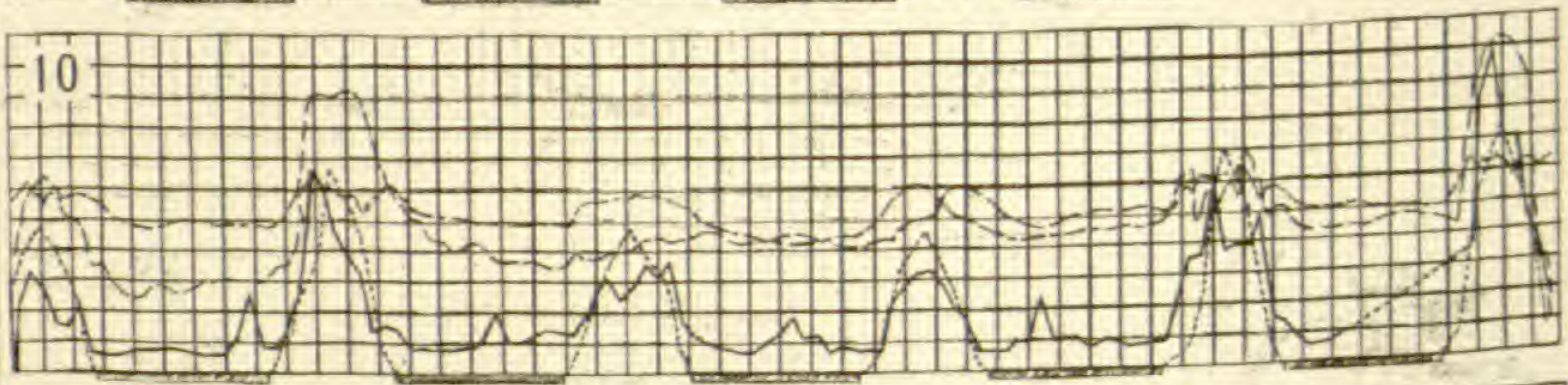


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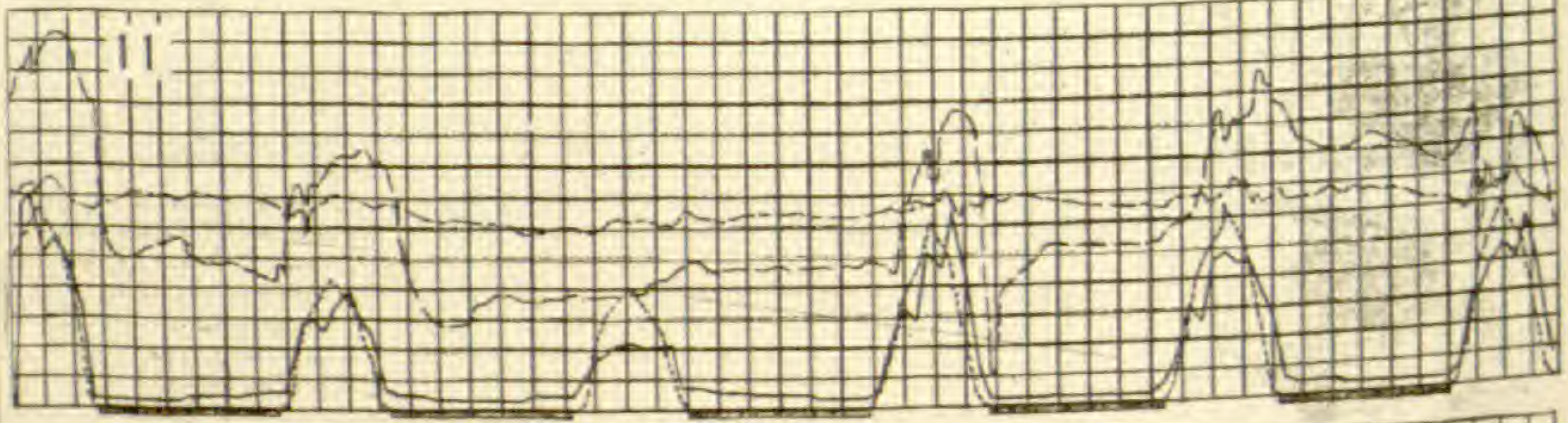


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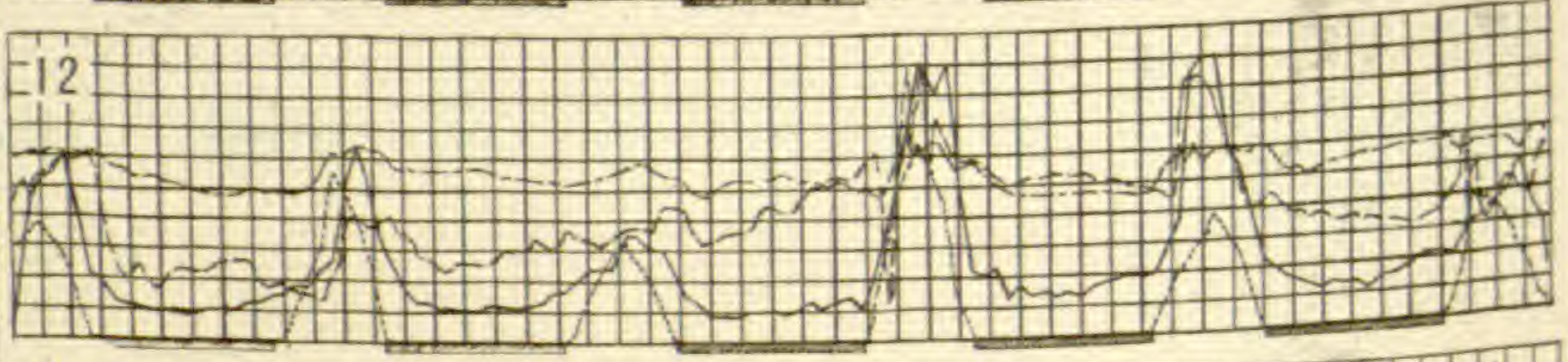

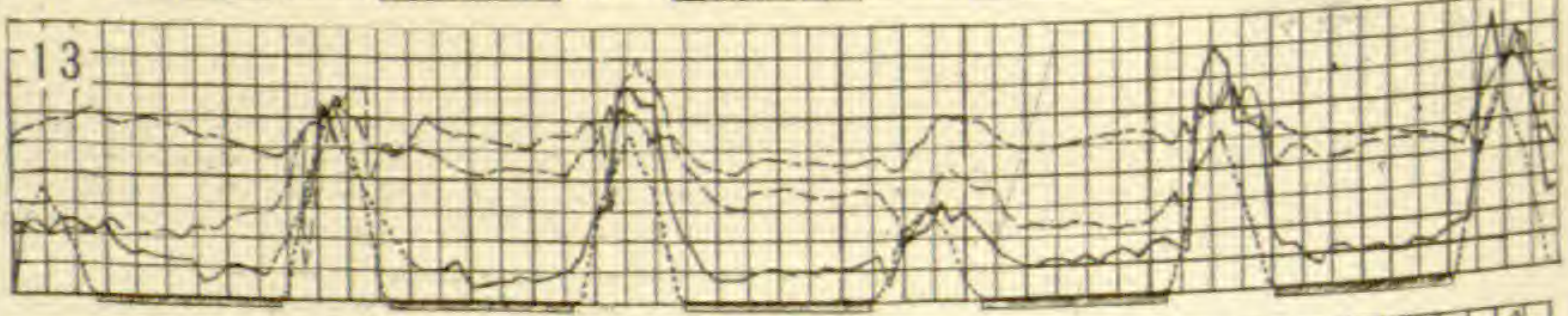


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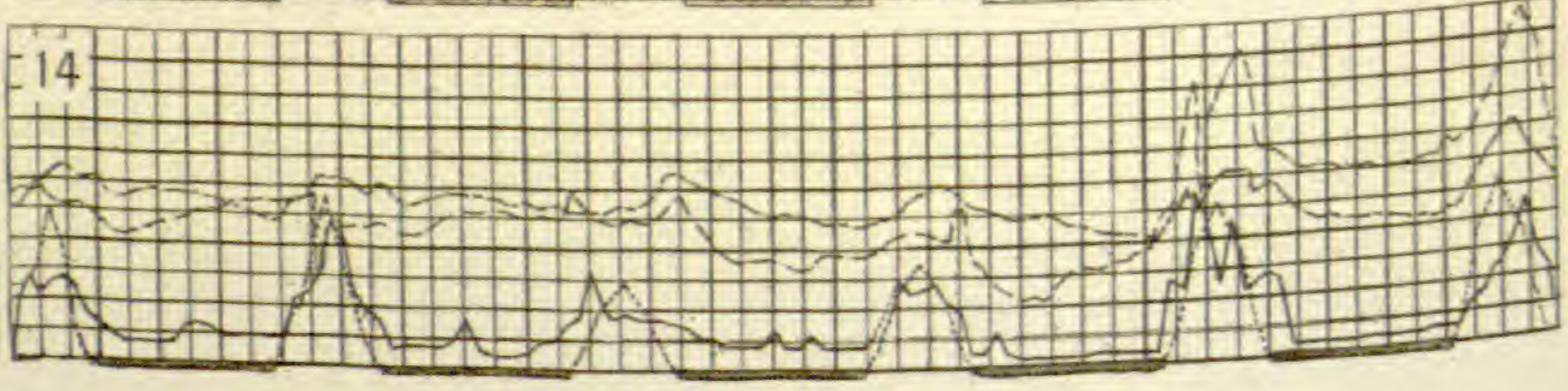

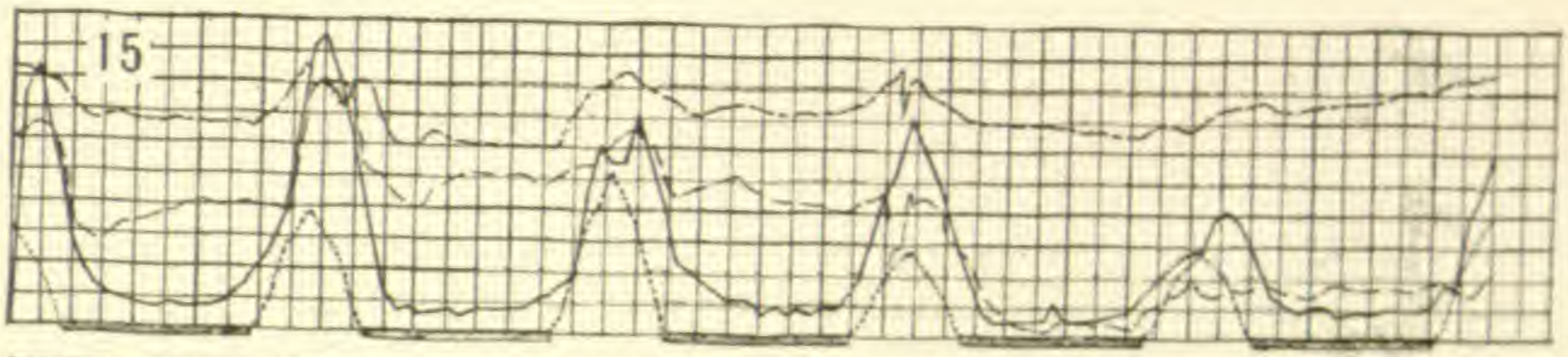


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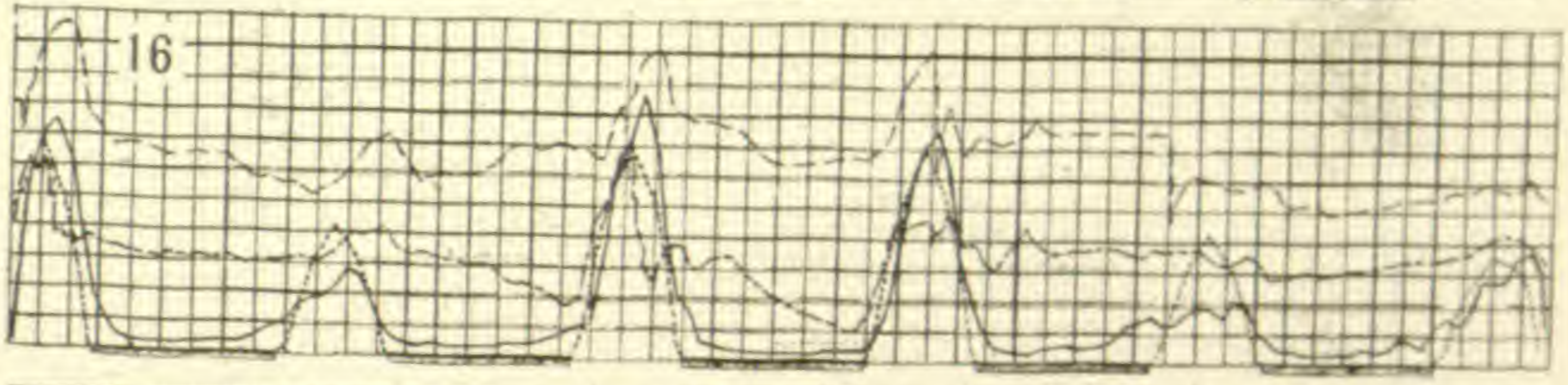


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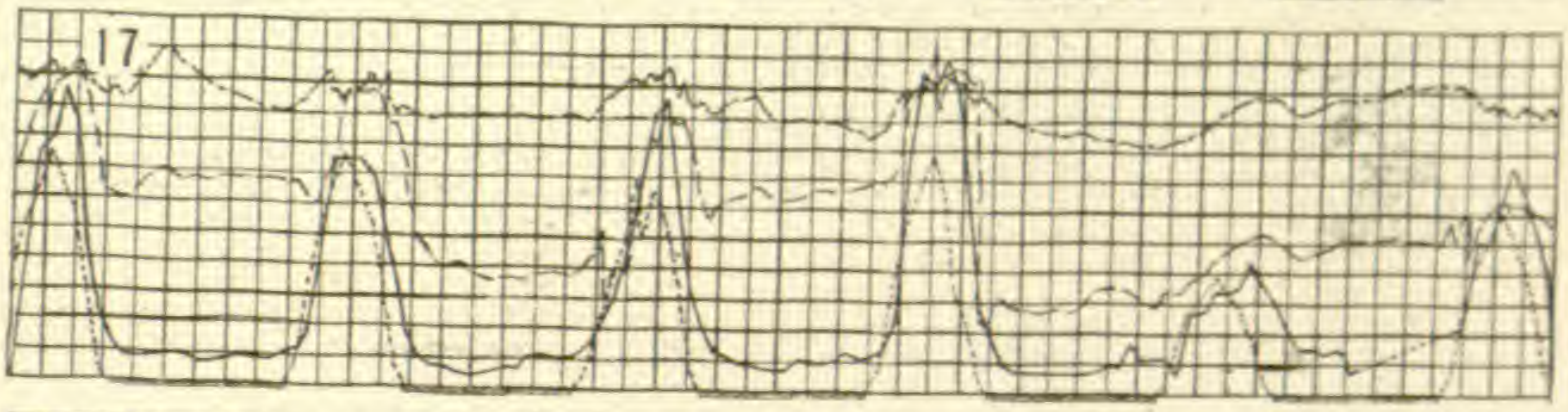


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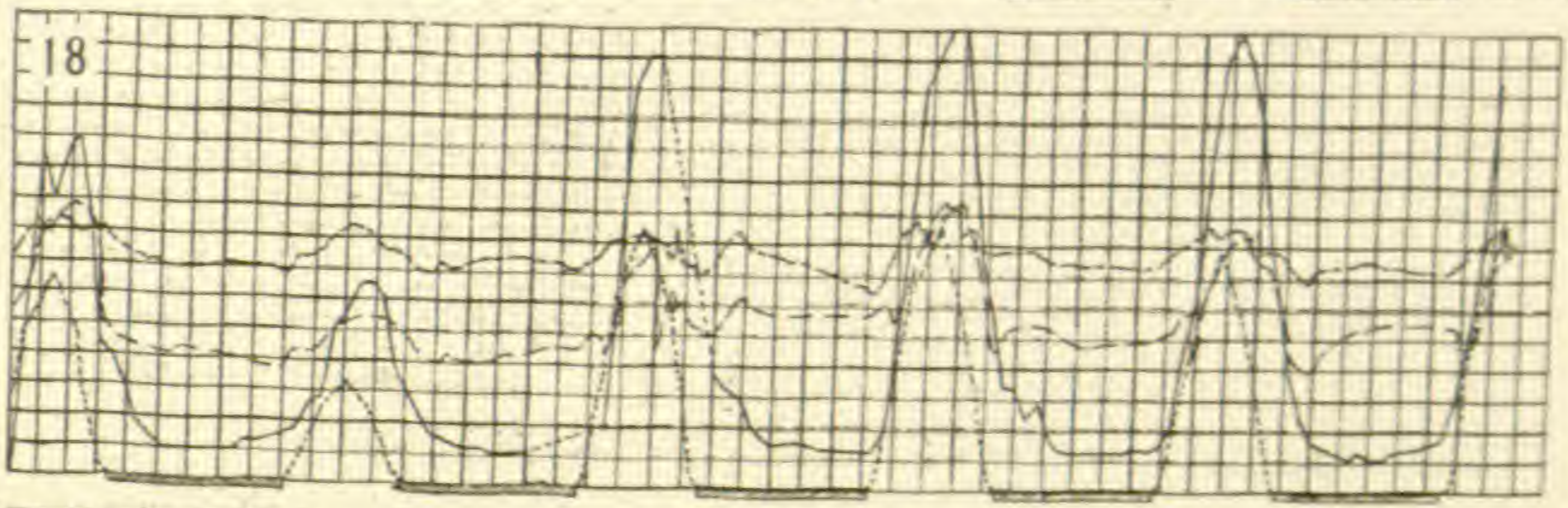


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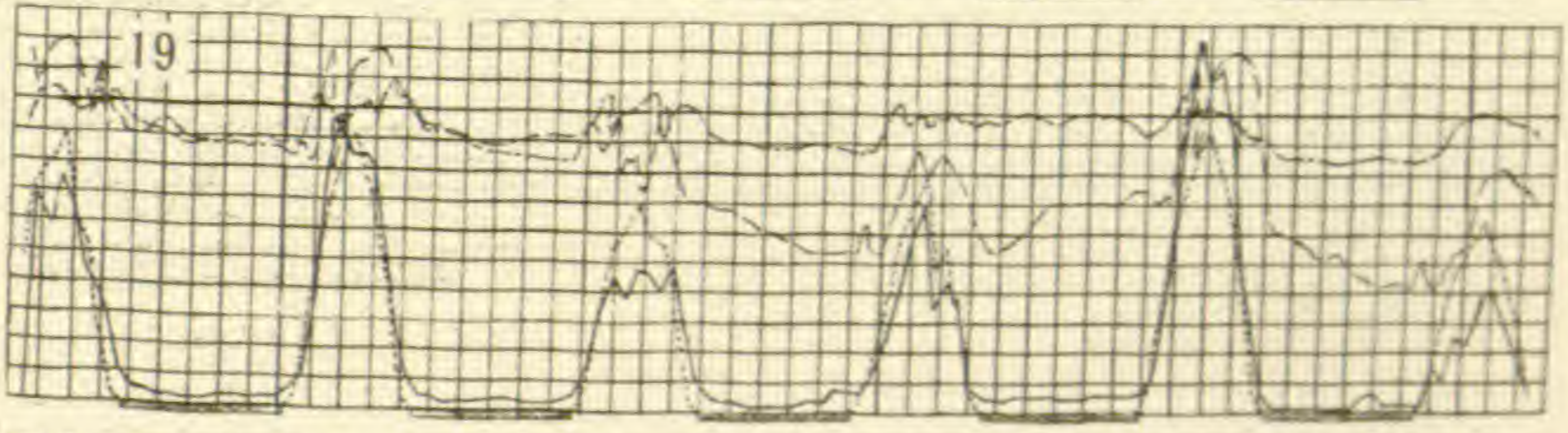


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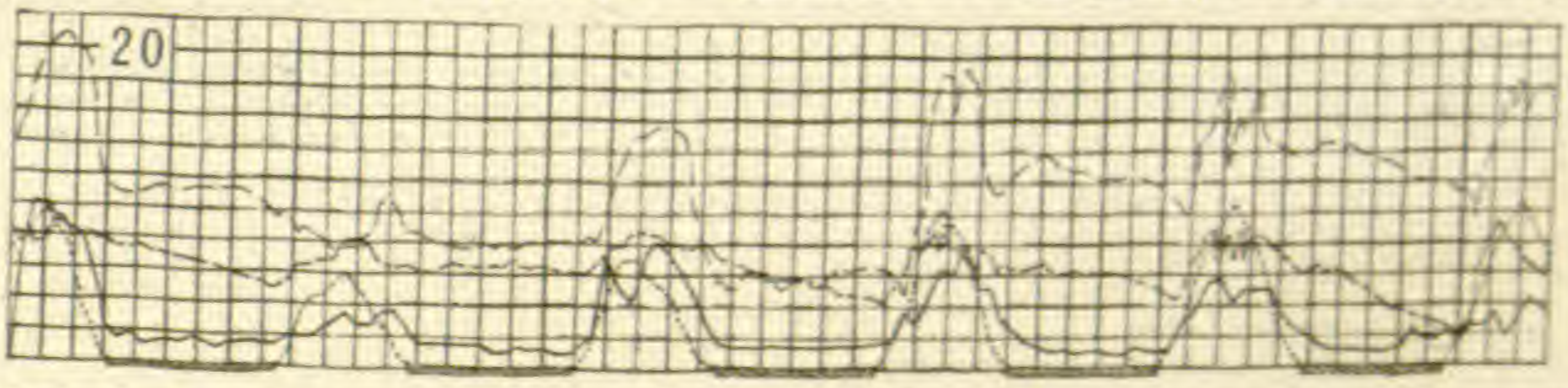


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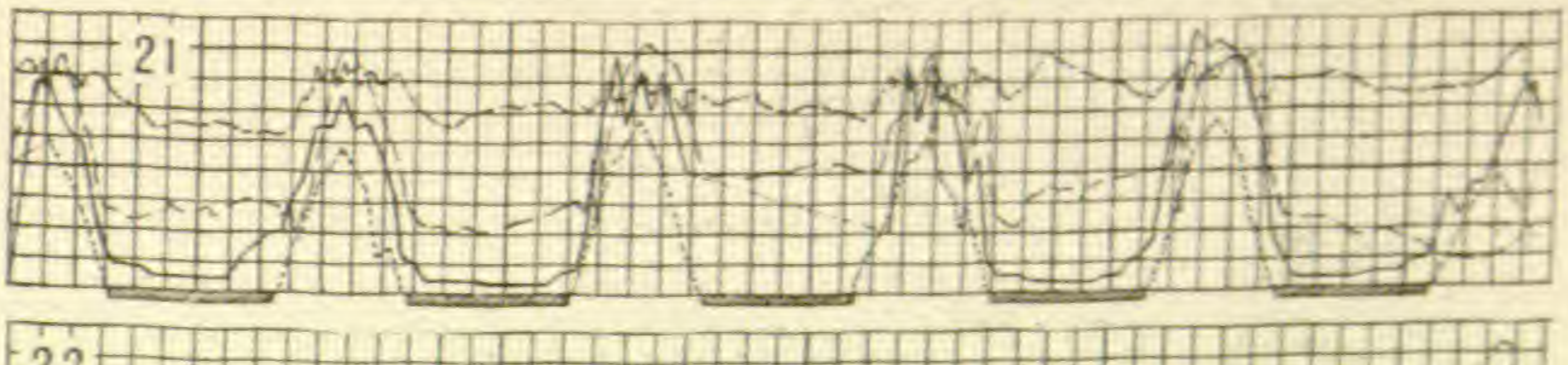


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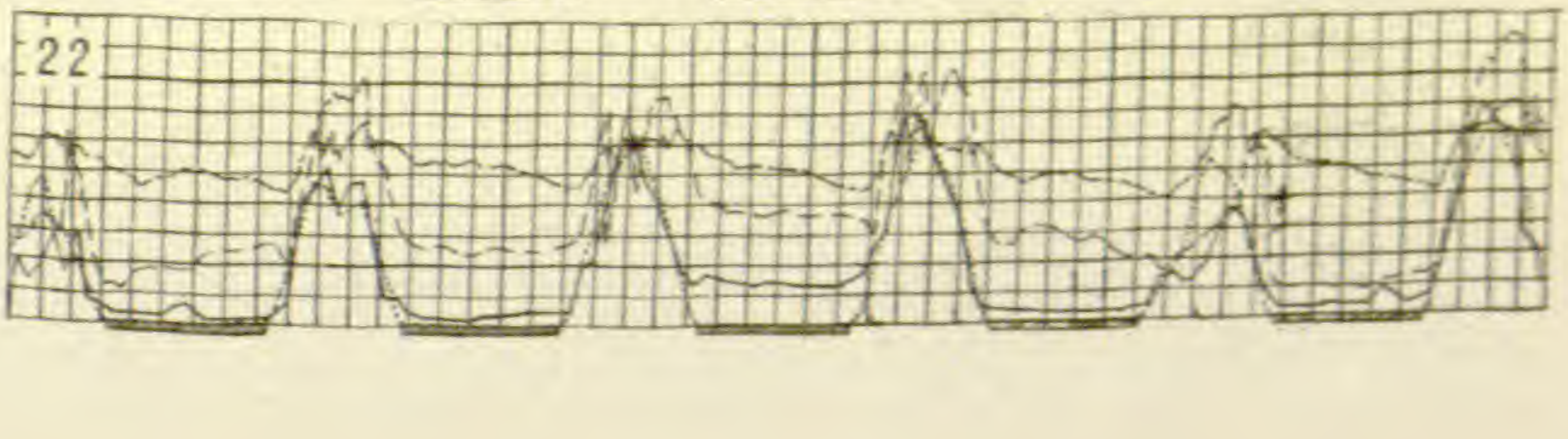


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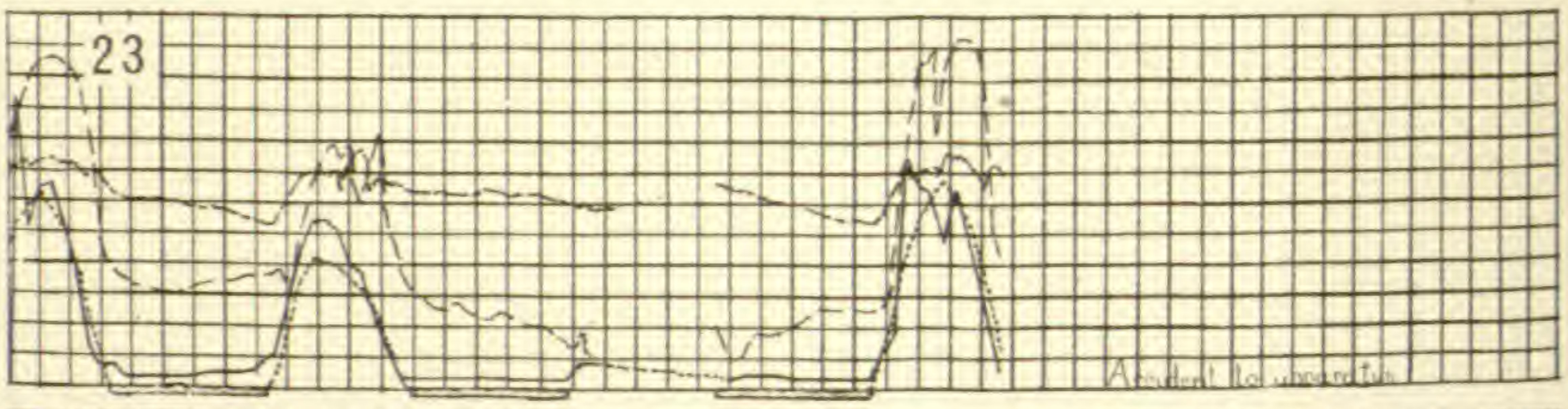
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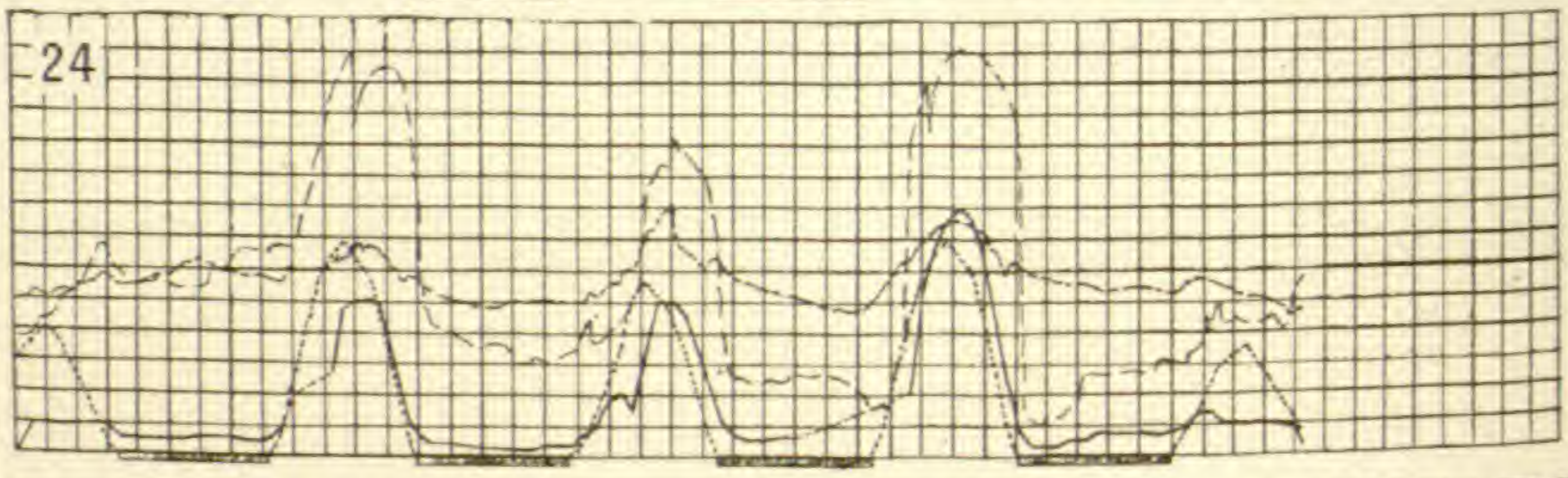
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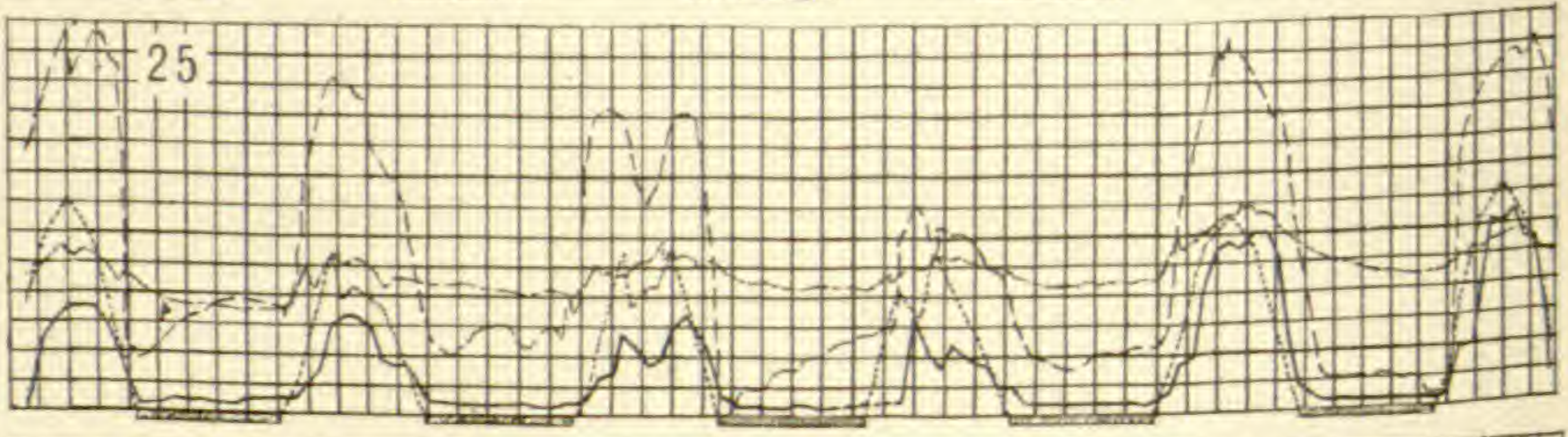
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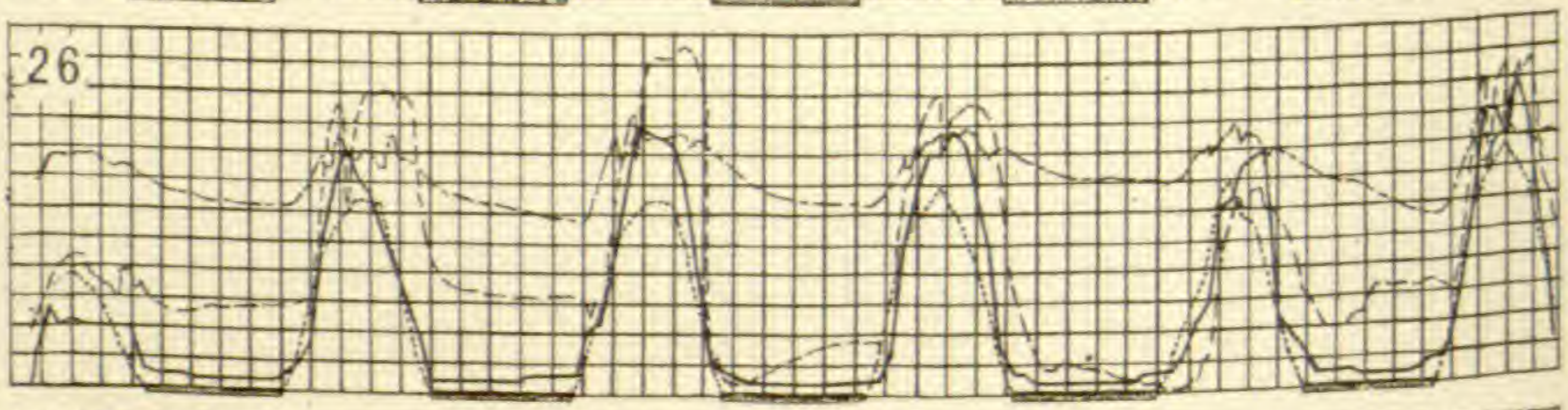
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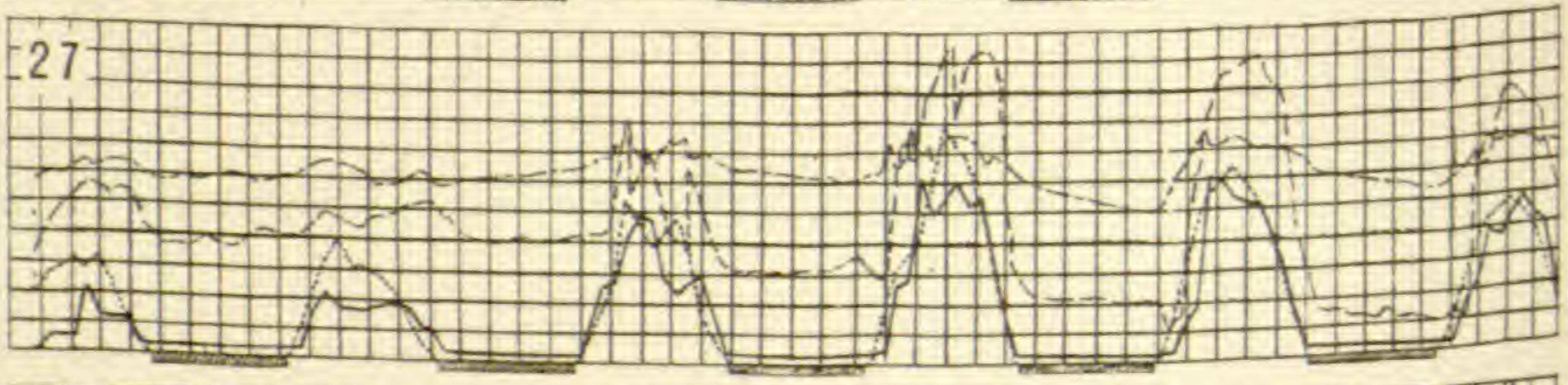
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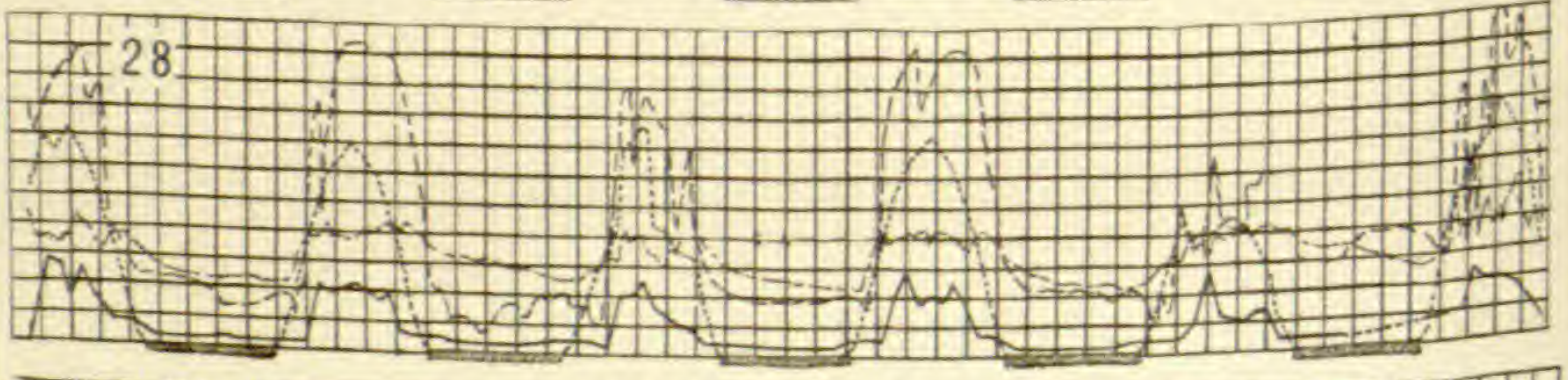
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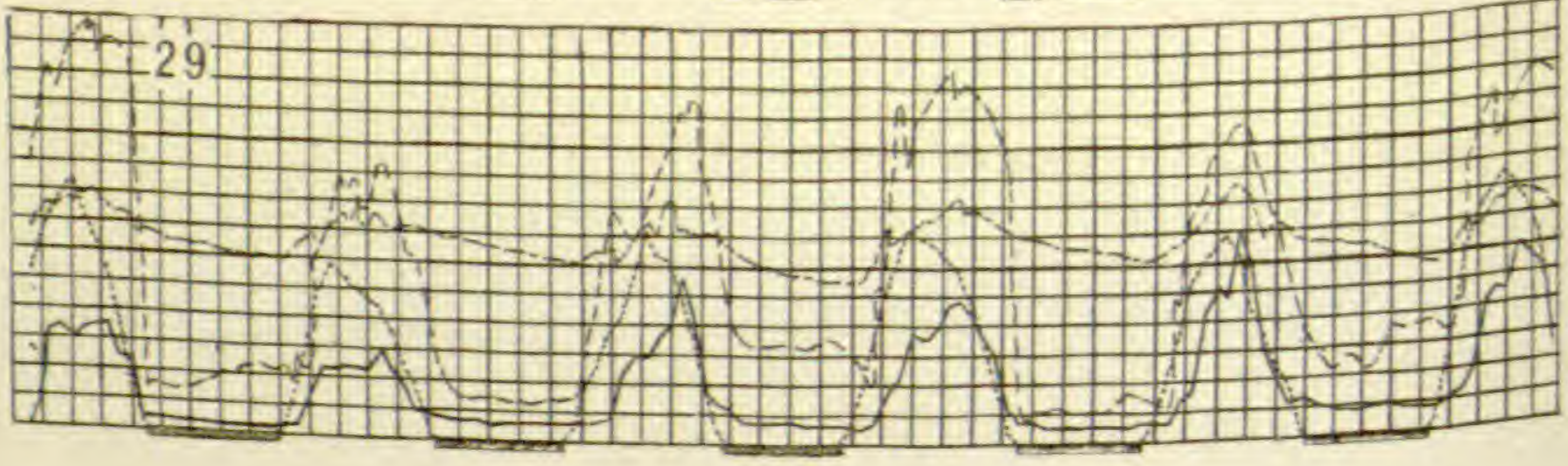
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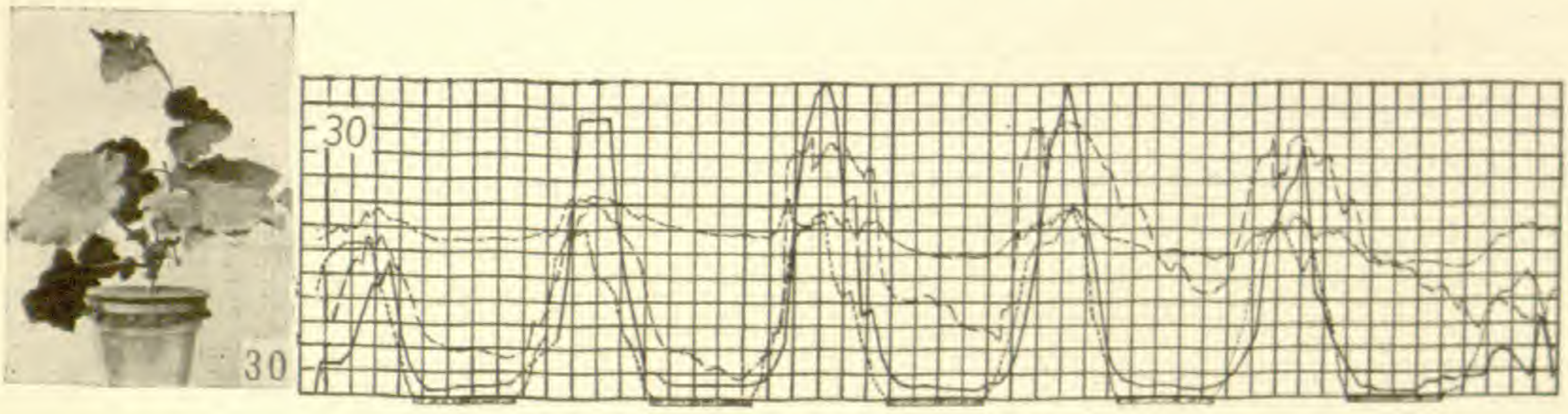

EXPLANATION OF GRAPHS

T=temperature — · · — · · —   D=dryness — — — —
L=light · · · · · · · ·   Tr=transpiration————

The values given for *T* should be read in the seventh vertical space from the base line; *D* in the fifth; *L* in the third; and *Tr* in the first.

1. *Zea Mays.* May 7–12. T 10–15; D 30–35; L 20–30; Tr 0–1.
2. *Lycopersicum esculentum.* May 13–18. T 15–20; D 30–35; L 20–30; Tr 0–1.
3. *Salvia involucrata.* May 20–25. T 10–15; D 35–40; L 20–30; Tr 0–1.
4. *Senecio Petasitis.* May 28—June 2. T 10–15; D 35–40; L 20–30; Tr 0–2.
5. *Cestrum elegans.* June 3–8. T 20–25; D 35–40; L 20–30; Tr 0–½.
6. *Ipomoea purpurea.* June 10–15. T 10–15; D 30–35; L 20–30; Tr 0–½.
7. *Euphorbia pulcherrima.* Oct. 22–27. T 20–25; D 30–35; L 20–30; Tr 0–1.
8. *Phaseolus vulgaris.* Oct. 29—Nov. 3. T 15–20; D 40–45; L 20–30; Tr 0–1.
9. *Impatiens Holstii.* Nov. 5–10. T 20–25; D 40–45; L 10–15; Tr 0–½.
10. *Ricinus communis.* Nov. 12–17. T 20–25; D 40–45; L 10–15; Tr 0–½.
11. *Abutilon striatum.* Nov. 19–24. T 15–20; D 30–35; L 10–15; Tr 0–½.
12. *Heliotropium peruvianum.* Nov. 26–31. T 20–25; D 40–45; L 10–15; Tr 0–1.
13. *Cineraria stellata.* Dec. 6–12. T 20–25; D 50–55; L 10–15; Tr 0–1.
14. *Primula sinensis.* Dec. 13–19. T 20–25; D 40–45; L 10–15; Tr 0–½.
15. *Chrysanthemum frutescens.* Jan. 14–19. T 10–15; D 50–55; L 10–15; Tr 0–2.
16. *Pelargonium zonale.* Jan. 21–26. T 30–35; D 40–45; L 10–15; Tr 0–2.
17. *Fuchsia speciosa.* Jan. 28—Feb. 2. T 0–5; D 40–45; L 10–15; Tr 0–1.
18. *Tropaeolum majus.* Feb. 4–9. T 10–15; D 45–50; L 10–15; Tr 0–1.
19. *Pelargonium domesticum.* Feb. 11–16. T 0–5; D 30–35; L 10–15; Tr 0–1.
20. *Coleus Blumei* var. Feb. 18–23. T 50–55; D 20–25; L 20–30; Tr 0–1.
21. *Ficus elastica.* Feb. 25—Mar. 2. T 15–20; D 55–60; L 20–30; Tr 0–½.
22. *Pelargonium peltatum.* Mar. 4–9. T 20–25; D 50–55; L 20–30; Tr 0–1.
23. *Vicia Faba.* Mar. 11–20. T 15–20; D 40–45; L 20–30; Tr 0–½.
24. *Senecio mikanioides.* Mar. 18–23. T 20–25; D 30–35; L 10–15; Tr 0–1.
25 *Helianthus annuus.* Mar. 25–30. T 25–30; D 35–40; L 20–30; Tr 0–2.
26. *Lupinus albus.* Apr. 1–6. T 10–15; D 40–45; L 20–30; Tr 0–½.
27. *Hedera Helix.* Apr. 8–13. T 15–20; D 40–45; L 20–30; Tr 0–½.
28. *Tradescantia zebrina.* Apr. 15–20. T 30–35; D 40–45; L 20–30; Tr 0–½.
29. *Begonia argentea guttata.* Apr. 22–27. T 30–35; 15–20; DL 20–30; Tr 0–½.
30. *Cucurbita Pepo.* Apr. 29—May 4. T 15–20; D 30–35; L 20–30; Tr 0–1.

Briefly they bring out these facts: there are two daily extremes, a maximum loss around noon when the sunlight is most intense, heat usually the greatest, moisture least in the atmosphere, but a good supply of water in the soil around the roots; the minimum loss occurs some time during the night when the temperature is low, the atmospheric moisture approaches saturation, the darkness is complete, and in most plants the stomata are closed. The curve from 8 to 9 in the morning sometimes suddenly drops or rises. This is due either to the time lost when the plant is watered and rebalanced or to the falling of sunshine directly upon the plant. The drop in the curves between February and March 18 just after noon is due to the shading of the plant from the direct afternoon sun by an intervening house.

The effects of cool, cloudy, moist days are well brought out in the curve of *Phaseolus vulgaris*, where such conditions prevailed the first half of the week, tending to check transpiration; while the brighter, warmer, drier, latter half of the week increased the loss of water. The same differences are seen equally well in the curves of *Pelargonium domesticum* and *P. peltatum*, Fuchsia, and Tropaeolum; and are more strikingly shown in those of *Senecio mikanioides*, *Lupinus albus*, *Zea Mays*, *Senecio Petasitis*, *Cucurbita Pepo*, *Lycopersicum esculentum*, and *Abutilon striatum;* but the last-named plant and Chrysanthemum were at no time in brilliant sunlight.

In general the transpiration curve supplements the tabulated results, showing in addition the effects of physical conditions. The important fact brought out by the curves as a whole is the extreme sensitiveness of transpiration to even slight changes in external conditions, a fact already mentioned above in connection with the variations shown under "standard" conditions. So great, indeed, is this sensitiveness that it seems out of all proportion to the direct physical changes and suggests the possibility that the relation between conditions and transpiration is not purely physical, as it is apparently now considered by some students, but is indeed one which involves the action of the conditions as stimuli. But this is a separate matter and I have been concerned merely with transpiration as a fact, quite regardless of its explanation. Furthermore, I have not tried to separate the influence of the conditions nor to show, when several cooperate, which produces the greatest effect. I have taken them as they came; their separation is a separate investigation.

We consider now the results of this study from the practical point of view of the teacher, as to which plants are best for the demonstration of transpiration. While the columns designated "M^2HG average" give some plants a greater average transpiration, the columns of "M^2HG minimum and maximum" really offer a better choice of material, because they show the great range possible for each plant. *Helianthus annuus* stands first in amount of transpiration, but it is open to objection in that it is not kept in greenhouses and must be grown expressly several weeks before needed for use in the classroom· *Vicia Faba*, *Lupinus albus*, *Lycopersicum esculentum*, *Ipomoea purpurea*, and *Cucurbita Pepo*, although they show a high rate of transpiration, are open to the same objection. The first choice falls, then, in order of respective excellence to *Chrysanthemum frutescens*, *Tropaeolum majus*, *Pelargonium domesticum*, *Fuchsia speciosa*, *Senecio Petasitis*, *Senecio mikanioides*, *Pelargonium zonale*, *Heliotropium peruvianum*, and *Pelargonium peltatum*. They are best for two reasons: their first value lies in the fact that they lose large amounts of water; the second, and no less important, is that in addition to their being easily obtained at any time of year from the greenhouse, they are grown in many homes. An objection to *Euphorbia pulcherrima* and *Impatiens Holstii*, moderate transpirers, is that they lose many leaves just at maturity; this is true also of the heliotrope. Of least value are *Primula sinensis*, *Coleus Blumei*, *Hedera Helix*, *Cestrum elegans*, *Begonia argentea*, and *Tradescantia zebrina*, which, though common, have a very low rate of transpiration.

In addition to the value given by the tables for the different kinds of plants, it will be of some interest to note the average transpiration for these plants taken collectively. The result may be considered a general expression of the amount of transpiration of ordinary plants growing in any greenhouse. This quantity is 48.732 or in round numbers about 50 grams per hour per square meter of surface for the day time, and 8.898, or in round numbers 10 grams for the night—about five times as much on the average per hour per square meter by day as by night.

NORTHAMPTON, MASS.

BRIEFER ARTICLES

LUCIEN MARCUS UNDERWOOD

(WITH PORTRAIT)

The editors of the BOTANICAL GAZETTE wish to make this brief record of the life and work of a distinguished American botanist and an intimate personal friend. The papers prepared for the memorial meeting of the Torrey Botanical Club, held January 29, 1908, have been published in the *Bulletin* of that club (**35**:1–43. 1908), so that a full account is not necessary here.

Professor UNDERWOOD was born October 26, 1853, at New Woodstock, N. Y., and died at Redding, Conn., November 16, 1907. He graduated at Syracuse University in 1877, and held the following college and university positions: Hedding College (1880), Illinois Wesleyan University (1880–1883), Syracuse University (1883–1890), De Pauw University (1890–1895), Alabama Polytechnic Institute (1895–1896), Columbia University (1896–1907).

His chief work was with the Pteridophytes, and the repeated editions of *Our native ferns* bore testimony to his early activity and to the popularity of the book. This work laid the foundation for the larger and more serious interest in the group which bore abundant fruit in his maturer years. The Hepaticae were also included in the range of his professional interest; and the Fungi also shared in his contributions. His bibliography, as published in the memorial proceedings referred to, includes 212 titles, 188 of which are botanical; and among these 78 deal with Pteridophytes, 31 with Hepaticae, and 29 with Fungi.

Great as is the loss to American botany, the personal loss will be felt still more keenly by his colleagues. He was eminently a companionable man, and at every botanical gathering which included him he was a center of sprightly conversation and good feeling. These qualities, and his untiring and unselfish devotion to his work and to his students, developed for him an amount of personal affection and loyalty that was his chief possession. His place in American botany was sufficiently unique to remain vacant; and his personality will be still more impossible to duplicate.

A CONVENIENT TRAVELING BALANCE

(WITH TWO FIGURES)

In carrying out certain lines of investigation involving more or less travel, or the establishment of temporary stations remote from well-equipped laboratories, the lack of a compact and readily transportable balance, of a fair degree of sensitiveness, often seriously hampers the progress of the work. The ordinary balance inclosed in its glass case is too cumbrous and fragile to be readily transported, yet no dependable weighing can be made except upon a suitable balance properly protected.

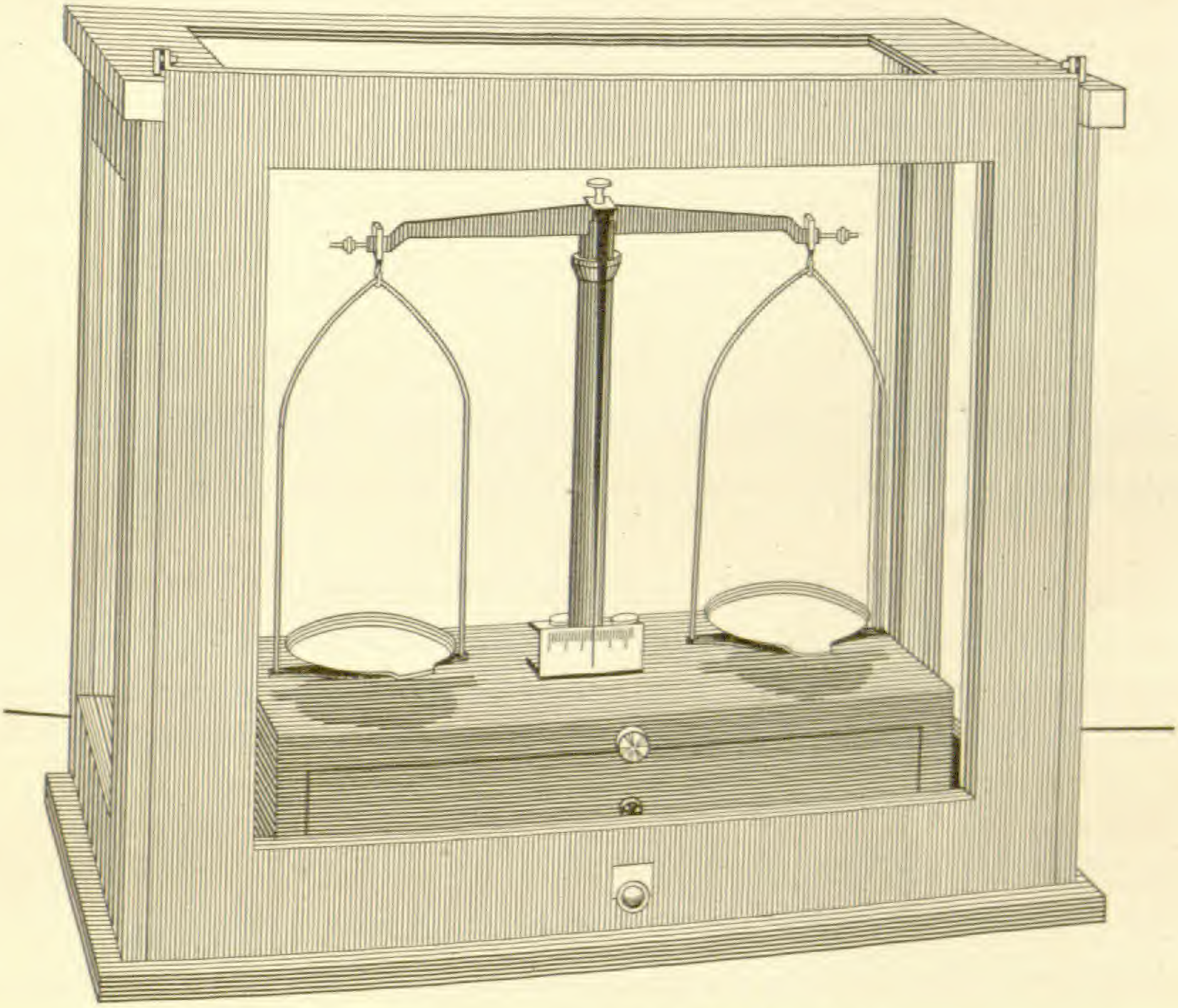

FIG. 1.— Balance set up within the closed case.

In preparing an outfit of apparatus for field use I have devised a convenient form of "knock-down" balance with collapsible case, which in great measure solves the question of a practicable field balance.

The balance proper and the case are entirely independent. From a reliable instrument maker I ordered a good type of laboratory balance fitted with the usual accessories but without the glass case. The pillar is fastened to the box with screws having milled heads and is readily removable. All the metal parts of the balance can be detached and packed safely in the drawer of the box.

The case for this balance is made in six sections, of which those forming the ends are secured in place on the base by dowel pins. The joints of the case are made dust proof by means of a tongue-and-groove fitting. The door is hinged at the upper corners by means of pivot hinges and swings outward. After all the sections are in place, complete stability is secured by means of small hooks and screw-eyes which lock the various parts together. Instead of glass, clear celluloid is used, which renders the case practically non-breakable.

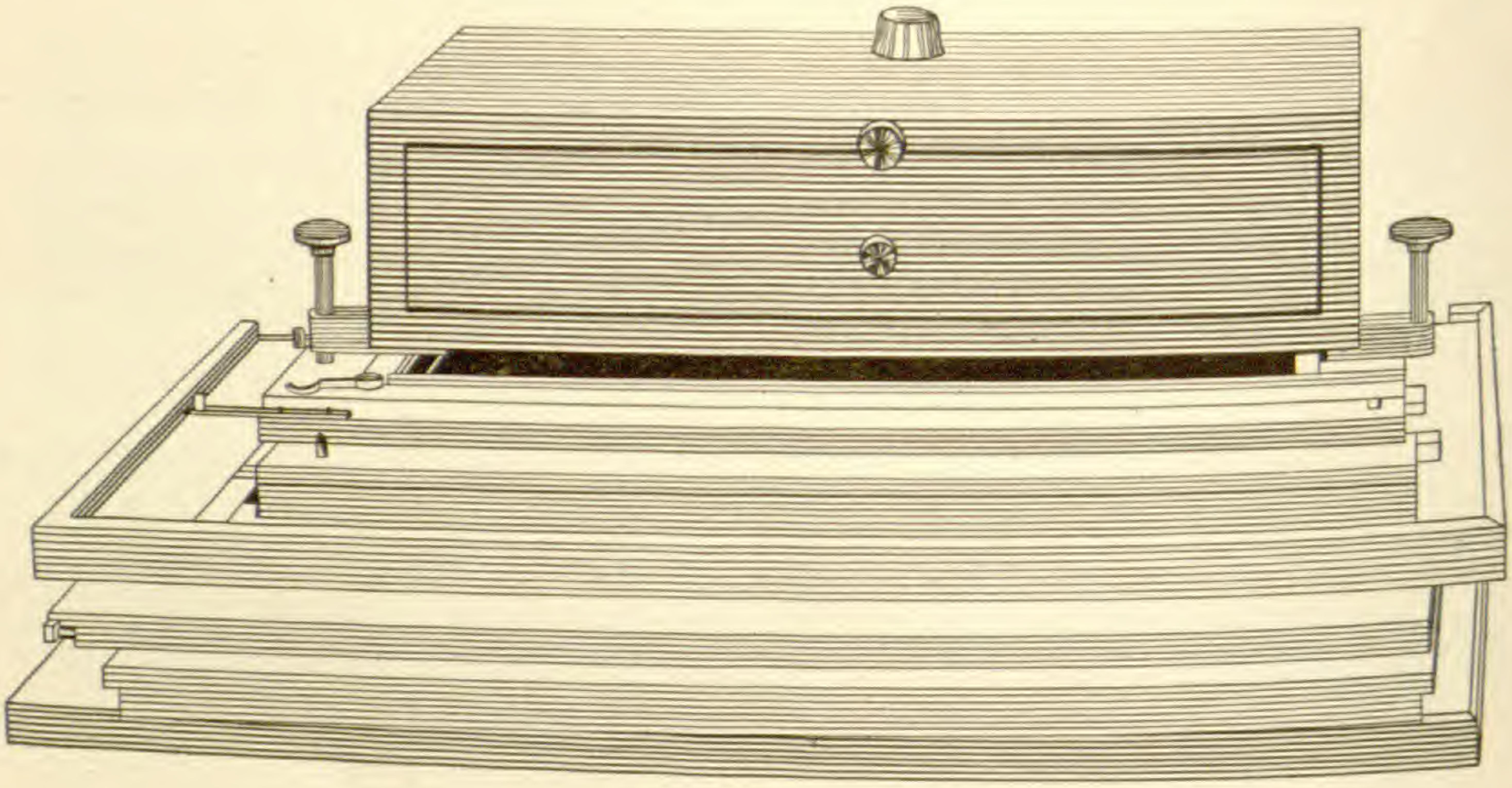

FIG. 2.—Case collapsed and balance packed.

Fig. 1 shows the balance set up within the case which is closed; *fig. 2* shows the case collapsed and balance packed. The total weight of balance and case is approximately ten pounds, and in the collapsed form may readily be carried in a trunk or even in a suit case.—W. W. STOCKBERGER, *Bureau of Plant Industry, Washington, D. C.*

THE TOXIC ACTION OF CERTAIN ORGANIC PLANT CONSTITUENTS.—A CORRECTION

THE EDITORS regret that in the article by SCHREINER and REED in the February number of this journal, vol. 45, pages 80 and 90, *figures 2* and *6*, illustrating the action of cholin and of cumarin, were interchanged. All of the figures appear without legends. These have now been supplied by the authors and are as follows:

FIG. 1.—Wheat grown in: (1) Freshly prepared solution of tyrosin, 16 parts per million; (2) discolored solution of tyrosin, originally containing 16 parts per million.

FIG. 2.—(Applies to block printed as *fig. 6*, p. 90.) Wheat grown in: (1) Control in pure distilled water; (2) solution of cholin, 1000 parts per million; (3) 500 parts; (4) 100 parts; (5) 25 parts; (6) 5 parts; (7) 1 part.

FIG. 3.—Wheat grown in: (1) Solution of pyrocatechin, 1000 parts per million; (2) 500 parts; (3) 100 parts; (4) 25 parts; (5) 1 part; (6) control in pure distilled water.

FIG. 4.—Wheat grown in: (1) Solution of phenol, 250 parts per million; (2) resorcin, 250 parts; (3) phloroglucin, 250 parts; (4) phenol, 100 parts; (5) resorcin, 100 parts; (6) phloroglucin, 100 parts.

FIG. 5.—Wheat grown in: (1) Solution of vanillin, 1000 parts per million; (2) 500 parts; (3) 100 parts; (4) 25 parts; (5) 1 part; (6) control in pure distilled water.

FIG 6.—(Applies to block printed as *fig. 2*, p. 80.) Wheat grown in: (1) Control in pure distilled water; (2) solution of cumarin, 1 part per million; (3) 25 parts; (4) 50 parts.

FIG. 7.—Wheat grown in: (1) Solution of esculin, 1000 parts per million; (2) 500 parts; (3) 100 parts; (4) 25 parts; (5) 1 part; (6) control in pure distilled water.

CURRENT LITERATURE

BOOK REVIEWS

Chemistry of the higher fungi

Investigations on the chemistry of fungi began about a century ago, and have been prosecuted with various aims. Sometimes the endeavor has been to isolate unknown substances, and especially poisonous or medicinal ones; sometimes to ascertain the nutritive value of edible species; and sometimes to contribute to the general knowledge of this group. Though the separate studies have been numerous, hardly more than a beginning has yet been made, as shown by the fact that out of the immense number of species, the chemistry of only four is known with even approximate completeness.

ZELLNER, who himself has contributed much to the subject, has collated the present data in a monograph of some 250 pages.[1] He excludes from his consideration, by the adjective "higher," the bacteria, molds, and yeasts, recognizing that this is nothing more than a convenient discrimination. The matter is not arranged by species or groups of fungi, but by groups of substances, such as mineral constituents, fats, alcohols, amino-acids, tannins, coloring matters, toxins, etc. The largest sections are those which treat of fats, bases, carbohydrates, and enzymes. To compensate for the disadvantages of this mode of presentation, the author furnishes a complete index of the names of species mentioned, which unfortunately he has separated from the index of substances mentioned. While an index is a good thing, two are never better than one.

In the way of generalizations the author gathers together, at the close of his volume, the information in regard to the four species, whose composition has been fairly determined. It may be interesting to know that these are *Amanita muscaria* L., *Polyporus officinalis* Fr., *Claviceps purpurea* Tul., and *Aethalium septicum* L. He also attempts to distinguish by their chemical characteristics the following groups, whose composition is dependent upon the substratum: (1) saprophytes (living in humus), (2) coprophytes, (3) wood dwellers, partly saprophytic, partly parasitic, (4) plant parasites, (5) animal parasites.

Regarding the higher fungi as a whole, ZELLNER notes these chemical peculiarities: the cell membrane consists chiefly of chitin, or an allied substance, fungin, true cellulose and true wood material never having been found in fungi; they likewise contain no starch, but dextrin-like carbohydrates or glycogen are widely distributed, though in variable amounts; the fats are mostly rich in free fatty acids, associated with bodies of the ergosterin group and mostly also lecithins; basic

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[1] ZELLNER, J., Chemie der höheren Pilze. 8vo. pp. vi + 257. Leipzig: Wilhelm Engelmann. 1907. *M* 9.

bodies, allied to trimethylamin, are widely distributed, but no true alkaloid has yet been found. The composition of fungi and animals shows some striking resemblances.

In gathering together the data regarding the chemistry of the higher fungi, in citing the literature, and in describing methods of isolation and identification, ZELLNER supplements acceptably the related sections of CZAPEK'S *Biochemie der Pflanzen* and facilitates further studies in this field.—C. R. B.

"Sleep" movements

In a monograph of some two hundred pages PFEFFER[2] describes his recent investigations on the so-called "sleep" movements of leaves. For these researches he has planned ingenious methods of securing the self-registration of the movements of simple leaves, or the folding of a number of leaflets. In addition to the clever arrangements of levers, he has devised several forms of registering apparatus, and methods for continuous and intermittent illumination, in which the disturbing effects of varying temperature are eliminated, and at will a sudden or gradual illumination is secured, by electric tungsten or mercury lamps, without sparking at the contacts. These methods and apparatus will doubtless prove of very great service, not only in the study of "sleep" movements, but also wherever, as in this case, optical methods of record are not suitable.

The records published in this monograph, giving for the first time the autographs of leaves subjected to varying conditions of light and temperature, are likely to become classic illustrations. They show not only the so-called "sleep" movements, but also the independent autonomous movements which often accompany them, and the interesting rhythmic movements that persist for some time when the leaves are kept in uniform conditions. The work is one which will take its place with other monographs of this author as a permanent contribution to plant physiology. The contribution, however, lies rather in the useful apparatus and in the direct results recorded than in any theoretical considerations which are deducible.

The "sleep" movements, whether they are executed by growth or turgor, are herein definitely shown to be photonastic or thermonastic reactions, called forth by the daily change of illumination or temperature, or by the co-operation of both factors. That being the case it is to be regretted that the author does not adopt some more suitable term than the misleading *Schlafbewegungen*. When under constant conditions the "sleep" movements gradually disappear in 3-5 days through after-movements of diminishing amplitude, there often remain autonomous movements of much shorter rhythm, which are quite independent of the others. Many specific peculiarities in the responses are described, which cannot be here enumerated. Thermonastic responses show similar peculiarities and the photonastic reactions seem bound up, to a certain extent, with them, since in most leaves thermonastic responses may be induced when the photonastic ones have

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[2] PFEFFER, W., Untersuchungen über die Enstehung der Schlafbewegungen der Blattorgane. Abhl. Math.-phys. Kl. König. Sächs. Gesells. Wiss. 30:259-472. *figs. 36.* 1907. Separately of B. G. Teubner, Leipzig. *M* 8.

been excluded, and *vice versa*. The natural rhythm of response may be increased or diminished, provided the latent period of the organ is less than the daily period of light and darkness.

This work is one that will be needed in every active laboratory, so that any more detailed statements of its conclusions would be superfluous.—C. R. B.

NOTES FOR STUDENTS

Plant breeding.—Bulletins of the various Agricultural Experiment Stations usually deal with the practical side of agricultural problems, and there has not been at all times a recognition of the practical value of theoretical matters emanating from pure scientific sources.

One can only commend the purpose of Dr. E. M. EAST[3] in issuing a bulletin in which a well-written review of the development of the evolution idea is given, along with the more recent developments in our knowledge of variations and of heredity. In the main the treatment is well balanced, but in some respects there is too obvious a leaning on MORGAN'S recent publications. Particularly does it seem out of place, in so brief a paper, to present MORGAN'S theory of the impurity of the gametes—a theory which has lost practically all the slight support it seemed to have when first propounded.

It is noted on p. 68 in the discussion of the Belgian-hare cross, as an illustration of "masked" characters, that the older explanation is retained, based upon the supposed presence of a black factor in the albino. CASTLE[4] has given a more satisfactory explanation of such cases by assuming an "agouti factor" which distributes the pigment in distinct regions in each hair, giving a grey effect. The Belgian hare of the experiment cited had both dominant characters, namely pigment and agouti, and the albino lacks them both. The advantage of CASTLE'S explanation lies in the fact that it brings such cases into harmony with the "presence and absence" hypothesis which has found such wide support in other cases and to which the author of the bulletin himself subscribes.

The section on "Methods of plant improvement" is doubtless the most valuable part of the paper. Its scope may be indicated by the subheads: "The selection of fluctuations," "Isolation of elementary species," "Judging plants by their progeny," "Correlated characters," "Improvement by hybridization." Under the first of these subheads a valuable summary is given of the results gained by HOPKINS and his associates at the Illinois Experiment Station in the breeding of maize for high and low protein and high and low oil content. The crop averages for ten years are given, showing that in this time the maximum separation of the extremes with respect to these characters seems to have been attained.

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[3] EAST, E. M., The relation of certain biological principles to plant breeding. Conn. Agric. Exp. Sta., Bull. 158. pp. 93. *figs. 6.* 1907.

[4] CASTLE, W. E., On a case of reversion induced by cross breeding and its fixation. Science N. S. 25:151–153. 1907.

On p. 62 the "Knight-Darwin law" that "nature abhors perpetual self-fertilization" is amended to read, "nature resists any sudden change in long-established conditions." A suggestion having the same import was made some years ago by HAYS,[5] but this seems to have been overlooked by Dr. EAST. HAYS says, "possibly DARWIN'S law would more broadly cover the truth if expressed thus: nature abhors a radical change which would require species to cross in much closer or in much more radical relationship than is their long-established habit."

The last ten pages preceding the summary discuss the technique of maize breeding, giving an insight into the great complexity of the problems to be solved in breeding this crop, owing to the occurrence of fluctuations and numerous elementary species, and the injurious effects of too close inbreeding. The author's experience in maize breeding, as an associate of HOPKINS at the Illinois Station, and his continued efforts along similar lines at the Connecticut Station, have thoroughly qualified him to write on this subject, and his discussion will doubtless prove of great value to maize breeders; but the reviewer questions the correctness of the assumption that the isolation of pure strains of maize is the proper aim of those who would secure the greatest possible improvement in this crop.

It is believed that this excellent bulletin will have such a reception that other stations will follow Dr. EAST'S example in disseminating advanced scientific information.—G. H. SHULL.

The latent life of seeds.—Two rival doctrines have been used heretofore, with equal success, to account for the latent life of seeds. The one holds that life is completely suspended, all the exchanges, both of matter and of energy, being arrested. The other holds that the phenomena of metabolism are greatly diminished, but that they are never entirely suppressed. Both of these theories have been supported by experimental evidence, and the question has called forth a very large number of investigations. These antagonistic views have led BECQUEREL to a further study,[6] with the idea that the previous conflicting results depended upon neglected properties of the integuments. He has demonstrated the impermeability for gases of the integuments of seeds of a considerable number of species; and it seems GOLA[7] simultaneously had discovered impermeability for water to be a common character.

These facts make it necessary to go over previous experiments, perforating the integuments or removing them. Seeds which resist the action of alcohol, ether, and chloroform, when their integuments are intact, succumb when these agents are brought into actual contact with the embryo. But low temperatures, even to −210°, do not have any appreciable effect on germination if the protoplasm has already undergone thorough desiccation. The existence of gaseous exchanges,

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[5] HAYS, W. M., Plant breeding. U. S. Division of Physiology and Pathology, Bull. No. 29. pp. 72. *figs. 21.* 1901. (See pp. 38, 39.)

[6] BECQUEREL, PAUL, Recherches sur la vie latente de graines. Ann. Sci. Nat. Bot. IX. 5:193–320. 1907.

[7] As cited by BECQUEREL: GOLA, J., Mémoire sur la physiologie [in another place, Recherches sur] des téguments des graines. Acad. [Sci.] Turin. 1905.

heretofore attributed to respiration, was confirmed. But it is clearly shown that the fixation of oxygen and the evolution of CO_2 have no relation to the life of the seed, and that a direct oxidation probably takes place with equal activity in live and dead seeds. Thus the gaseous exchanges of the integuments, compared with those of the decorticated seeds from which theycame, were frequently, weight for weight, very much larger. Further, these exchanges in the isolated integuments, compared with those of the entire seeds of the same species whose integuments are impermeable, are absolutely the same. Where the integument is permeable, however, the gaseous exchanges of the embryo are added to those of the integuments. None of these changes take place unless the air is moist and the protoplasm contains still a certain proportion of water.

BECQUEREL has also shown that no perceptible gaseous exchanges take place in the embryo when completely dehydrated, and that it can bear for a long time an atmosphere of carbonic acid, nitrogen, or a complete vacuum without losing its power to germinate. In fact, he is convinced that the loss of germinative power is dependent upon the changes permitted by the hygroscopicity of the seeds, and the only ones in which longevity is to be looked for are those with an impermeable integument.

Incidentally he discusses the longevity of seeds *in extenso*, and himself made experiments upon almost 500 species, belonging to 30 of the most important families of monocots and dicots, whose age was accurately known from the records of the *Muséum d'Histoire Naturelle* of Paris. Only four families had any viable seeds. Eighteen species of Leguminosae (87 to 28 years old), three species of Nelumbium (56 to 18 years), one Lavatera (64 years), and a Stachys (77 years) germinated.

BECQUEREL seems to have reduced the question to this dilemma: since there is no appreciable metabolism for as much as two years' time, in perfectly dry yet viable seeds, either their life-processes are wholly anaerobic, intracellular, and very slow, or life is completely suspended. He has begun an experiment of ten years' duration to determine whether the dried protoplasm is stable in a vacuum as perfect as can be obtained. He has inclosed in exhausted vials dry seeds of buckwheat and of wheat, with perforated integuments. These vials will be kept in darkness and will be examined with a spectroscope for gases every year, and at the end of ten years the seeds will be planted. This experiment promises to be a crucial one, if (as is to be expected) no gases are set free, and the seeds live. If it turns out otherwise, the choice between retarded life or suspended life for seeds will remain a speculative one.—C. R. B.

Apogamy and apospory in ferns.—HELENE WORONIN,[8] working in GOEBEL'S laboratory, has published the results of an experimental study on apogamy and apospory in ferns. The study included six species; *Trichomanes Kraussii*, *Pellaea flavens*, *P. nivea*, *P. tenera*, *Notochlaena Eckloniana*, and *N. sinuata*.

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[8] WORONIN, HELENE, Apogamie und Aposporie bei einigen Farnen. Flora 98: 101–162. *figs. 72.* 1907.

Three species of Cheilanthes (*tomentosa*, *persica*, and *fragrans*) and five species of Notochlaena (*Marantae*, *squamosa*, *trichomanoides*, *vellaea*, and *lanuginosa*) were studied to induce apospory and apogamy, but without success, excepting one case of *N. Marantae*, which she says is a very rare one.

The paper first gives a description of *Trichomanes Kraussii* as to its general morphology, structure and development of antheridia, apogamy, and apospory. Then there follows an account of apogamy and apospory in three forms of Pellaea (*flavens*, *nivea*, and *tenera*) and two species of Notochlaena (*Eckloniana* and *sinuata*). Next there is given the result of apogamous and aposporous development in *Pellaea flavens* under culture both in darkness and on sandy soil. Last there is an account of the result of experiments on regeneration, using the two apogamous species of Pellaea (*nivea* and *flavens*), one apogamous form of Notochlaena (*Eckloniana*), and two normal species (*Gymnogramme farinifera* and *N. Marantae*).

From the detailed account a few points of interest may be summarized as follows: The prothallia of *Trichomanes Kraussii* consist of both filamentous and flattened parts; archegonia fail to develop; antheridia never attain maturity, apogamous embryos develop from the filamentous part, or from the margin or surface of the flattened part; at the outset the apogamous embryo consists of several cell layers which the author thinks to be homologous with the archegoniophore; from this structure a leaf process first appears, and then the stem apex develops independently of the former; the appearance of the root is delayed. Apospory is also induced in the same species by cultivating cuttings of leaves on loam; often the aposporous tendency prevails so far that antheridia are formed directly from the margin of the leaf.

The three forms of Pellaea (*flavens*, *nivea*, and *tenera*) and two species of Notochlaena (*Eckloniana* and *sinuata*) are all apogamous. No archegonia ever develop, and in *N. sinuata* no antheridia are ever formed. Generally speaking, in these five forms the apogamous structure is formed first as a leaf on the under surface of the sinus of the prothallium from the apical meristem. The stem apex then appears quite independent of the leaf; either between the leaf and prothallium or under the leaf; the root appears later.

In *Pellaea flavens*, grown in darkness, there is induced an apogamous structure, at first appearing as a leaf. After continued cultivation in darkness apospory was induced; from the arrested leaf grown under the sinus of the prothallium a new prothallium is formed which bears antheridia and develops also apogamous structures. It was not possible to distinguish the boundaries of the prothallial and apogamous structures. In sand cultures of *Pellaea flavens* there were found leaf buds on the arrested leaf grown under the sinus of the prothallia. Apogamous structures also developed from the same prothallium.

As a result of regeneration there appeared several interesting cases. When cut fragments of the sinus region of prothallia are put on the soil, further continuous development is checked, but new structures arise. From these structures often prothallia are formed again. The prothallia aposporously produced develop antheridia.—S. YAMANOUCHI.

Summation of light stimuli.—It is well known that stimuli, too brief to produce a visible reaction, if repeated at intervals not too long, lead to a response, just as continuous stimulation for a sufficient time does. WIESNER found that when intermittent light stimuli followed each other within double the period of their duration, reaction began as soon as though the plants had been continuously stimulated. Generalizing from this he concluded that summation of the stimuli occurred without loss during the intervals. TALBOT'S law, however, which holds with extreme exactness for the human eye, indicates that the effect of an intermittent light can only equal that of a constant one of lower intensity. Thus, if a light of intensity i acts for x seconds, at intervals of $3x$ seconds, its stimulating effect will be equal to that of a light of $i/4$ intensity. Obviously if this law be valid for heliotropic reactions of plants, reaction time, which alone WIESNER determined, cannot be a correct index of the effects of light as a stimulus. NATHANSOHN and PRINGSHEIM showed that this is so, and further undertook to make an extended comparison of the effects of constant and intermittent light stimuli.[8] Using such lights on opposite sides of seedlings of Brassica and Avena, they find that TALBOT'S law is valid for heliotropic phenomena within reasonable limits. Only when the frequency and duration of the illumination was greatly reduced, as to 1⅛ minutes duration with darkness 3⅜ minutes, did the point of physiological indifference depart from the optical one (determined by the photometer) in favor of the intermittent light. Thus they established that there was, for plants as for the eye, a so-called critical periodicity. When intermittent stimuli are too slow, the eye sees a flickering; the plant responds by pendulum-like swings instead of a steady curvature. They also found the law valid within wider limits with weaker illumination than with strong.

In an elaborate theoretical discussion they propose to account for the observed phenomena of summation thus: They assume, with HELMHOLTZ, that the primary excitation is photochemical, and that its effect is proportional to the product of the intensity by the time of illumination. This produces a certain excitation, which is steadily maintained in constant illumination, but falls in the dark interval of intermittent illumination. In both cases a counter-reaction is assumed to be released, which antagonizes the primary reaction, and must be overcome if the latter is realized visibly. With constant illumination the algebraic sum of the light excitation and the counter-reaction determines the maximum effect. In intermittent light the counter-reaction is effective in the dark intervals in reducing the effect of each excitation, the remainder being added to by the next impulse, which serves at the same time to heighten the counter-excitation, and so on. As we understand it, the case for constant illumination might be represented thus: $a-b=c$, a being the total direct excitation, b the total counter-excitation, and c the response, with intensity i. At the intensity $2i$, and alternate equal periods of light and darkness, the case is this: $(x-y)+[x-(y+k)]+n=c$; where x is

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[9] NATHANSOHN, A. und PRINGSHEIM, E., Ueber die Summation intermittierende Lichtreise. Jahrb. Wiss. Bot. 45:137–190. 1907.

the direct excitation of each stimulus, *y* the counter-excitation set up thereby, *k* the increment of *y*, *n* any number of additional terms, and *c* the response. No good reason appears for assuming a counter-reaction. Partial or complete recovery from the primary excitation in the intervals of stimulation is a simpler assumption and would seem equally to account for the facts. Further details must be sought in the paper itself.—C. R. B.

Edaphic steppes in Sweden.—The "alfvar" is a name given in Sweden to some remarkable formations that occur chiefly on the island of Öland, and to a lesser degree on the island of Götland, and in the mainland province of Vestergötland. The name "alfvar" is applied to extensive treeless plains, whose underlying rock is a Silurian limestone. While there are several plant formations, the alfvar presents general ecological unity, and it has an extremely xerophytic stamp. It is somewhat similar to the "garigue" of southern Europe, and to certain rock formations of Servia. The physiognomy is that of a steppe, and yet there is anything but a steppe climate in Sweden. There is no question that the alfvar is determined by edaphic causes in the main (such as the dryness and temperature changes of the rocks, which are everywhere at or near the surface, and the poverty of the scanty soil), reinforced by strong insolation and constant winds. The alfvar is very distinct in every way from the heath. Such a region is relatively rich in species, of which more than half are glacial or subglacial, and about a third are representatives of the oak flora. Six per cent. belong to the climatic steppe, and only 2 per cent. to the beech forest flora. This flora is doubtless a relict of a much more widespread flora in the period succeeding glaciation. There are two general aspects, the dominant aspect of the steppe, and the lesser aspect of the meadow. The herbaceous plants commonly dominate, although shrubs are frequently conspicuous. The leading character plants of the steppe are *Mollia tortuosa, Helianthemum oelandicum*, and *Cynanchum vincetoxicum*. The chief shrubs are *Juniperus communis* and *Potentilla fruticosa*. In less xerophytic places, *Festuca ovina* assumes a prominent place. There are often vast areas of Cynanchum almost alone, giving almost an exact picture of certain climatic steppes. Perhaps the most interesting part of the paper is the discussion of nanism. The author distinguishes facultative dwarfs, due to environmental causes, and constitutional dwarfs. Constitutional dwarfs (the group to which the term nanism more properly applies) differ from facultative dwarfs not only in breeding true, regardless of conditions, but also in a much greater qualitative reduction. Constitutional dwarfs show reduction in all organs, not chiefly in aerial vegetative organs; roots and rhizomes are reduced, while they are rarely reduced and often actually increased in facultative dwarfs. Constitutional dwarfs also show reduced floral organs, their internodes are fewer as well as shorter, and their leaves are reduced in number as well as in size. Most of the dwarfs are annuals, although some show a certain lability as to their duration. Species commonly perennial or biennial, for example, may become annual in very dry situations. The usual "protective" structures of xerophytic vegetation occur here in high degree, and are regarded by WITTE[10] as of advantage in checking transpiration. The alfvar was very fully treated from this standpoint some years ago by GREVILLIUS. Some plants commonly herbs here become half-shrubs, e. g., *Gypsophila fastigiata*. There is an excellent study made of the underground organs, which are frequently neglected.—H. C. COWLES.

Lateral roots.—NORDHAUSEN has studied the direction and growth of lateral roots under the influence of external factors, particularly those which arise from the removal of portions of the main root or its wounding.[11] It is well known that when a millimeter or less of the primary root is cut off, regeneration of the tip occurs; but when larger portions are removed, one or more of the lateral roots change their angle with the main axis, as it were to substitute themselves for it. NORDHAUSEN can only say, as one result of his studies, that this reaction on the part of the lateral roots is "dependent upon the intensity and quality of internal conditions which bear more or less relation to the needs of the plant," which of course is an empty form of words. It was already known that decapitation within the growth zone results in a very active substitution response. If the cutting exceeds this, however, whether much or little, says NORDHAUSEN, this response is minimal or wanting, provided a rather long piece of the primary root remains; but shortening the stump beyond a certain amount again increases the response. The alteration of the angle of the substitute roots with the main axis rests, contrary to the conclusions of BRUCK and CZAPEK, upon both geotropic and autotropic factors, of which the former is determinative. This substitution of lateral roots is independent of actual wounding, since it can be brought about by merely checking the growth, especially of the main axis. A partial response follows, even without disturbance or operation on the growing point, if certain tracts within the central cylinder (not only in the phloem but especially in the xylem) are interrupted by lateral wounding. The disturbance of nutrition here plays only a subordinate rôle. NORDHAUSEN agrees with MCCALLUM in assuming the existence of specific inhibitory stimuli, regulating the formation of organs in intact plants. Certain roots (Lupinus, Phaseolus) respond to a temporary past reduction in the water supply by altering the angle of lateral roots, which at the time of the difficulty had not even appeared, though when they develop they are under normal conditions. Lateral wounding of the main root leading to direct or indirect affection of the pericambium, even before the lateral roots become visible, produces a traumatropic deviation of these from the wounded side. The transmission of the excitation, easier in the acropetal than the basipetal direction, may produce sympathetic response of more distant lateral roots.

The influence of curvature of the main root upon the form and lateral deviation of the side roots, determined by NOLL and explained (?) as morphaesthesia, is due, according to NORDHAUSEN, to changes in the tension of the central cylinder,

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[10] WITTE, H., Till de Svenska alfvarväxternas ekologi. pp. 119. Upsala. 1906.

[11] NORDHAUSEN, M., Ueber Richtung und Wachstum der Seitenwurzelen unter dem Einfluss äusserer und innerer Faktoren. Jahrb. Wiss. Bot. 44:557–634. 1907.

particularly of the pericambium. He finds these effects producible by injuries, depression of turgor, etc., which need only act in the past, while the growth of the lateral roots takes place under regular conditions.—C. R. B.

Soil fertility.—Two recent bulletins from the Bureau of Soils deal with the question of the factors affecting soil fertility. One, by LIVINGSTON and others,[12] demonstrates the presence in unproductive soils of substances deleterious to plant growth. He adds the evidence gathered up to the close of his work that the toxic substances are apparently produced by the plants themselves growing in soil and water cultures. He finds that such toxic substances can be dissolved partly in a watery extract of soil and may be removed from such an extract by shaking with finely divided insoluble solids. This leads to the further suggestion, partly supported by experiment, that stable manure and green fertilizers are beneficial rather by their action on the soil constituents, than by either the salts or the organic matter put at the disposal of the plants. Incidentally it was found necessary to devise methods for securing non-toxic distilled water. The most satisfactory water was prepared by shaking ordinary distilled water with fine, clean carbon black or precipitated ferric hydrate and filtering.

Developing the idea suggested by LIVINGSTON'S work, SCHREINER and REED[13] show that the toxic substances originate from the plants themselves, diffusing from their roots. This they demonstrate by direct experiment with seedling wheat and other plants, and support by adducing experiments and observations which have been accumulating for years, and can be best explained by their experimental results. It appears, for example, that the excreta from no other roots were so harmful to wheat as its own, and that the excreta from oats were more harmful to wheat than those from the less closely related corn and cowpea. The natural succession of plants, the deleterious effects of grass sod on apple trees, oak-openings, fairy rings, crop rotation, the effects of good tillage, can all be rationally explained in the light of the facts adduced.

SCHREINER and REED have also published the substance of this bulletin in another paper which requires only citation.[14]—C. R. B.

Items of taxonomic interest.—A. A. HELLER (Muhlenbergia **2**:269–338. 1907), in an enumeration of plants collected in the coast region of California during 1907, describes new species in Limnia (Claytonia), Lathyrus, Lupinus, Trifolium, Amsinckia, Stachys (3), and Plectritis (2); and also proposes Heleniaceae, Anthemidaceae, and Senecionaceae as new families.—E. O. WOOTON and P. STANDLEY

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[12] LIVINGSTON, B. E. Further studies on the properties of unproductive soils. U. S. Dept. Agric., Bureau of Soils, Bull. 36. pp. 71. *pls. 7.* 1907.

[13] SCHREINER, O., and REED, H. S., Some factors influencing soil fertility. U. S. Dept. Agric., Bureau of Soils, Bull. 40. pp. 40. *pls. 3.* 1907.

[14] SCHREINER, O., and REED. H. S., The production of deleterious excretions by roots. Bull. Torr. Bot. Club **34**:279–303. 1907.

(Bull. Torr. Bot. Club **34**:517–520. 1907) have described 2 new species of Androsace from New Mexico.—M. L. FERNALD (Rhodora **9**:221–226. 1907) has described 3 new species and 2 new varieties of Salix from Eastern America.—K. YENDO (Jour. Coll. Sci. Univ. Tokyo **21**: art. 12. pp. 174. *pls. 18.* 1907) has published (in English) a full account, with excellent plates, of "The Fucaceae of Japan" (61 species), including new species in Cystophyllum (2), Coccophora, and Sargassum (2), and also a new genus (*Ishige*).—J. BRIQUET (Botanisk Tidskr. **28**:233–248. 1907), in describing the Labiatae from the Central Asian and Persian collections of OVE PAULSEN, describes a new genus (*Paulseniella*) in Stachyoideae.—S. KAWAMURA (Jour. Coll. Sci. Univ. Tokyo **23**: art. 2. pp. 12. *pls. 5.* 1907) has described a new genus (*Miyoshia*) of Ascomycetes parasitic on the stems of a species of bamboo.—C. WARNSTORF (Hedwigia **47**:113–127. 1908) has described the following new species of Sphagnum from the U. S.: *S. Mehneri* (Alaska), *S. Faxonii* (Mass.), and *S. riparioides* (Alabama).—A. W. EVANS (Torreya **7**:225–229. 1907) has described a new genus (*Leucolejeunea*) of Hepaticae, segregated from Archilejeunea and containing 5 species.—H. D. HOUSE (*idem* 233–235) has discovered that the generic name Shortia cannot be maintained for the interesting *S. galacifolia* of the southern Appalachians, on account of an earlier Shortia of RAFINESQUE; accordingly the name *Sherwoodia* is proposed and the names of the one American and the three Asiatic species are transferred.—W. W. EGGLESTON (*idem* 235, 236) has described a new Crataegus from New Mexico.—A. NELSON and P. B. KENNEDY (Muhlenbergia **3**:137–143. 1908) have described new species from the Great Basin in Lepidium, Boisduvalia, Chylisma, Oreocarya, Phlox, Plantago, and Symphoricarpos.—F. RAMALEY (Univ. Col. Studies **5**:47–63. 1907), in a list of the woody plants of Boulder County, Col., including the ranges, enumerates 112 species in 50 genera.—TH. VALETON (Bull. Depart. Agric. Ind. Néerland. 10. pp. 70. 1907), in a list of Papuan plants, describes 21 new species.—N. L. BRITTON (Bull. Depart. Agric. Jamaica **5**: suppl. 1. pp. 19. 1907) has published a list of the sedges of Jamaica, enumerating 96 species, 37 of which belong to Cyperus.—G. MASSEE (Trans. N. Z. Inst. **39**:1–49. *pls. 1, 2.* 1907), in his second paper on the fungus flora of New Zealand, presents the Polyporeae (9 gen., 47 spp.), Hydneae (4 gen., 8 spp.), Thelephoreae (10 gen., 36 spp.), Clavarieae (2 gen., 11 spp.), and Tremellineae (9 gen., 15 spp.).—OAKES AMES (Philipp. Jour. Sci. **2**:311–337. 1907) has published a list of the orchids of Mount Halcon and vicinity, Mindoro, 93 species being named, 39 of which are new.—C. S. SARGENT (Rep. Mich. Geol. Survey **1906**:515–570. 1907) has published an account of the species of Crataegus in Southern Michigan, recognizing 55, of which 24 are described as new.—J. M. GREENMAN (Field Columb. Mus. Publ. Bot. Ser. **2**:247–287. 1907) has published the results of his study of recent collections from Mexico, Central America, and the West Indies. The species number 92, of which 35 are described as new, one of them constituting a new genus (*Goldmania*) of Coreopsideae. Of Senecio 28 species are presented, 7 of which are new, and 18 of which are fully characterized for the first time.—J. M. C.

Anatomy of Tmesipteris.—Miss SYKES[15] has investigated this interesting genus from material sent by Mr. A. P. W. THOMAS of New Zealand, comprising the two species *T. tannensis* and *T. lanceolata*. The genus consists of epiphytic species on tree ferns in New Zealand, Australia, and Polynesia. Only adult plants have ever been obtained, and the gametophyte is entirely unknown. Naturally the investigation by Miss SYKES has to do chiefly with the anatomy, and an outline of her results is as follows, in part confirmatory of previous work.

In the rhizome there occurs a protostele, which has usually two exarch protoxylem groups, but in passing to the aerial branch, pith arises in the center of the stele and quickly expands to form a large tissue. This is a case of a protostele passing directly into an ectophloic siphonostele, without the intermediate stage of an amphiphloic siphonostele; and all this occurs at a level at which no leaves have yet arisen. It was discovered that the course of the vascular bundles as described by BERTRAND for a sterile branch is exactly similar to that found in a fertile branch; that is, the single bundle entering the axis branches into three, the two lateral traces supplying the leaves, and the central one representing the vascular supply of the apex. In the fertile branches the central bundle supplies the synangium, and indicates to the author that the so-called "sporophyte" is cauline in nature, consisting of an axis bearing two leaves, and at its apex a synangium formed of two masses of sporogenous tissue that have fused over the tip. Attention is called also to the essential similarity in the formation of leaf and branch traces, it being claimed that the presence of a gap depends simply on the greater length of time elapsing between the division of a xylem group to form a trace and the departure of that trace from the stele. In fact, if the so-called "sporophyll" is really a sporophyll, the exit of its trace results in a leaf-gap; but according to Miss SYKES this is a branch-gap. The conclusion as to relationship, apparently inevitable in all such pieces of work, is that the Psilotaceae had better be retained as a separate division of Pteridophytes, the Psilotales.—J. M. C.

Rôle of certain elements.—The precise physiological rôle which the essential chemical elements play in plant life has long been an attractive subject for investigation. With advancing chemical knowledge the methods of experimentation have been greatly improved. It must be said that the older experiments have little value, and it is doubtful whether even the newest have very much, because the chemistry of the proteids is still such an enigma. REED has undertaken the study of the effects of four elements, potassium, phosphorus, calcium, and magnesium, upon certain filamentous algae, protonemata of mosses, prothallia of ferns, root tips of seed plants, and filaments of Basidiobolus.[16] His technique contains certain improvements; at the same time, on the score of the solutions used, it is

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[15] SYKES, M. G., The anatomy and morphology of Tmesipteris. Annals of Botany **22**:63–89. *pls. 7, 8. figs. 13.* 1908.

[16] REED, H. S., The value of certain nutritive elements to the plant cell. Annals of Botany **21**:501–543. *figs. 2.* 1907.

open to some objections, which, however, may be more theoretical than practical. From his observations on plants grown in the absence of certain salts, he draws conclusions as to the rôle of a particular element—a time-honored but nevertheless unsafe process. Some of these observations coincide with those of earlier observers, and others are to be added to the long list of specific effects previously recorded after like experiments. We cannot enumerate the results in detail. Assuming REED'S and all others as valid, no one is yet in a position to interpret this immense mass of data, many of them conflicting, and to frame any generalizations.

In his discussion REED clearly recognizes that the elements may either enter into the composition of some organ or substance in the cell, or without doing this permanently may act as catalysers, or in some other way may condition certain reactions. It is the varied possibilities within these categories that render useless at present all conclusions regarding the rôle of an element. The causal nexus is too intricate to be analyzed until far more knowledge of cell chemistry is available.—C. R. B.

Respiration and potassium cyanid.—Incited by the studies of animal physiologists on the effect of hydrocyanic acid and cyanids upon animal respiration, SCHROEDER set out to determine the effect of potassium cyanid upon the respiration of *Aspergillus niger.*[17] Of course "respiration" here means the intake of O_2 and the output of CO_2, processes which are quite independent of one another, and SCHROEDER'S results furnish further evidence, if any were needed, of this independence. Such investigations can hardly yield, as the author hopes, satisfactory "conclusions as to the chemism of vital functions," until it is possible to make a much more exact analysis of the fixation of O_2 and the evolution of CO_2 than is yet possible. Enough is known regarding the diverse sources of CO_2, however, to minify the value of superficial researches upon such obscure phenomena. The investigations themselves are extensive and thorough enough, but they necessarily deal with superficial phenomena.

SCHROEDER finds both the fixation of O_2 and the evolution of CO_2 strikingly reduced by potassium cyanid. The production of CO_2 is practically stopped, but the consumption of oxygen is not. The author is uncertain "whether this small intake of O_2 is to be considered as a vital process, or a purely chemical phenomenon," a phrase which indicates an unfortunate state of mind regarding "vital" processes. He does not think that the cessation of the evolution of CO_2 is a valid mark of death. (Obviously not, since it is well known that neither its evolution nor cessation has any definite relation to death.) The further distinctions which he makes between the action of HCN on the respiratory process "as a primary action . . . . and not as a phenomenon of death," seem quite invalid in view of our ignorance of the details of dissimilation.—C. R. B.

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[17] SCHROEDER, H., Ueber den Einfluss des Cyankaliums auf die Atmung von *Aspergillus niger*, nebst Bemerkungen über die Mechanik der Blausäure-Wirkung. Jahrb. Wiss. Bot. **44**:409–481. *figs. 2.* 1907.

Compositae of Southern California.—H. M. HALL[18] has brought together our present knowledge of the Compositae of Southern California, an area about the size of Pennsylvania. The 445 species and varieties are distributed as following among the tribes: Eupatorieae (9), Astereae (113), Inuleae (25), Ambrosieae (18), Heliantheae (34), Madieae (29), Helenieae (86), Anthemideae (19), Senecioneae (25), Cynareae (17), Mutisieae (2), Cichorieae (68). These numbers would be largely increased if the author were not very conservative in his conception of species. As it is, the contribution becomes a manual of readily recognized forms, which are further brought within easy reach by the numerous well-constructed keys. It is unusual to find in so extensive a contribution that only three new species have been described, two in Aster and one in Lessingia. However, there is large recognition of varieties, many new ones being proposed; and new combinations are frequent. The reduction of numerous names to synonymy may be a service or may add to the confusion, dependent upon the care with which the types were studied. In any event, this type of monograph for so interesting a region is to be commended.—J. M. C.

Nuclear division in Zygnema.—ESCOYEZ[19] has published the results of cytological studies on Zygnema. The conclusions are as follows: (1) A chromatin network forms chromosomes by gradual concentration. (2) The nucleolus contains no chromatin substance and does not furnish any morphological elements to the chromosomes. (3) The chromosomes are not formed by the fusion of four groups of chromatin granules, as was claimed by Miss MERRIMAN. (4) The chromosomes split longitudinally in the metaphase as in typical mitosis. (5) In the telophase the chromosomes first become massed at the poles and then vacuolated. The anastomosed structure of the nucleus consists entirely of chromosomes. (6) The nucleolus is not formed by the confluence of chromosomes, but appears quite independent of the chromatin network. (7) There is formed no continuous spirem, and the chromosomes evidently keep their individuality. (8) Pyrenoids and chromatophores divide simply by constriction, and the division is independent of that of nucleus. The division of the two pyrenoids does not necessarily occur simultaneously.—S. YAMANOUCHI.

The double leaf trace.—MISS THOMAS[20] has brought together the facts in reference to the double leaf-trace, so characteristic of the ancient fern and older gymnosperm series, and well marked in the living cycads. This condition is claimed to exist in the "double bundle" of the cotyledons of angiosperms, and is of very frequent occurrence in modern ferns. A phenomenon of such wide occurrence suggests "the existence of a common cause of a fundamental and primitive character," and this the author finds in dichotomous branching; which means

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[18] HALL, H. M., Compositae of Southern California. Univ. Calif. Publ. Bot. **3**:1–302. *pls. 1–3.* 1907.

[19] ESCOYEZ, EUD., Le noyau et la caryocinèse chez le Zygnema. La Cellule **24**:356–366. *pl. 1.* 1907.

[20] THOMAS, ETHEL N., A theory of the double leaf-trace founded on seedling structure. New Phytologist **6**:77–91. *figs. 4.* 1907.

that the double leaf-trace "is neither more nor less than an early dichotomy of the vascular system of the leaf." This persistent dichotomy of the leaf-trace, long after dichotomy has disappeared from the leaf, is used to explain the irregular dichotomy of the leaves of Cycadofilices, the dichotomy of the early leaves of modern ferns, the splitting of cotyledons in polycotyledony; and "it finds its latest expression in the 'double bundle' of the cotyledons of angiosperms." This view certainly helps to connect the angiosperms with the same old fern stock that gave rise to the gymnosperms.—J. M. C.

Effect of electricity.—A very concise summary of the work previously published upon the effect of electricity on the growth and development of plants is furnished by PRIESTLEY[21] as the preface to a brief account of some recent experiments on a large scale by NEWMAN near Bristol, Gloucester, and Evesham. The current used was of high tension, distributed by overhead wires from which depended metallic points. In general the results show a very decided increase in the quantity, or quality, or earliness of the crop. Thus year-old strawberries showed 80 per cent. increase, Canadian Red Fife wheat 39 per cent. The electrified wheat produced a better baking flour and consequently sold at 7.5 per cent. higher prices. Electrified beets not only showed 33 per cent. increase in the crop, but contained over 14 per cent. more sugar. Currents traversing the earth produced occasionally increase in rate of growth, but often had no definite effect. The physiological action of the current is not clear. PRIESTLEY rejects POLLACCI'S view that it enables the green plant to elaborate starch in the dark.—C. R. B.

A theory of photosynthesis.—GIBSON outlines[22] thus a photoelectric theory of photosynthesis, which he is to elaborate later in cooperation with two colleagues: The light rays absorbed by chlorophyll are transformed by it into electric energy which effects the decomposition of H_2CO_3, with the concomitant formation of an aldehyde and the evolution of oxygen. He finds a small quantity of formaldehyde present, as shown by the test of MULLIKEN, BROWN, and FRENCH, in all actively photosynthetic tissues, the amount being definitely related to the illumination. The maximum decomposition of CO_2 occurs in light equal to one-quarter direct sunlight. H.COH may be synthesized from CO_2 in the presence of water by a silent electric discharge, as LOEB has shown; and this GIBSON confirms. Electric discharges of sufficient intensity have already been found in adequately illuminated green tissues, and the light rays absorbed by chlorophyll are the ones which produce these currents. He promises in the forthcoming paper to connect formaldehyde with carbonic acid by a photolytic method which is above suspicion. —C. R. B.

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[21] PRIESTLEY, J. H., The effect of electricity upon plants. Proc. Bristol Nats. Soc. IV. 1:192–203. 1907.

[22] GIBSON, R. J. HARVEY, A photoelectric theory of photosynthesis. Annals of Botany 22:117–120. 1908.

NEWS

ABOUT 20,000 sheets were added to the Kew Herbarium during 1907, 7000 of which were purchased.

R. H. LOCK, Cambridge University, has been appointed assistant director at the Royal Botanic Gardens of Peradeniya, Ceylon.

DR. CHARLES J. CHAMBERLAIN, University of Chicago, spent the month of March in Mexico in further study of Dioon and Ceratozamia.

AT THE MEETING of the British Association in Dublin next September, FRANCIS DARWIN will be general president, and F. F. BLACKMAN president of the section of botany (K).

THE SWEDISH ACADEMY OF SCIENCES has granted to Professor C. F. O. NORDSTEDT 1000 kronor ($270) for the purpose of printing a supplement to his *Index Desmidiacearum*.

DR. MAX KÖRNICKE, *privat-docent* in the University of Bonn, has been appointed to succeed Professor L. JOST as the professor of botany in the Agricultural College at Bonn-Poppelsdorf.

PROFESSOR SERGIUS NAWASCHIN, of the University of Kieff, completed on March 3 last twenty-five years of service as a teacher. The Society of Naturalists of Kieff on that day elected him an honorary member and arranged for further celebration of the occasion.

DR. J. N. ROSE, of the U. S. National Herbarium, left Washington March 17 for an extended trip through Texas, New Mexico, Arizona, southern California, and northern Mexico, for the purpose of studying Cactaceae in the field. His headquarters are at the Desert Laboratory at Tucson, Arizona.

"L'ASSOCIATION INTERNATIONALE DES BOTANISTES" will hold its next meeting at the University of Montpellier, beginning June 7, 1908, the meetings of standing committees being announced for the preceding day. Most attractive excursions are being planned. Members who intend to be present are urged to notify Professor FLAHAULT as soon as possible.

THE HERBARIUM of Dr. OTTO KUNTZE has been secured for the New York Botanical Garden, through the generosity of Mr. ANDREW CARNEGIE. It is estimated that the collection contains over 30,000 specimens, from all parts of the world. To American botanists the greatest interest of this collection is that it contains a large number of type specimens of tropical American and South American species.

A BIOGRAPHICAL SKETCH, with portrait, of the late GEORGE E. DAVENPORT, who died November 27, 1907, at the age of 74 years, is published in *Rhodora* (**10**:1–9. 1908), prepared by F. S. COLLINS. A list of his botanical writings includes 109 titles. Mr. DAVENPORT's contributions to the BOTANICAL GAZETTE began with the second volume, and in the early volumes 28 contributions are credited to him.

GEBRÜDER BORNTRAEGER announce the inception of a new journal, *Zeitschrift für induktive Abstammungs- und Vererbungslehre*, under the editorship of Dr. E. BAUR (Berlin) and the general direction of CORRENS, HAECKER, STEINMANN, and VON WETTSTEIN. It will appear in octavo parts of variable size (how many are to form a volume is not stated, nor is the price announced), and is to contain contributions to the theory of evolution, whether from the field of paleontology, biology of living forms, taxonomy, physiological chemistry, or experimental evolution.

THE BOTANICAL PROGRAMME (February 14) of the Wisconsin Academy includes the following titles: Nuclear division in the vegetative cells of *Gentiana detonsa*, by R. H. DENNISTON; The blepharoplast in the development of the antherozoid in ferns, by RUTH F. ALLEN; Variations in macrospore formation in *Smilacina stellata*, by FRED MCALLISTER; Nuclear division and spore formation in Geoglossum, by HALLIE D. M. JOLIVETTE; Nuclear structure and nuclear division in *Chrysomyxa ledi*, by R. A. HARPER; The development of the male cells of a moss, by C. E. ALLEN.

DR. FRIEDERICH KÖRNICKE, Geheimer-Regierungsrat and professor in the Landwirthschaftliche Akademie at Bonn from 1867 to 1898, when he retired as *emeritus*, died on January 16, at the age of 80. He was a native of Pratau, near Wittenberg, studied under KUNTH, LINK, and ALEXANDER BRAUN in Berlin, where he served as assistant for seven years. After three years in St. Petersburg he went in 1859 to the agricultural school at Waldau, and in 1867 to Bonn. He was the author of monographs on Eriocaulaceae, Rapataceae, and Marantaceae. His important work, *Die Arten und Varietäten des Getreides*, was published at Bonn in 1885.

PROFESSOR W. A. KELLERMAN, in charge of the Department of Botany at Ohio State University, Columbus, died on March 8 in Guatemala from an attack of malarial fever, at the age of 57 years. Last December he went to Central America with a party of students to collect parasitic fungi and to study tropical vegetation, and this sudden death is a sacrifice to scientific zeal. His publications include such books as *The flora of Kansas,* a text book entitled *Elementary botany*, and numerous papers; but he was chiefly interested in the *Journal of Mycology*, of which he was editor and proprietor, and in the more recently established *Mycological Bulletin*, to which he held the same relation. He was a native of Ohio, graduated at Cornell University in 1871, received his doctor's degree at the University of Zurich in 1881, and held positions in a state normal school of Wisconsin, the State Agricultural College of Kansas, and the State University of Ohio, where he was in charge of the botany since 1891. Professor KELLERMAN was not only an enthusiastic student of his chosen subject, but he was also a man of fine and genial presence, who attached to himself all those who came into personal relations with him.

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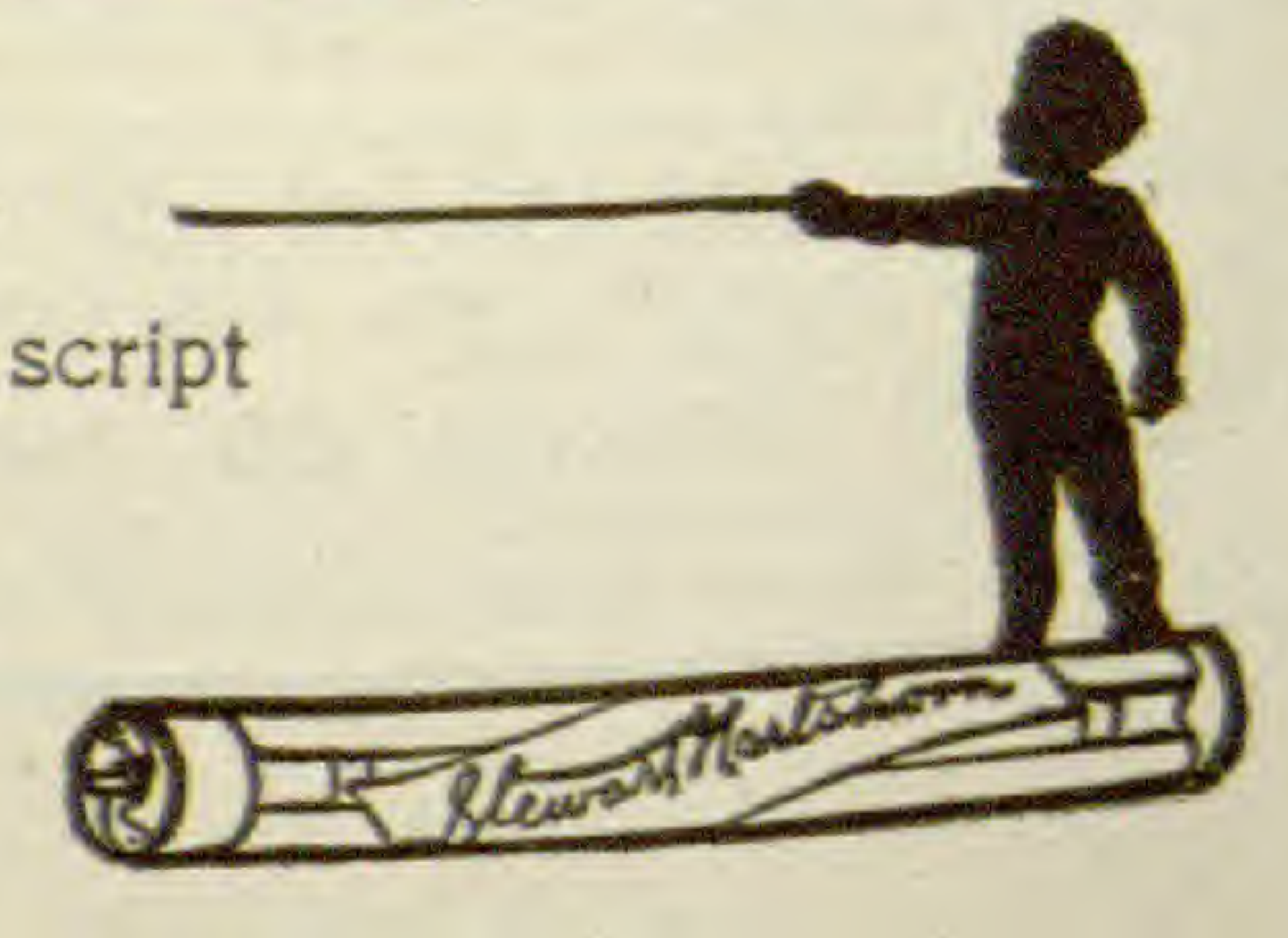
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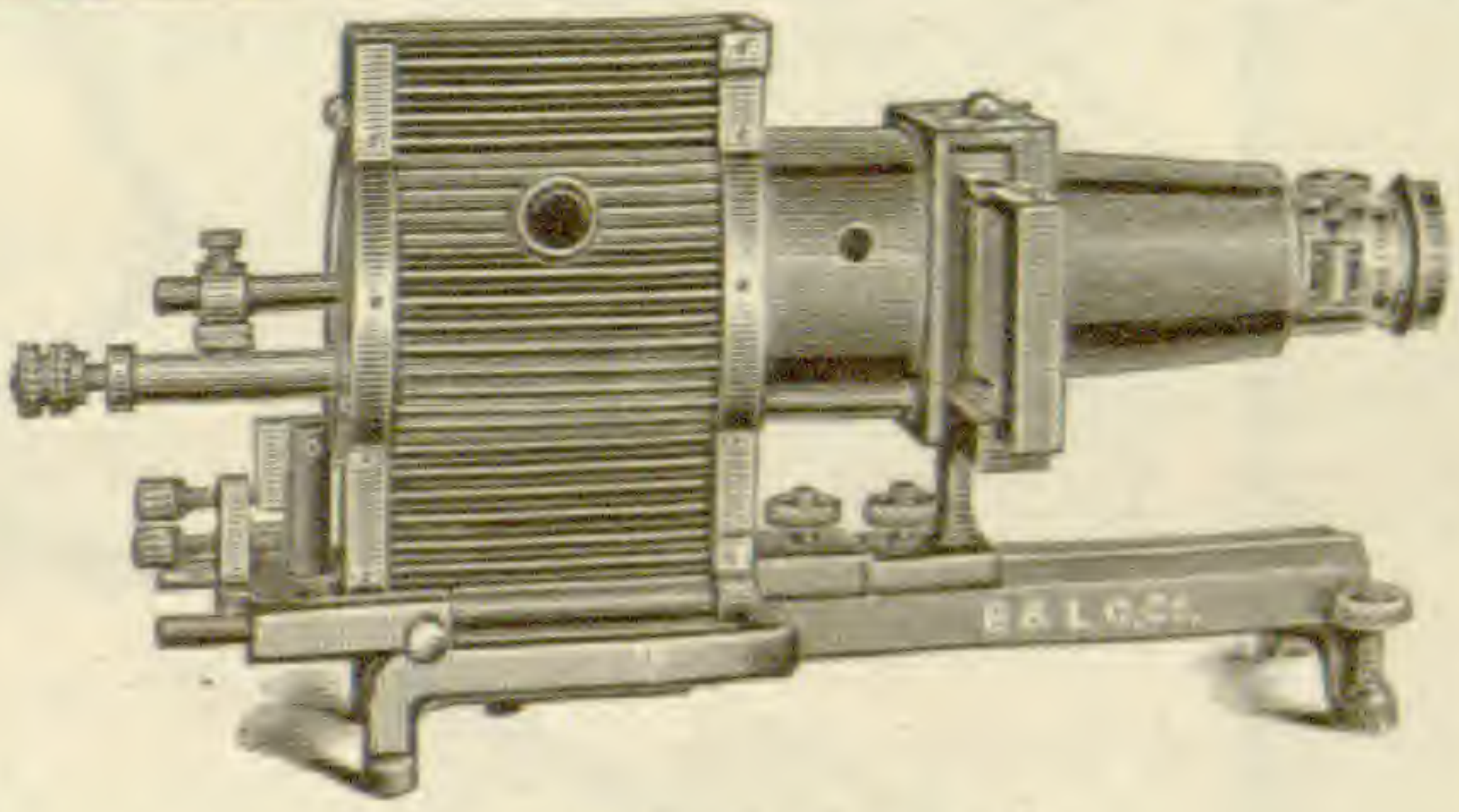
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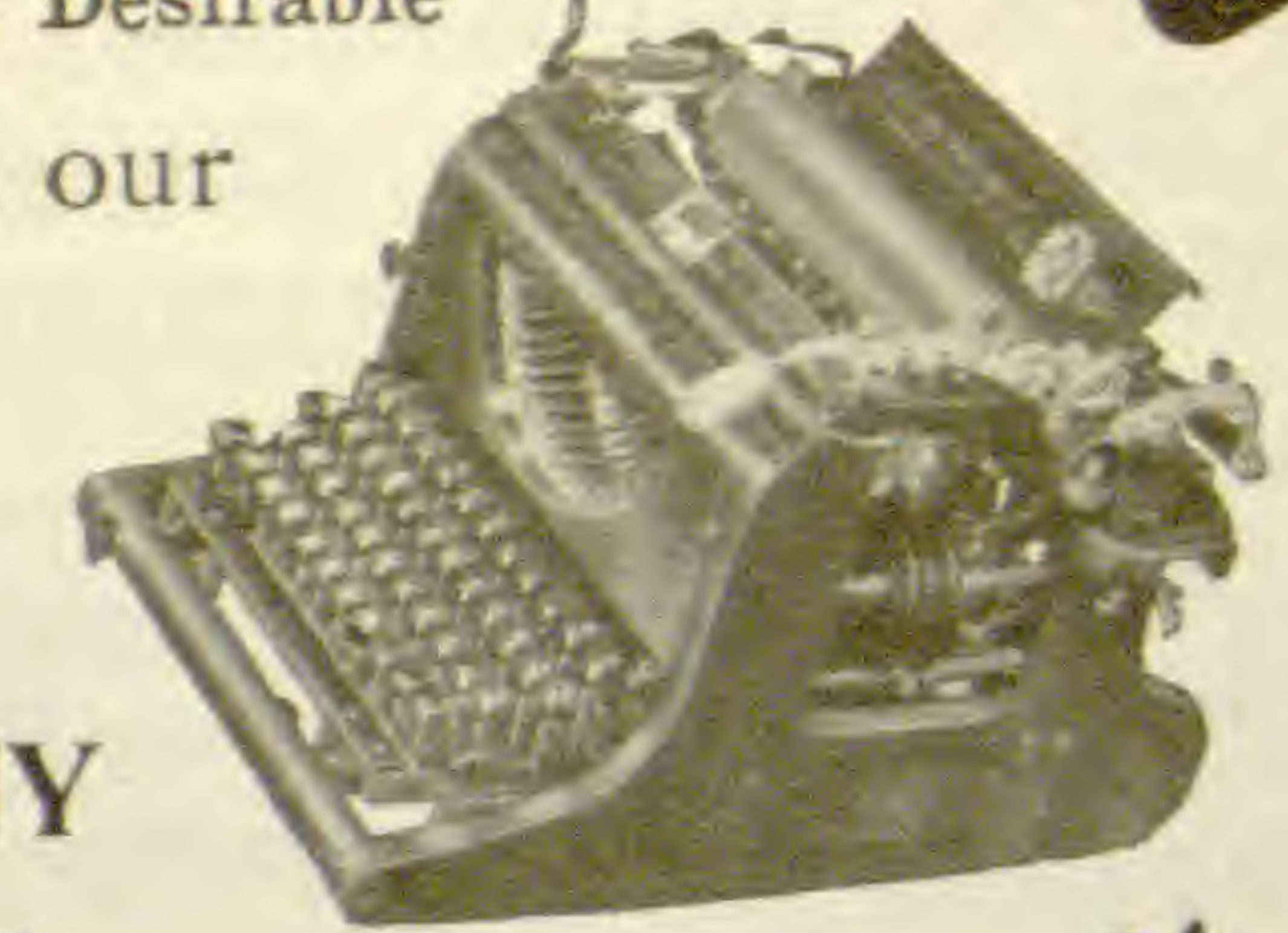

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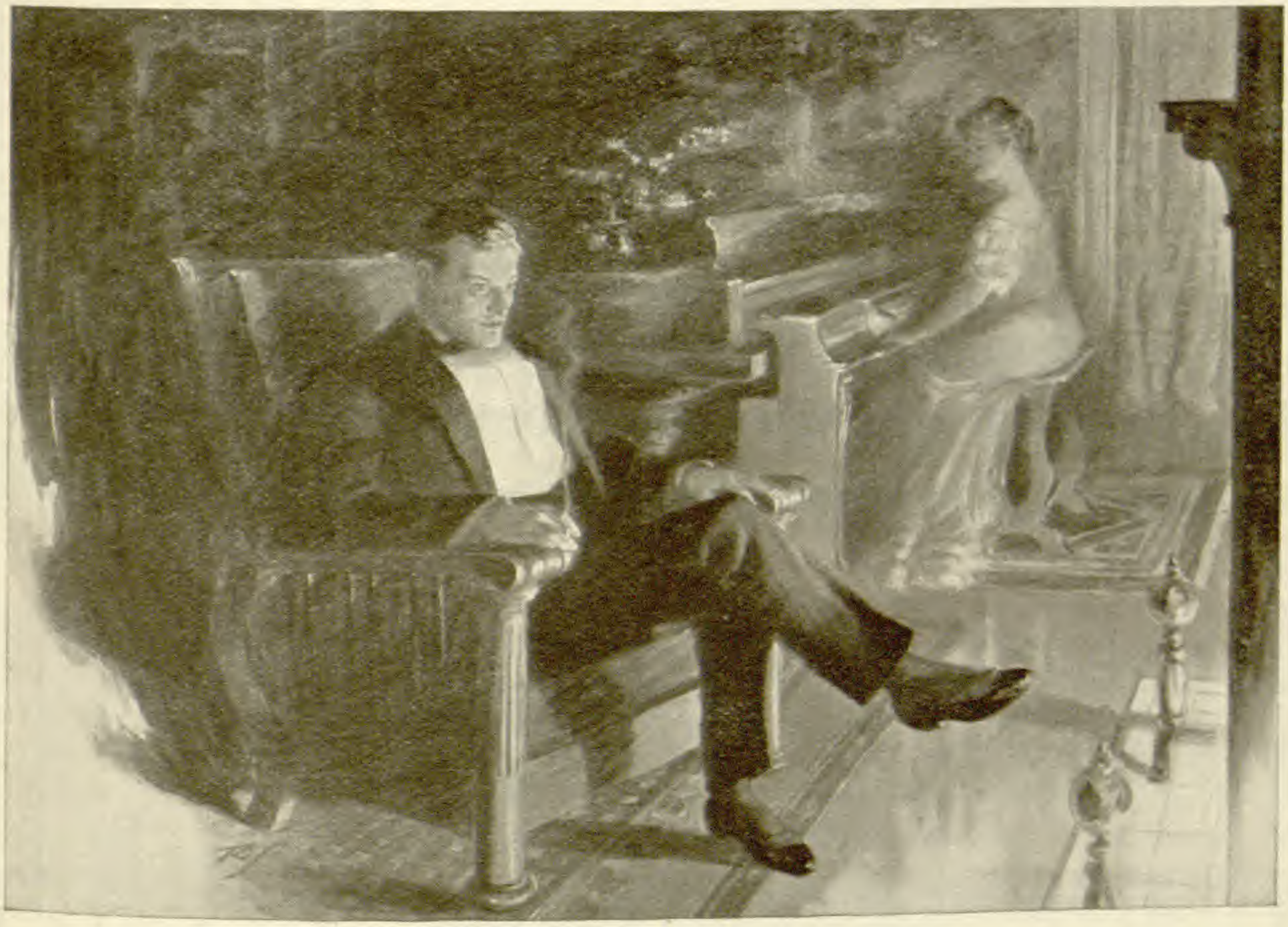

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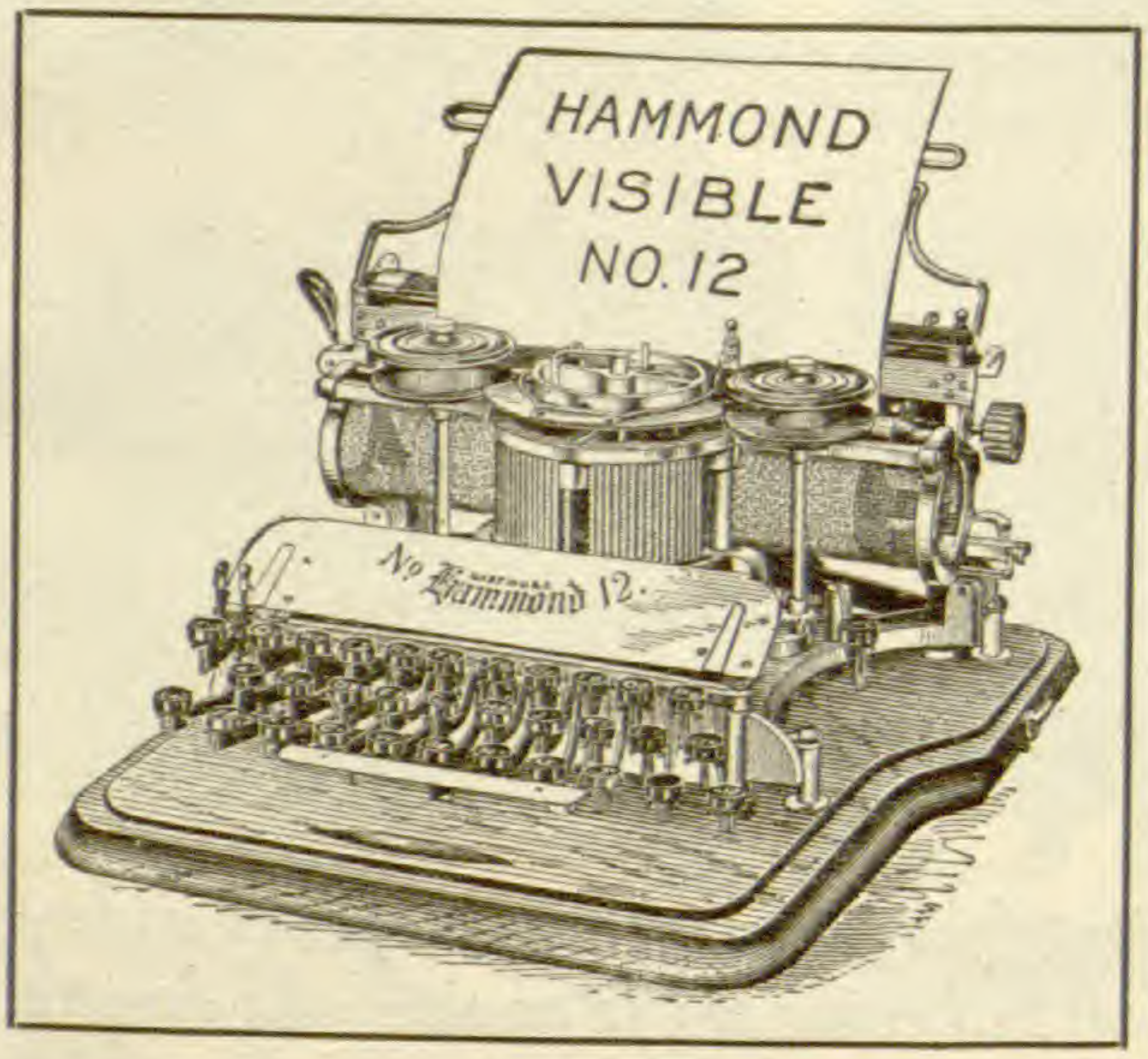
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THE
BOTANICAL GAZETTE

May 1908

Editors: JOHN M. COULTER and CHARLES R. BARNES

CONTENTS

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A Monthly Journal Embracing all Departments of Botanical Science

Edited by JOHN M. COULTER and CHARLES R. BARNES, with the assistance of other members of the botanical staff of the University of Chicago.

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CONTENTS FOR MAY 1908

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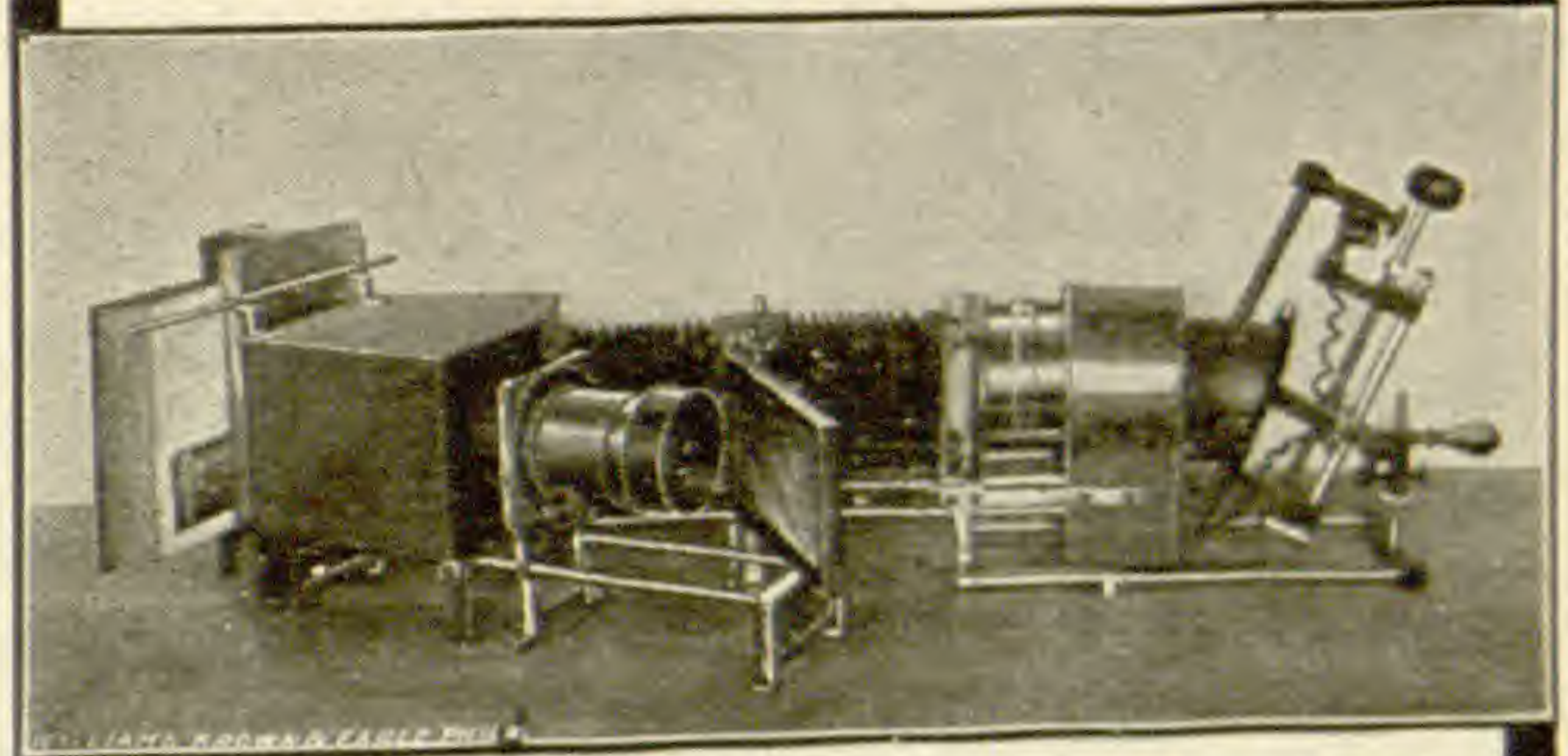

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Heretofore the University of Chicago has contributed approximately $2,000 annually toward the support of the Botanical Gazette and it is not probable that the amount of this subsidy can be increased. At the same time, the cost of publication has been increasing, until the editors are now compelled to decide whether the number of pages shall be reduced or the subscription price increased.

On the basis of material standards, where the manufacturing cost lies, it appears that the Botanical Gazette has been giving 47 per cent. more pages, 380 per cent. more plates, and 50 per cent. more text figures, for 23 per cent. less money than the average of five European journals of the first rank.

A tabulated comparison of the number of pages, figures, and plates, and the domestic subscription prices of these journals for 1906 and 1907 is submitted for your information. They have been selected because they are of the same general type as the Botanical Gazette.

Journal	Year	Vol. no.	No. pages	No. plates	No. text figures	Price (domestic)
Botanische Zeitung	1906	64	*816	8	0	$6.00
	1907	65	*816	5	39	6.00
Flora	1906	96	544	5.	258	5.00
	1907	97	430	14	134	5.00
Jahrbücher für wissenschaftliche Botanik	1906	43	628	5	82	7.00
	1907	44	724	7	63	9.00
Annales des Sciences Naturelles, Botanique	1906	3, 4	766	11	257	6.00
	1907	5, 6	745	12	304	6.00
Annals of Botany	1906	20	474	35	43	7.50
	1907	21	543	19	41	7.50
Botanical Gazette	1906	41, 42	983	46	177	5.00
	1907	43, 44	907	44	187	5.00
Average for above five journals			648	12	122	6.50
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It is evident from this comparison that the Botanical Gazette is far below the standard of price set by these journals; and the cost of manufacture abroad is decidedly less than in this country.

The advice of numerous botanists has been sought and freely given, and with great unanimity their opinion favors the maintenance of the present standard of size (80 pp., which we have not always been able to maintain), with an increased subscription price, for it seems evident that the pressure for publication is increasing rather than diminishing. Furthermore, these botanists have suggested a more rigid selection of original papers, a greater compression of these papers in text and illustrations, a franker expression of opinion in reviews, and the abandonment of the department of "News." These suggestions will be adopted.

In view of the whole situation, it has been decided to increase the annual subscription to $7.00, in the belief that the amount represents a fair charge for the service rendered. It is not expected, however, that the increased income from subscriptions will be sufficient (together with the present subsidy) to do more than enable us to maintain the present size and to improve, where necessary, the illustrations.

The new rate will apply to subscriptions beginning with the July number (1908) and thereafter.

VOLUME XLV NUMBER 5

BOTANICAL GAZETTE

MAY 1908

APOGAMY IN NEPHRODIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 109

SHIGÉO YAMANOUCHI

(WITH PLATES IX AND X, AND THREE TEXT FIGURES)

Introduction

The term apogamy was proposed by DE BARY in 1878, following FARLOW's discovery (**21, 22**) that in *Pteris cretica*, under artificial culture, the sporophyte is developed from the gametophyte with the suppression of the sexual act. Since this discovery, the apogamous development of a sporophyte as a vegetative outgrowth from the gametophyte in pteridophytes, together with the phenomena of parthenogenesis, where the sporophyte is developed from an unfertilized egg, has been described in many forms.

FARLOW (**22**), in contrasting the apogamous embryo with the normal one, notes the following four points: (1) the apogamous embryo is intimately connected with the prothallium in such a way that one cannot decide where the one begins and the other ends; (2) there is formed no foot or equivalent organ; (3) the vascular bundle of the sporophyte is in direct connection with vessels which lie wholly in the prothallium; (4) the order of evolution is different, a leaf arising first and becoming tolerably well developed before the root and afterward the stem make their appearance.

FARLOW's investigation was followed by an extensive study of DE BARY (**1**) on a number of forms in Polypodiaceae, in which he described a similar sporophytic growth in *Aspidium Filix-mas cristatum* and *A. falcatum*. He records various conditions of the development of sexual organs in apogamous prothallia: in *Aspidium*

Filix-mas cristatum archegonia are apparently absent, in *Pteris cretica* they never fully developed; although all prothallia bear more or less numerous antheridia.

SADEBECK in the following year reported apogamy in *Todea africana* (in SCHENK'S *Handbuch der Botanik* **1**:233. 1879). And later apogamy was found in *Osmunda regalis* and *Ceratopteris* (LEITGEB **38**); *Todea rivularis, T. pellucida* (STANGE **63**); *Doodya caudata* (STANGE **63**, HEIM **29**); *Trichomanes alatum* (BOWER **6**); *Selaginella rupestris* (LYON **43**); *Trichomanes Krausii, Pellaea flavens, P. nivea, P. tenera, Notochlaena Eckloniana, N. sinuata, N. Marantae, Gymnogramme farinifera* (WORONIN **78, 79**); and in some others.

LANG'S study (**36**) of the apogamous development of the sporophytes on prothallia of several forms of Polypodiaceae is the most detailed contribution on apogamy in pteridophytes. The paper presents a discussion of the phenomenon in relation to alternation of generations, and adds detailed descriptions of the results of cultivating prothallia grown from spores, for a period of two years and a half in the following fourteen forms: *Aspidium aculeatum* Sw. var. *multifidum* Woll, *A. angulare* Willd. var. *foliosum multifidum,* var. *acutifolium multifidum* (no apogamy seen), *A. frondosum* Lowe; *Athyrium nipponicum* Mett., var. *cristatum, A. Filix-foemina* Bernh. var. *percristatum* Cousens, var. *cruciato-cristatum,* var. *coronatum* Lowe; *Nephrodium dilatatum* Desv. var. *cristatum gracile, N. Oreopteris* var. *coronans* Barnes; *Polypodium vulgare* L. var. *grandiceps* Fox; *Scolopendrium vulgare* Sm. var. *ramulosissimum* Woll, var. *marginale.*

According to his account, the apogamous growth resulted from artificial cultures, watered entirely from below and exposed to direct sunlight, important departures from the normal conditions surrounding fern prothallia. The asexual sporophytic outgrowth from the prothallia present some minor differences in different individuals, but, taken as a whole, they arose in the following ways: as leaves, roots, and ramenta directly on the prothallia or on a cylindrical process from the prothallium; as a continuation of the process as a leaf; as sporangia on the process from the prothallium; as tracheids in the prothallia or in the middle lobe and cylindrical process.

It seems hard to draw from these experiments any more precise conclusion than that the normal life-history is checked at a critical period (fertilization) and that the plant is forced into an expression of vegetative activity. Apogamy brings forward also the theory of the homologous origin of alternation as contrasted with the antithetic. The theory of the homologous origin of the alternation of generations, as held by PRINGSHEIM (**55, 56**) and SCOTT (**61**), is discussed by LANG in his studies on apogamy. He is inclined to the opinion that apogamy and apospory in ferns support the homologous view, since the prothallium can so readily take on sporophytic characters and the sporophyte can develop the gametophyte aposporously. He recognized, however, that all speculations on these points must be tentative until the actual nuclear conditions in apogamy and apospory have been ascertained.

The investigations mentioned above have greatly extended our knowledge concerning the phenomena of apogamy and apospory, and some of them have contributed much to elucidate the structural features involved, but cytological details of apogamy in pteridophytes remained unknown until last year, when there appeared two papers, one by FARMER and DIGBY (**24**) and the other by STRASBURGER (**68**).

FARMER and DIGBY'S paper is one of the most important of recent contributions to apogamy in ferns. Their preliminary note (**23**), published four years ago, announced the discovery of nuclear fusions in the vegetative cells previous to the apogamous sporophytic outgrowths from prothallia. The final paper deals with the results of their studies on apogamy and apospory in the following seven forms: *Lastrea pseudo-mas* vars. *polydactyla* Wills., *polydactyla* Dadds, *cristata apospora* Druery; *Athyrium Filix-foemina* vars. *clarissima* Jones, *clarissima* Bolton, and *unco-glomeratum* Stansfield; and *Scolopendrium vulgare* var. *crispum Drummondae.*

All the prothallia of the five forms used for their investigations, excepting the two *polydactyla* varieties, were aposporus outgrowths, either on peripheral cells of unmatured sporangia, from sori of sterile sporangia, or on apices, surface, or margins of pinnae. In two forms —var. *unco-glomeratum* and var. *cristata apospora*—the aposporous prothallia were induced artificially by pegging detached pinnae down on damp soil. The two varieties *polydactyla* of Lastrea, producing ordinary spores and prothallia, were obtained by sowing the spores.

In these apogamous prothallia antheridia are always produced in profusion and sperms are matured in every case, but the development of archegonia is different in different forms: sometimes no archegonia are produced (var. *polydactyla* Wills. and *cristata apospora*), or some are formed but do not attain maturity (var. *clarissima* Jones), and in these two cases sporophytes appeared apogamously as vegetative outgrowths; in other cases an egg is produced in the normal way, but there is no fertilization, the embryo being developed either from the unfertilized egg (var. *clarissima* Bolton and Scolopendrium) or endogenously in connection with an archegonium (*unco-glomeratum* Stansfield).

So far as the number of chromosomes is concerned, these seven cases of apospory and apogamy may be placed in three categories: (1) in the two vars. *polydactyla*, in which sporogenesis shows the ordinary reduction, a doubling of chromosomes is attained by the fusion of vegetative nuclei instead of by fertilization; (2) in four forms—three varieties of *Athyrium Filix-foemina* and a Scolopendrium—sporogenesis is omitted from the life-cycle, prothallia arise directly from abortive sporangia or pinnae, and sporophytes develop apogamously from the prothallia, the approximate sporophytic number of chromosomes being retained throughout the cycle; (3) in var. *cristata apospora*, in which apospory and apogamy regularly follow each other, the approximate number of chromosomes through the life-cycle is 60, and the authors believe that in this case the sporophyte may retain the original gametophytic number of chromosomes, the suggestion being based upon the fact that the number 60 approximates 72, the gametophyte number of the type species *Lastrea pseudo-mas*.

Throughout the investigation the authors met a variable number of chromosomes in different parts of an individual, and they express the belief that such variations are not due entirely to errors of counting, but that they correspond to fluctuations in the number actually present in the different regions. Their general conclusions are as follows: there is no necessary correlation between the periodic reduction in the number of chromosomes and the alternation of generations. Fertilization and reduction, however, are recognized as holding a very definite relation to one another, but without any *a priori* grounds for assuming any necessary connection between either of them and any other feature in the life-history; and therefore the problem of alternation must be settled by an appeal to evidence other than that derived from the facts of mitosis.

STRASBURGER (**68**) studied apogamy in Marsilia from the cytological standpoint. Previous to his work, parthenogenesis in Marsilia had been described by two observers. Almost ten years ago SHAW (**62**) found normal parthenogenesis, as it was called by the author, to be of frequent occurrence. He also isolated megaspores of *Marsilia Drummondii* from microspores before the sperms matured, and over 50 per cent. of the isolated female prothallia produced embryos, while not more than 69 per cent. of those which were mixed with male prothallia produced embryos. Four years later NATHANSOHN (**48**) induced parthenogenesis in *Marsilia vestita* and *M. macra.* He found that it was possible to stimulate the egg cell to a parthenogenetic development by exposing the germinating megaspores to a temperature of 35° C. for 24 hours, and allowing them to continue their development at a temperature of 27° C. As a result, about 7–12 per cent. of the spores gave rise to parthenogenetic embryos. Under lower temperatures the egg developed an embryo only after fertilization. No nuclear conditions were noted by SHAW or NATHANSOHN.

In parthenogenesis in seed plants, a reduction of chromosomes does not occur in the formation of the embryo sac, so that the egg nucleus contains the diploid number, which naturally obviates any necessity for the act of fertilization. Considering parthenogenesis in seed plants, there arises naturally a question as to how the egg nucleus in Marsilia, which usually establishes a new sporophyte after a normal act of fertilization, could have developed parthenogenetically into the sporophyte. A clear explanation of this question from the cytological standpoint was made by STRASBURGER in his recent paper (**68**).

STRASBURGER states that the classification into species in Marsilia cannot always be relied upon. He finds that megaspores of BRAUN'S *Marsilia Drummondii* develop embryos habitually by parthenogenesis, and that in three species—*M. vestita, M. aegyptica,* and *M. quadrifolia*—embryos are formed only after fertilization.

In *Marsilia Drummondii* he finds that the nuclei of the prothallia in the megaspores contain 32 chromosomes, the diploid number, as found in the root tips and other vegetative structures of the sporophyte.

One or two ventral canal cells always persist without disorganizing, which may also prevent the entrance of sperms and check the act of fertilization.

In *Marsilia vestita*, the species worked by NATHANSOHN, STRASBURGER tried NATHANSOHN'S method of inducing parthenogenetic growth, but did not obtain a single parthenogenetic embryo. In this species the sporophytic number 32 is reduced to 16 in sporogenesis, and hence under natural conditions an embryo should develop only after fertilization. He found similar normal conditions in *M. quadrifolia*, *M. elata*, and *M. hirsuta*, whose chromosome number is the same as that of *M. vestita*.

According to his detailed account of sporogenesis in *Marsilia Drummondii*, the number of megaspore mother cells is less than 16, the usual number in normal forms, and sometimes only four. In diakinesis 32 chromosomes appear, and in the metaphase there were observed two kinds of mitotic figures, one of heterotypic type with 16 bivalent chromosomes, and the other of vegetative type with 32 univalent chromosomes. In both cases a second division follows, so that two kinds of megaspore tetrads are formed, one with the $2x$ or diploid number of chromosomes, and the other with the x or haploid number. The proportion of these two kinds of spores differs in each individual form; for instance, in GOEBEL'S material he found the megaspores with the diploid number only. In microsporogenesis there was observed a tendency toward forming a heterotypic figure, but no mature sperms developed; two species, *M. macra* and *M. Nardu*, behaved similarly.

Such a megaspore forms a prothallium whose nuclei have the diploid number of chromosomes, which pass to the egg nuclei, so that the sporophytic number of chromosomes is maintained throughout the life-history, as in cases of parthenogenesis known among seed plants; however, the case found in *Marsilia Drummondii* by STRASBURGER, where a tetrad division occurs not accompanied by chromosome reduction, seems to be a condition never described before, because most of the cases of parthenogenesis known in seed plants are characterized by the omission of the tetrad division as well as the accompanying reduction.

STRASBURGER calls the phenomenon in Marsilia apogamy, maintaining the principle stated in his paper on parthenogenesis in EUALCHEMILLA (**66**), that the asexual development of an embryo from the gametophyte with the diploid number of chromosomes, whether the embryo comes from an egg or a vegetative cell, should be regarded as apogamy; while the term parthenogenesis should be reserved for the asexual development of an egg with the haploid number of chromosomes and consequently capable of being fertilized. WINKLER (**76**) opposed STRASBURGER'S view concerning the application of the terms apogamy and parthenogenesis. The difference of opinion concerns not only the question of terminology, but also involves theoretical views regarding the significance of the number of chromosomes, which will be considered later.

The present investigation on apogamy in *Nephrodium molle* Desv. was undertaken in the hope of adding something to our knowledge concerning the cytological interpretation of the phenomenon of apogamy. As was stated in the preliminary note, the writer was convinced of the necessity of understanding beforehand the nuclear conditions throughout the whole normal life-history of this species. Consequently, first sporogenesis, and second spermatogenesis, oogenesis, and fertilization were studied. These results were published in two preceding papers (**81, 82**).

Material and methods

All of the apogamous prothallia used in this investigation were raised from ordinary spores, secured from the same material used for the study of sporogenesis, which was collected from the greenhouses of the Hull Botanical Laboratory, of Lincoln Park, and of Washington Park, Chicago.

Spores were sown upon sterilized soil consisting of a mixture of vegetable mold and sand, placed in the greenhouse, and kept growing with special care since October 1906. Some of the prothallia presented certain peculiarities, one being different from the rest in the same pots, but in general the differences held no relation to apogamous development. Antheridia and archegonia were produced in profusion. Nuclear conditions in the vegetative cells, as well as in spermatogenesis, oogenesis, and fertilization, were studied in this material and the results were published in the preceding papers (**81, 82**).

In a number of pots placed in saucers filled with water like the rest, watering from above was avoided and the cultures were exposed to direct sunlight after the prothallia had developed two or three cells. The excessive evaporation from the soil was regulated carefully, so as not to permit condensation on the prothallia, and allow fertilization. Thus the prothallia were kept growing for a long period in dryness and in exposure to direct sunlight, the temperature of the room being kept at 28°–32° C. No fungi or lower algae developed in the pots.

The rate of growth of these prothallia when compared with that of those placed in normal conditions was quite slow. Antheridia appeared earlier than under normal conditions and were very numerous. About five or six weeks after the prothallia of two or three cells were examined, there was observed a peculiar thickening in the cushion region of some of the prothallia which reached the cushion condition earlier than the rest. This thickening was determined afterward to be the initiation of an apogamous sporophytic outgrowth. During the next three or four weeks the growth of the sporophyte was rather rapid, and at the end of that time it had become leafy. Fixation of the prothallia was made during all stages of development.

The killing and fixing of the material, with washing, imbedding, cutting, and staining, were done by the method used in the study of spermatogenesis, oogenesis, and fertilization.

This investigation was begun in October 1906, at the suggestion of Professor JOHN M. COULTER and Dr. CHARLES J. CHAMBERLAIN, and I wish to express my sincere gratitude to these gentlemen for their kind advice and criticism. I am also under obligation to the other members of the botanical staff for many courtesies.

Description of the apogamous prothallia

VEGETATIVE MITOSIS

The prothallia which produce sporophytic outgrowths apogamously do so under the influence of artificial culture. The mitoses which occur up to the 30–50-celled stage are exactly similar to those in normal prothallia, but beyond that stage the morphological structure seems to become influenced by the artificial conditions. The growth becomes very slow, and the cells show a tendency to increase greatly in size, while under normal conditions mitosis would occur before such a size had been reached. Probably for this reason mitotic figures are less frequently met during the growth of such prothallia. As the cell increases in size, its nucleus grows large and the mitotic figure is generally larger than in normal prothallia of this species.

It was not difficult to find stages of mitosis in the vegetative cells, and their comparatively large size facilitated the accurate counting of chromosomes. The resting nucleus contains a delicate reticular structure consisting of a mixture of ragged clumps and slender threads of chromatin. Nucleoli with conspicuous peculiarities of form are always present; sometimes there are two to several isolated round nucleoli scattered irregularly within the nucleus, and sometimes part of them are arranged into a group or groups. They are likely to be mistaken for chromatin nucleoli, but after a close examination of serial stages in the development of the chromosome it is clear that they lie entirely free from the chromatin network and do not seem to contribute any material to the chromosomes by direct transformation. Cell contents beside the nucleus and cytoplasm are not so abundant as in normal cases, the cell cavity consisting largely of vacuoles.

In prophase, the spirem is developed from the chromatin reticulum (*fig. 4*) as described for normal prothallia. The metaphase (*fig. 5*) and anaphase (*figs. 6, 7*) show no peculiar deviation from the typical mitosis. The number of chromosomes is 64 (*fig. 8*) or 66. When two daughter nuclei are reconstructed, a cell plate is laid down between them which finally divides the mother cell into two cells. In this material the binucleate condition was seldom observed, so that it may be claimed that the telophase of mitosis in the vegetative cell is always followed by cell division, and that there is no migration of the nucleus of one cell to an adjacent one.

As stated before, mitosis in the vegetative cells of the normal prothallia and in those reproducing apogamously agree except as to the axis of the spindle, which does not hold any regular relation to the surface of the prothallia, the cell walls being laid down in various directions. This is quite contrary to the condition in normal prothallia, in which, at least up to the appearance of the archegonium initial, the walls are formed somewhat regularly, being more or less perpendicular or parallel to the surface of the prothallium.

SPERMATOGENESIS

Every apogamous prothallium bears antheridia in profusion, the antheridium initial being formed earlier than in normal prothallia of similar size. The formation of the primary spermatogenous cell, which takes place as in normal prothallia, is studied more readily on account of the comparatively large size. The mitoses from the primary spermatogenous cell to the formation of spermatids and sperms showed no deviation from spermatogenesis in normal prothallia. During these mitoses 64 or 66 chromosomes could always be counted. The peculiar structures which were observed within the cytoplasm in the primary spermatogenous cell of the normal prothallia were also present here, but they seemed to be undoubtedly plastids.

The sperms are actively motile and are attracted by 0.01 per cent. solution of sodium malate. From the similarity of the genetic development, morphological structure, and characteristic response to the chemotactic stimulus, it is clear that sperms formed in such prothallia can function when conditions permit.

An irregularity in the axis of mitoses was observed during the cell divisions within the antheridium: in most cases the first wall which divides the primary spermatogenous cell vertically is followed by two or three vertical walls parallel to the first, before any transverse division takes place.

OOGENESIS

While antheridia and functional sperms are formed in abundance, archegonia are rare in these prothallia. The power of forming archegonia seems to be almost suppressed; and the cushion region where archegonia generally arise is very often covered with antheridia instead of archegonia. In extremely rare cases, however, there appears an archegonium initial, from which a central cell is cut off as in normal prothallia. The central cell either remains without any further division and imbedded below a superficial cell, or develops into canal cells and an egg cell, the projecting neck cells being poorly developed. The central cell, when it remains undivided, grows to a considerable size, with a corresponding increase in the size of its nucleus, which finally assumes a form similar to that of an egg, but it always appears collapsed. When canal cells and egg are formed, they also appear collapsed. The collapsed appearance of the central, canal, and egg cells is likely induced by the artificial treatment of the prothallia rather than by fixing reagents.

Whether the egg in such a collapsed condition is still capable of fertilization, is questionable, but the writer is inclined to believe that it is incapable of functioning. No case of a sperm having entered an egg was found.

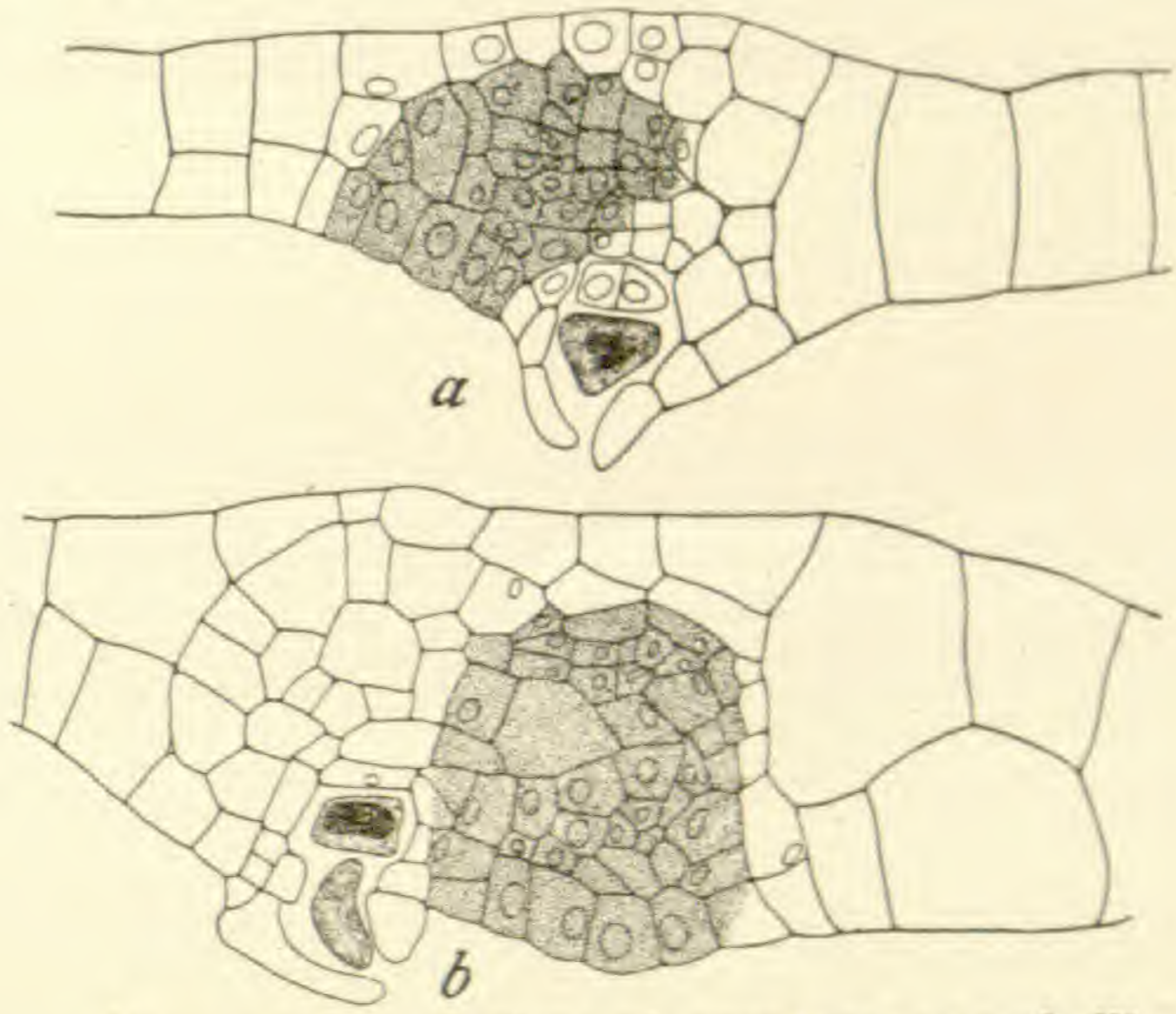

FIG. 1.—Two sections of apogamous prothallia, showing two different stages in the development of archegonia; the shaded regions represent sporophytic outgrowths; *a*, archegonium with central cell; *b*, archegonium with canal cells and egg.

Not only is the formation of archegonia extremely rare, but when formed they are very much belated. In all cases in which their formation was observed, apogamous sporophytic outgrowths were already in an advanced stage of development (*figs. 1a, b*). Hence it is reasonable to suppose that in the apogamous prothallia a tendency to develop sporophytic outgrowths becomes predominant when the power of forming archegonia becomes weak.

SPOROPHYTIC OUTGROWTHS

As described before, during the early development of prothallia, mitoses occur in the vegetative cells just as in normal prothallia, except that the mitotic figures are comparatively large. The mitoses continue in the vegetative cells and there are organized prothallia of a single cell layer in thickness. The general outline of the prothallia does not show any peculiarities which might be regarded as characteristic of apogamous prothallia as distinct from normal ones.

Sporophytic outgrowths begin very early from cells in the region where later the cushion arises, so that the development of the sporophytic outgrowth and the gradual completion of the cushion proceed side by side for a while. When the prothallium has assumed the characteristic heart shape, with a cushion near the sinus and an extensive lateral growth of prothallial tissue on either side, the sporophytic outgrowth is usually in a well-advanced stage. The main features of the formation of this outgrowth and of the gradual completion of the cushion are as follows:

Previous to the formation of the cushion, mitoses take place in rapid succession in the vicinity of the sinus, partition walls always being laid down perpendicular to the surface of the prothallium and parallel to one another, so that the cells formed are very narrow. Some of these mitoses in different stages are represented in *figs. 9–11*. Mitosis continues and cell plates are laid down parallel or oblique to the surface of the prothallium, the ultimate result being the initiation of a thick cushion region (*figs. 12, 13*).

One of the superficial cells in the cushion region begins at once to increase considerably in size, the increase being accompanied by an excessive growth of its nucleus. The nucleus in the resting condition contains a reticulum of ragged clumps and slender threads of chromatin, from which the spirem of the prophase is established (*figs. 14, 14a*). Two or more nucleoli are always present. Successive stages of the mitosis following the early prophase were examined (*figs. 15–17*), which were exactly similar to those of typical mitosis in the vegetative cells of normal prothallia. In the telophase of this mitosis, when the two groups of daughter chromosomes have reached the poles, a little irregularity in the form of the chromosomes is observed, but the number of chromosomes, before they had become aggregated into a mass, was always 64 or 66 (*figs. 18a, b*).

Consequently, it is perfectly clear that, so far as the chromatin is concerned, no change has occurred in the nucleus of these prothallia up to the formation of the superficial cell. In the late telophase a cell plate is laid down perpendicular to the surface of the prothallium, so that there are formed two superficial daughter cells arranged side by side (*fig. 19*).

A number of mitoses follow in the same way, and thus there is established in a certain region of the superficial layer a group of parallel cells. The increase of the superficial cell is remarkable in apogamous prothallia. In ordinary prothallia, if such a growth in size ever occurs in a superficial cell of the cushion region, it is an archegonium initial; however, the archegonium initial never attains such conspicuous development, surpassing all the other superficial cells in that region. Moreover, the development of the archegonium generally occurs after the cushion region has extended over a comparatively large area and attained some thickness. On the other hand, the increase in size of the superficial cell in an apogamous prothallium commences at an early stage and is associated with the gradual formation of the cushion. The accompanying figures will illustrate the situation. In *fig. 2, a, b* is shown the cushion region, displaying different stages of archegonium development in normal prothallia. *Fig. 3* represents eight successive sections of the same

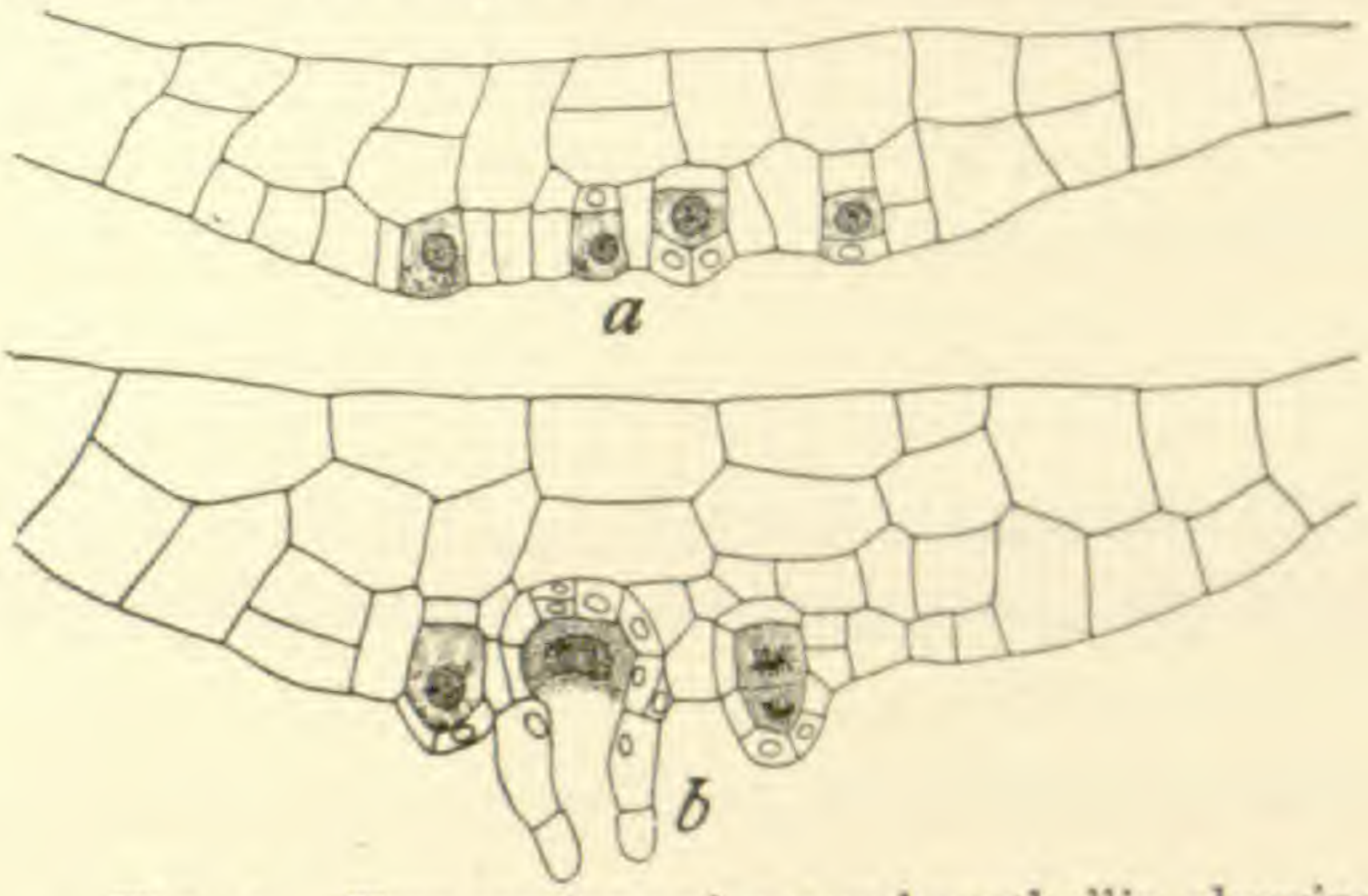

FIG. 2.—Two sections of normal prothallia, showing different stages in the development of archegonia.

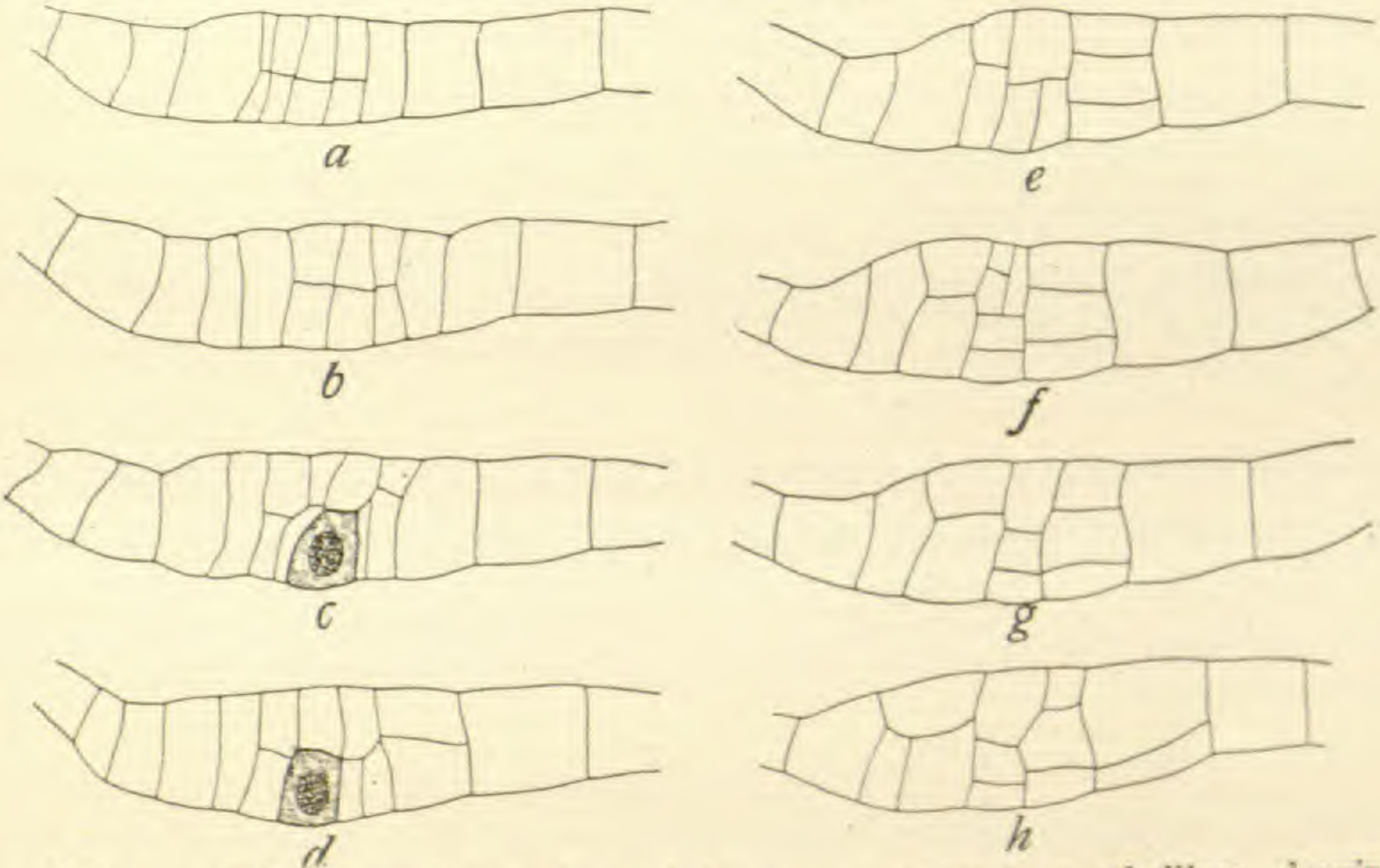

FIG. 3.—Eight successive sections of the same apogamous prothallium showing a single superficial growing to considerable size in a young cushion.

prothallium, which indicate that a single superficial cell has grown to a considerable size in a young developing cushion region. The remarkable increase in size and the characteristic contents made these cells very conspicuous. Sooner or later, from one of these superficial cells an apical cell is cut off by an oblique wall, and becomes the growing point of a leaf. Sometimes an apical cell appears very early, as shown in *figs. 20* and *21*. During these mitoses, the number of chromosomes is always 64 or 66.

While this process is in progress in the superficial region, cells beneath divide in various directions. Mitoses, of course, occur in various parts during the growth of the prothallia, but they occur more rapidly in the interior region which borders the group of superficial cells whose formation was described above.

Some of the characteristic features that distinguish these interior cells from the other prothallial cells are as follows: they are considerably smaller, probably due to their rapid successive divisions; the nucleus, in spite of the smallness of the cell, is large; the cytoplasmic contents are abundant and the plastids are small and few at this stage.

The mitoses in connection with the formation of this group of interior cells were studied. The general aspect of the mitotic figures in successive stages and the behavior of the chromosomes in these mitoses were similar to those of typical mitosis in the vegetative cells of normal prothallia. It was interesting to notice, however, that the narrowness of the cell cavity and the largeness of the nucleus showed a remarkable resemblance to the condition observed during the development of normal embryos later than the 32-celled stage (*figs. 22–29*). In telophase the number of chromosomes was always 64 or 66 (*fig. 27*).

The mitoses in the groups of superficial and interior cells continue as described above, and there is formed a structure which is in direct connection with the prothallial cells, the structure that later becomes an independent sporophyte. *Figs. 28* and *28a* represent the structure of the sporophytic outgrowth at a certain stage in the course of its development. One apical cell which is already differentiated in the structure is not shown in this section.

Thus the structure of the sporophytic outgrowth is brought about apparently by the cooperation of the mitoses of two different regions, one of which is a single superficial cell that has attained a conspicuous size, and the other the vegetative cells immediately beneath. If we trace further back the origin of the cells of these regions, they are found to be descendants from the vegetative cell or cells whose sister cells have organized the cushion region. It was impossible to detect a distinct period in which any change had occurred in the chromatin condition up to this development of the sporophytic outgrowth; practically the nucleus of the gametophyte has become directly the nucleus of the sporophytic outgrowth, without any nuclear fusion.

The sporophytic structure develops with repeated mitoses of the same sort as was described before: a leaf and a stem apex are developed from two apical cells which have appeared one after the other; a root initial is organized endogenously (*fig. 29*); scalariform vessels appear in the tissue connecting the leaf and stem origins with the root initial; and finally there is developed an independent leafy sporophyte.

From the foregoing it is clear that there is established a sporophyte with the haploid or x number of chromosomes in *Nephrodium molle*. This is the first instance yet known in plants, in which a sporophyte with the haploid number of chromosomes has been described.

Whether the sporophyte thus formed may produce spores has not yet been determined.

Discussion of cytological phenomena

APOGAMY

Since the first discovery of apogamy in *Pteris cretica*, instances of apogamy in pteridophytes have steadily increased until the phenomenon is now known in about fifty forms. No cytological studies were recorded until the appearance of the papers of STRASBURGER and of FARMER and DIGBY, already cited, but there had appeared several cytological studies of the apogamous development of the embryo in spermatophytes.

Apogamy (parthenogenesis and vegetative apogamy) is now known in spermatophytes for *Allium odorum* (TRETJAKOW **69**, HEGELMAIER **28**), Balanophora (TREUB **70**, LOTSY **39**), *Antennaria alpina*

(JUEL **30, 31**), several species of Alchemilla (MURBECK **44, 45, 46**; STRASBURGER **66**), *Thalictrum purpurascens* (OVERTON **53, 54**), *Gnetum Ula* (LOTSY **40**), several forms of Taraxacum (RAUNKIAER **57**; MURBECK **47**; JUEL **32, 33**), a number of species of Hieracium (OSTENFELD **50, 51**; MURBECK **47**; ROSENBERG **59**), and *Wikstroemia indica* (WINKLER **75, 76**). Among these contributions those of JUEL, MURBECK, OVERTON, STRASBURGER, WINKLER, and ROSENBERG present some very interesting cytological data.

JUEL (**30, 31**) made a comparative study of the parthenogenetic Antennaria and the normally fertilized *A. dioica*. In the latter a tetrad is formed from a megaspore mother cell, with the usual reduction of chromosomes, and the embryo sac is developed from one of the megaspores. There is a typical synapsis preceding a heterotypic mitosis, and the embryo sac is normal. The reduced number of chromosomes is 12–14 in the pollen mother cell and 20–24 in the integument. In the parthenogenetic *Antennaria alpina*, not only is the tetrad suppressed, but there is no trace of heterotypic and homotypic mitosis in the embryo sac. The number of chromosomes is 40–50 in the embryo sac and 45–50 in the integument. There is thus no reduction of the chromosomes during the formation of the embryo sac, and the egg retains the sporophytic number.

MURBECK (**44, 45, 46**) studied eight species of Alchemilla, chief attention being paid to the parthenogenetic *A. alpina*. In the parthenogenetic species of EUALCHEMILLA, he found that the embryo sac always developed from one megaspore of the tetrad produced from the megaspore mother cell through two successive mitoses, in which there seemed to be no evidence of a reduction of chromosomes. The number of chromosomes in these divisions is approximately 32–48, and this number is retained in the egg nucleus and the other nuclei of the embryo sac. He reports that the embryo of *A. sericata* is produced from a synergid. In the normal species *A. arvensis*, belonging to the section APHANES, he finds in the pollen mother cell 16 chromosomes—the reduced number. Two years later, MURBECK (**47**) in a short paper announced that embryos in *Taraxacum vulgare* Raunk. and *speciosum* Raunk. and *Hieracium grandidens*, *serratifrons*, and subsp. *crispatum* develop from flowers whose stamens have been removed, but he did not make any cytological studies. JUEL (**32, 33**) discovered a peculiar development of the embryo sac in the parthenogenetic *Taraxacum officinale.* The species produced pollen with a normal reduction of chromosomes, and 13 bivalent chromosomes were present in the heterotypic mitosis; but the megaspore mother cell undergoes a single mitosis and there are formed two daughter cells, the lower one of which develops into the embryo sac. This mitosis passes through the synapsis and leptonema stages as usual, but the heterotypic figure is not organized; it is a typical vegetative one with the univalent 24–26 chromosomes. The nuclear divisions in the embryo sac were not followed by JUEL, but, accepting the results of MURBECK, he believed that the egg nucleus retained 26 chromosomes—the sporophyte number.

OVERTON (**53, 54**) found a normal reduction in the pollen mother cells of *Thalictrum purpurascens* and showed that the number of chromosomes is 24 for the sporophyte and 12 for the gametophyte. The development of the embryo sac is of two types. In some cases a tetrad is formed from a megaspore mother cell, with all the phenomena of a reduction division; the lowest cell develops into the embryo sac. But many embryo sacs are formed in a different way. The first mitotic figure in the megaspore mother cell is not heterotypic, and shows 24 univalent chromosomes; and the same number is counted in the second division in the parthenogenetic embryo. He concludes that the sporophytic number (24) remains unchanged in the embryo sac in this case, and that the egg nucleus with the sporophytic number develops into the embryo parthenogenetically.

STRASBURGER (**66**) made an extensive study of numerous species of Alchemilla § EUALCHEMILLA, the group which furnished the material for MURBECK's important discoveries. Most of the forms in EUALCHEMILLA develop normal pollen and a reduction division was found both here and in *Alchemilla arvensis* of § APHANES. In the heterotypic mitosis in the pollen mother cell, STRASBURGER found 32 bivalent chromosomes, which MURBECK counted as 16. In the embryo sac development in apogamous species the two characteristic divisions of sporogenesis are cut out and no tetrad is formed. The nucleus of the megaspore mother cell emerges from synapsis with the sporophytic number of univalent chromosomes, and the ensuing division is typically vegetative and not heterotypic. The nuclei of the embryo sac thus contain the sporophytic number and parthenogenetic development of the egg takes place. STRASBURGER regards the parthenogenetic tendency of EUALCHEMILLA as associated with excessive mutability, which has weakened sexuality so that the process of fertilization is being displaced by apogamy.

WINKLER (**75, 76**) reports an interesting case of apogamy in *Wikstroemia indica*. He observed that seeds are produced apogamously, in spite of the fact that some pollen matures. The apogamous development of the embryo was proved by castration experiments. He describes a peculiar phenomenon in the cells of the tapetal layer, which usually contain two to six nuclei. These nuclei fuse into a huge nucleus, whose mitotic figure often shows over 100 chromosomes; but usually he counted 52 chromosomes. Although most of the pollen does not reach maturity, a tetrad division with reduction is present in sporogenesis, and in the heterotypic figure there are 26 bivalent chromosomes. The micropyle of the ovule is closed by the elongation of the inner wall of the ovary during the formation of embryo sac, which undoubtedly may have some relation to the apogamous development of the embryo. A megaspore mother cell in this form becomes directly an embryo sac, with the entire suppression of tetrad division. His material was not sufficient for a cytological study of the mitoses, and consequently he was unable to determine the entire absence of a reduction division in the embryo sac, but it seemed very likely that the egg which develops parthenogenetically may retain the sporophytic number of chromosomes.

ROSENBERG (**59**) presents the result of cytological studies on six species of Hieracium. He took up the species of Hieracium in which apogamy was proved by the experimental studies of OSTENFELD (**52**), and traced out their nuclear details. In *Hieracium excellens* the nucleus of the pollen mother cell, after it has come out from synapsis, presents a heterotypic figure with 17 bivalent chromosomes, but often with an irregularity in the number of the bivalent and univalent ones. When daughter halves of the bivalent chromosomes separate and become grouped to form the daughter nuclei, the univalent ones are left behind in the cytoplasm, as he had already observed in Drosera (**58**). In *H. flagellare* a normal heterotypic mitosis takes place in the pollen mother cell, the reduced number of chromosomes being 21. In these two species, the embryo sac develops after a tetrad division, as in normal cases; in rare cases the tetrad division with reduction is entirely cut out. The egg retaining the sporophytic number of chromosomes then develops the embryo parthenogenetically. But in the majority of cases he reports that while the normal development of the embryo sac is proceeding, an embryo sac from a cell quite near the tetrad (in *H. aurantiacum*), or in the integument, or in the chalazal region commences to develop. The normal embryo sac is then destroyed sooner or later by the encroaching embryo sac of vegetative origin. This development of an embryo sac from the nucellus is a new case, entirely different from those known in Funkia, Coelebogyne, Citrus, Opuntia, and *Alchemilla pastoralis*, for in the latter cases the embryo is produced directly from the nucellus, instead of through an intercalation of embryo sac formation, and hence the embryo is a vegetative bud from the sporophyte and entirely independent of gametophytic activities.

The papers of STRASBURGER and of FARMER and DIBGY, which are the latest contributions to the cytology of apogamy among pteridophytes, were briefly reviewed in the first part of this paper.

Summarizing the cytological facts in connection with apogamy among spermatophytes, as interpreted by different investigators, it seems evident that apogamy is closely associated with the suppression of sporogenesis in the megaspore mother cell. This necessarily results in no change in the chromosome number in the nucleus of the sporophytic generation, yet a structure is developed with the morphology of the gametophytic generation. Thus the embryo sac will contain the usual number of nuclei grouped in the typical manner, but these nuclei have the sporophytic number of chromosomes. From the facts of apospory, it seems probable then that the development of a gametophyte may result from an interference with the normal life-history and under conditions favorable to the gametophyte, even though the nuclei retain the sporophytic number of chromosomes.

If the doubling of chromosomes is that result of fertilization necessary to start the sporophyte generation, it is no surprise that either an egg with the sporophytic number or a vegetative nucleus with the same number may develop a sporophyte. In all the foregoing cases of apogamy this seems to be the situation.

The case of vars. *polydactyla* studied by FARMER and DIGBY is an instance in which the apogamous development of the sporophyte is not preceded by apospory. The prothallia in these forms are produced after normal sporogenesis and consequently they contain the gametophytic number of chromosomes. The authors claim to have found in certain vegetative cells the fusion of two nuclei, one of which has entered the cell from an adjacent one, and that the sporophyte develops from the region of the prothallium where this fusion occurs. They regard this fusion of two vegetative nuclei as a substitute for normal fertilization. A similar instance of the fusion of two vegetative nuclei is given by BLACKMAN (4) for Phragmidium, who regards (4, 5) the process as a reduced form of fertilization. The conjugated nuclei divide simultaneously through a long series of nuclear divisions, from the formation of aecidiospores to that of teleutospores, where the last pairs unite to form the single fusion nuclei within the teleutospores. There is much evidence that the period in the life-history characterized by the presence of the paired nuclei represents a sporophytic phase. Thus the fusion of the two nuclei in vars. *polydactyla* and the pairing condition of the two nuclei in Phragmidium may support as a working hypothesis the assumption that a nucleus with the sporophyte number of chromosomes is necessary for starting the sporophyte generation.

The condition shown in the apogamous Nephrodium is entirely different from anything yet recorded for plants. The prothallia are developed after normal sporogenesis and their nuclei retain the gametophytic number of chromosomes. The sporophyte then appears as a vegetative outgrowth from the prothallium, without any visible change in the nuclei, so that there is established a sporophyte with the gametophytic number of chromosomes. FARMER and DIGBY (24) have suggested that the number of chromosomes, approximately 60, which is found throughout the life-cycle in *Lastrea pseudo-mas* var. *cristata apospora* may be the gametophytic number in the type species, that is 72, and that this variety might have arisen from normal prothallia of the type species through apogamy. WILLIAMS (74) gives an instance of true apogamous development of the egg in Dictyota, which is as yet the only type among algae where the nuclear conditions of apogamy are known. In WILLIAMS' cultures the germination of the unfertilized egg with 16 chromosomes, the gametophyte number, presented many irregularities in the segmentation divisions and most of the young embryos died after four divisions. Dictyota, then, cannot be regarded as furnishing an instance similar to Nephrodium, since the apogamous developments are abortive and it was not determined whether the structures were sporophytic or gametophytic in nature.

As regards the application of the terms apogamy and parthenogenesis in the various cases observed, STRASBURGER'S principle (**66**) is based upon the number of chromosomes contained in the embryo asexually produced; that is, the asexual development of an embryo from the gametophyte with the diploid number of chromosomes, no matter whether it originates from an egg or a vegetative cell, he calls apogamy; and he regards an unfertilized egg with the diploid number as a vegetative cell. He would restrict the term parthenogenesis to the asexual development of an egg, with the haploid number of chromosomes, and with the capability of being fertilized. WINKLER'S (**76**) view is different. He applies the term parthenogenesis to the case of an asexual development of an egg cell, no matter whether it be haploid or diploid, and he proposes to restrict the use of the term apogamy to cases in which the sporophyte is formed as a vegetative outgrowth from the gametophyte. FARMER and DIGBY'S (**24**) terminology, though not similar, resembles WINKLER'S. The terms euapogamy and parthenogenesis are applied respectively to cases of asexual development of the sporophyte from vegetative cells and from an egg cell of a prothallium produced aposporously; and to the case where a sporophytic outgrowth is preceded by the fusion of two vegetative nuclei they apply the term pseudo-apogamy. The apogamy in Nephrodium, therefore, would be called apogamy by WINKLER, euapogamy by FARMER and DIGBY, and represents no category given by STRASBURGER.

ALTERNATION OF GENERATIONS

Since HOFMEISTER'S investigations we have known that the life-history of most plants involves a regular alternation of sexual and asexual generations. The subject has been discussed by many authors, such as CELAKOWSKY (**13, 14, 15**), SACHS (**60**), BRAUN (**10**), PRINGSHEIM (**55, 56**), VINES (**72**), DE BARY (**2**), BOWER

(**7, 8, 9**), VAIZEY (**71**), STRASBURGER (**65, 67**), BEARD (**3**), CAMPBELL (**11, 12**), SCOTT (**61**), LANG (**36, 37**), KLEBS (**34, 35**), HARTOG (**27**), COULTER (**18**), DAVIS (**19, 20**), WILLIAMS (**73**), BLACKMAN (**4, 5**), WOLFE (**77**), LOTSY (**41, 42**), GRÉGOIRE (**25**), CHAMBERLAIN (**16**), CHRISTMAN (**17**), OLTMANNS (**49**), HARPER (**26**), YAMANOUCHI (**80**), and many others.

Among thallophytes no generalization for the whole group is possible at present, partly because of extreme diversity, and partly on account of the meagerness of our knowledge regarding the life-cycle of the majority of the forms. Different opinions are held concerning the nature of the phenomena in various forms, and some even question the existence of an alternation of generations. However, it is now being gradually established by actual investigation, and quite recently cytological proof has been obtained from several forms, as Dictyota, Phragmidium, Nemalion, Polysiphonia, and some others.

Pteridophytes and bryophytes have been regarded as the best illustrations. Discussion in connection with the pteridophytes has not been in reference to the existence of alternation, but has centered about the question whether it is to be interpreted as of antithetic or homologous origin.

These two views represent different conceptions as to the origin of the sporophyte. Those who advocate the theory of antithetic origin regard the sporophyte of pteridophytes as a gradual elaboration from the zygote of some aquatic algal ancestor, a new phase having thus been intercalated in the life-history. This view was first clearly stated by CELAKOWSKY (**13, 14**). BOWER (**7, 8, 9**) supported it and endeavored to explain it as an adaptation to external conditions. STRASBURGER (**65**), restating the position in terms about identical with BOWER'S, based the theory upon nuclear details. Those who maintain the theory of homologous origin consider that the sporophyte arose as a modification of the gametophyte, and not as a new structure. PRINGSHEIM (**55, 56**), and more recently SCOTT (**61**), LANG (**36, 37**), COULTER (**18**), and others advocate the homologous theory. This theory is largely based upon the phenomena of apogamy and apospory and also to a certain extent upon experiments in regeneration.

When these two theories were proposed, cytological investigations had not yet developed, and even LANG'S admirable work did not touch any cytological particulars. Since the announcement of STRASBURGER'S view of the antithetic origin of alternation, the first to be based on cytological details, chief attention has been directed by many workers to the behavior of chromosomes during the reduction division in the normal life-cycle. As to the results of such accumulated studies, the various views are not readily grouped. However, the majority of cases confirms the view that the periodic reduction of chromosomes is necessary; in other words, the gametophyte with the x number of chromosomes is entirely distinct from the sporophyte with the $2x$ number, and the transition from one generation to the other is marked by the reduction of chromosomes in sporogenesis and the doubling of chromosomes in fertilization, in connection with which the predominant characteristics of one generation are entirely lost and the potentialities of forming the other generation are regained.

As stated above, the cytological work on apogamy and apospory has been chiefly among flowering plants; and quite recently our knowledge concerning these phenomena in ferns was extended by the contribution of FARMER and DIGBY (**24**) on Lastrea, Athyrium, and Scolopendrium, and of STRASBURGER (**68**) on Marsilia. According to these investigations, apogamy, whatever its cause may be, is always preceded either by apospory or the fusion of two vegetative nuclei, which seems to favor the view that the $2x$ number of chromosomes is necessary to establish the sporophyte. Apogamy and apospory, which have been the chief argument for the theory of homologous origin, now seem to support the theory of antithetic origin.

As a matter of fact, the nuclear condition in Nephrodium in the normal life-cycle confirms the antithetic theory; but apogamy in Nephrodium introduces a new situation. In this case apogamy is preceded neither by apospory nor fusion of vegetative nuclei, but the sporophytes are developed with the haploid number of chromosomes. If it might be questioned whether the situation in Nephrodium—in which the nucleus of the gametophyte with the x number can establish the sporophyte—may favor the idea of homologous origin, it must be remembered that we have such abnormal cases of apogamy and apospory in flowering plants, where the embryo sac (probably gametophytic) does not contain the characteristic x number of chromosomes, but always the $2x$ number.

Conclusion

According to the present interpretation of the value of chromosomes, in *Nephrodium molle* Desv. there is present an antithetic alternation of generations marked by the number of chromosomes.

Apogamy in Nephrodium presents a new situation, where the sporophyte is developed with the haploid number of chromosomes. This seems to be an abnormal case, but it must be admitted that it shows that the number of chromosomes is not the only factor which determines the characters of the sporophyte and gametophyte.

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NOTE.—After this paper was in type, STRASBURGER'S "Chromosomenzahlen, Plasmastrukturen, Vererbungsträger und Reduktionsteilung" (Jahrb. Wiss. Bot. **45**:479–568. *pls. 1–3.* 1908) appeared. It not only presents results of investigations on *Lilium Martagon* dealing with the chromosome situation during embryo sac formation and pollen tube development, but it also contains voluminous data concerning plasma structure, chromosomes as bearers of hereditary characters, and the phenomena of the reduction division, the discussion being based upon works of various invesitgators of both plant and animal cells.

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EXPLANATION OF PLATES IX AND X

The figures were drawn with the aid of an Abbé camera lucida, under Zeiss apochromatic obj. 1.5^{mm} N. A. 1.30, combined with compensating ocular 18; except *figs. 9, 10, 11, 12, 13, 14, 20, 21, 28a* drawn with compensating ocular 4; and *figs. 28, 26,* and text cuts drawn under combination of dry obj. 4^{mm} and ocular 4. The plates and text cuts are reduced to one-half the original size.

FIGS. 1–3 are in the text.

PLATE IX

FIG. 4.—Nucleus of vegetative cell of an apogamous prothallium; spirem formed; two nucleoli present.

FIG. 5.—Late prophase; long slender chromosomes present, previous to their arrangement in an equatorial plate.

FIG. 6.—Metaphase; two daughter halves of each chromosome just separated.

FIG. 7.—Late anaphase; daughter chromosomes grouped at two poles.

FIG. 8.—Polar view of late anaphase; 64 chromosomes present in a group.


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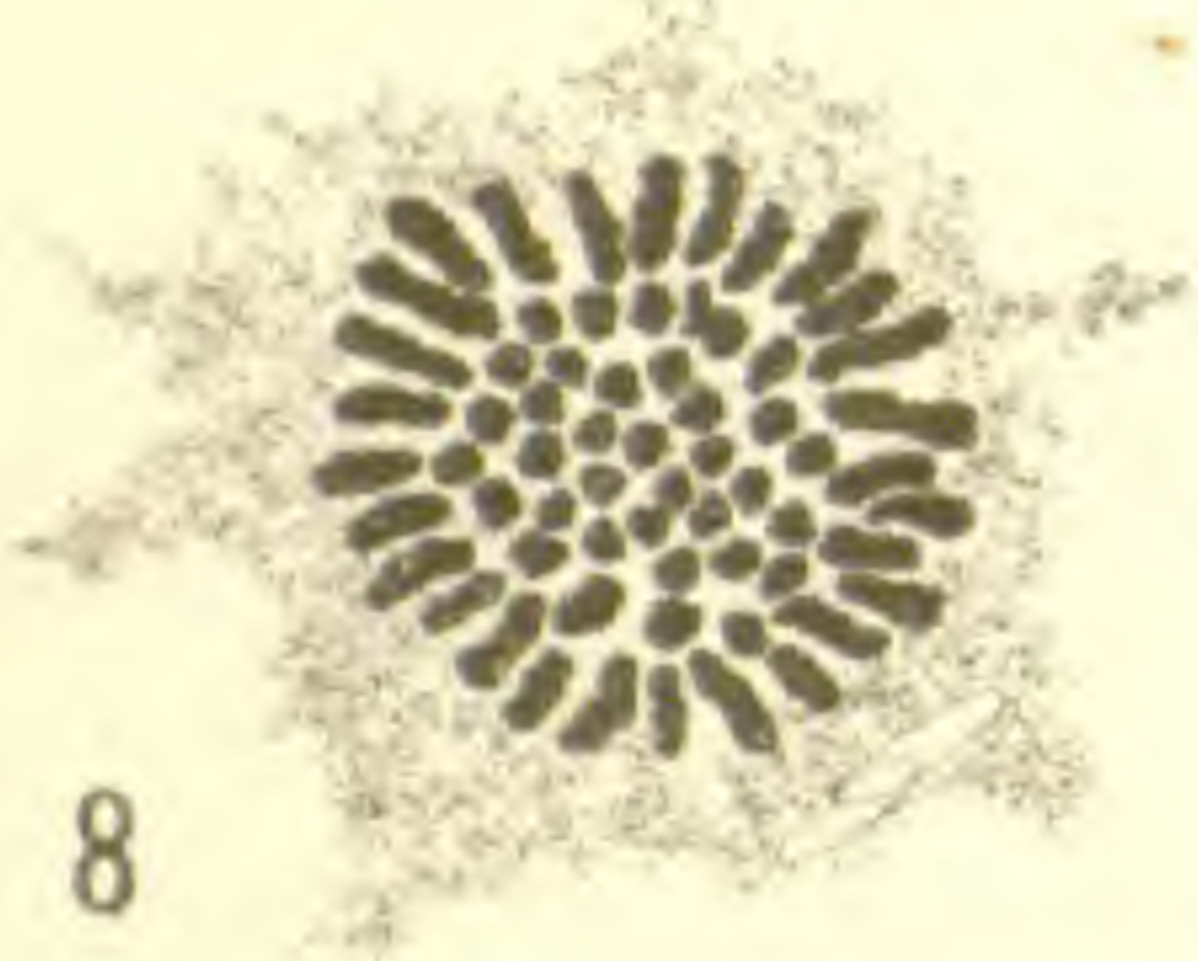
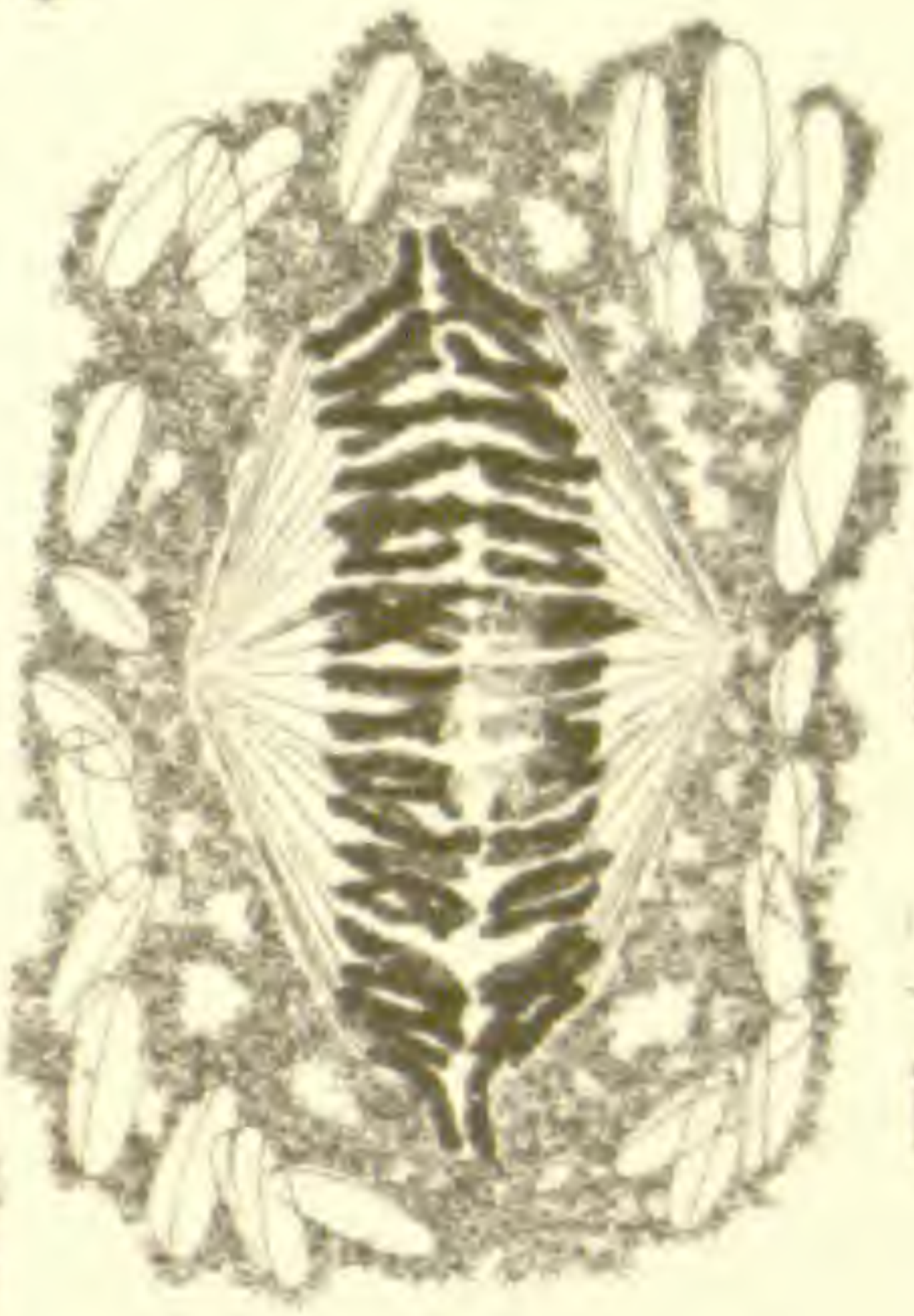

8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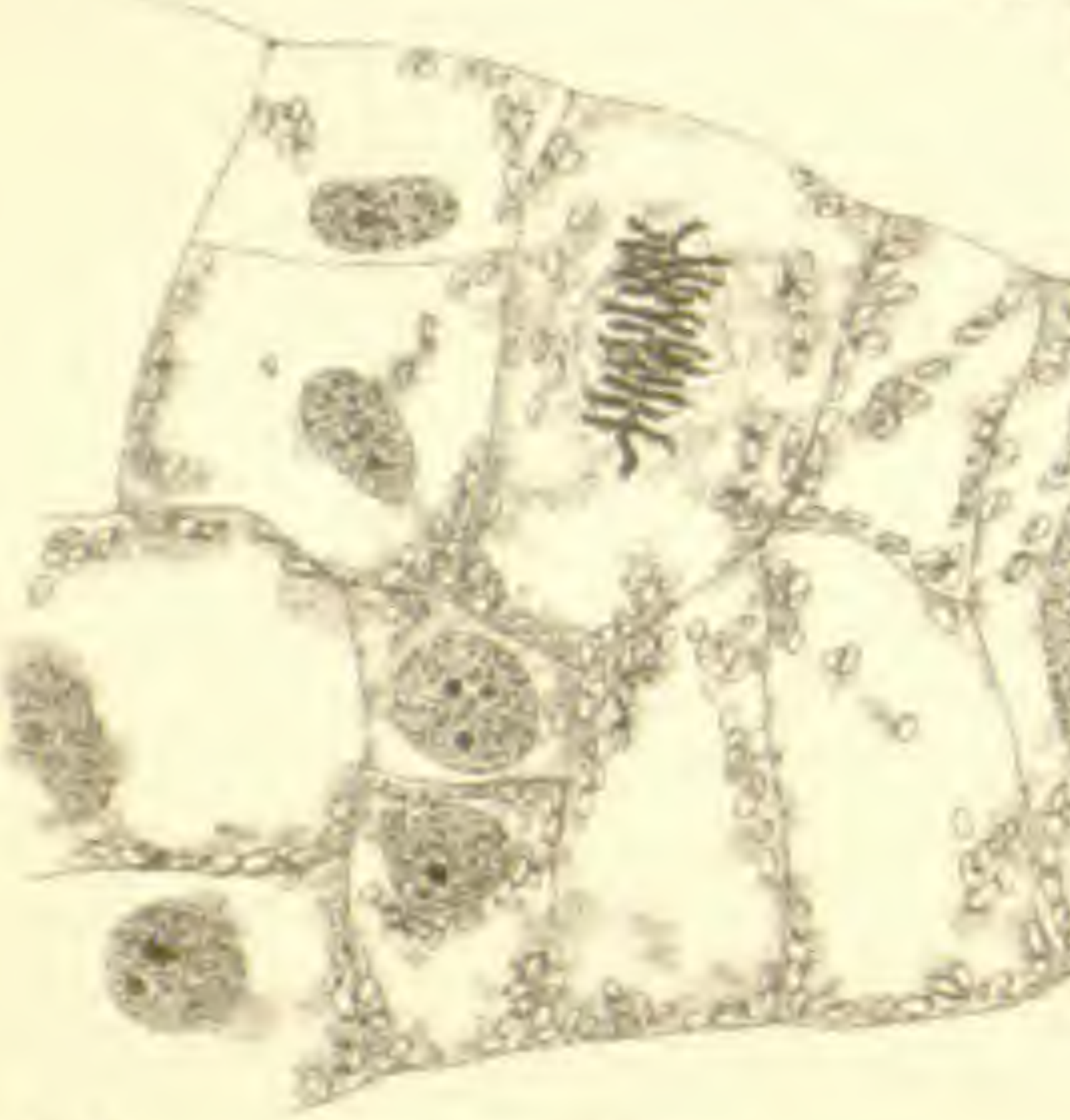
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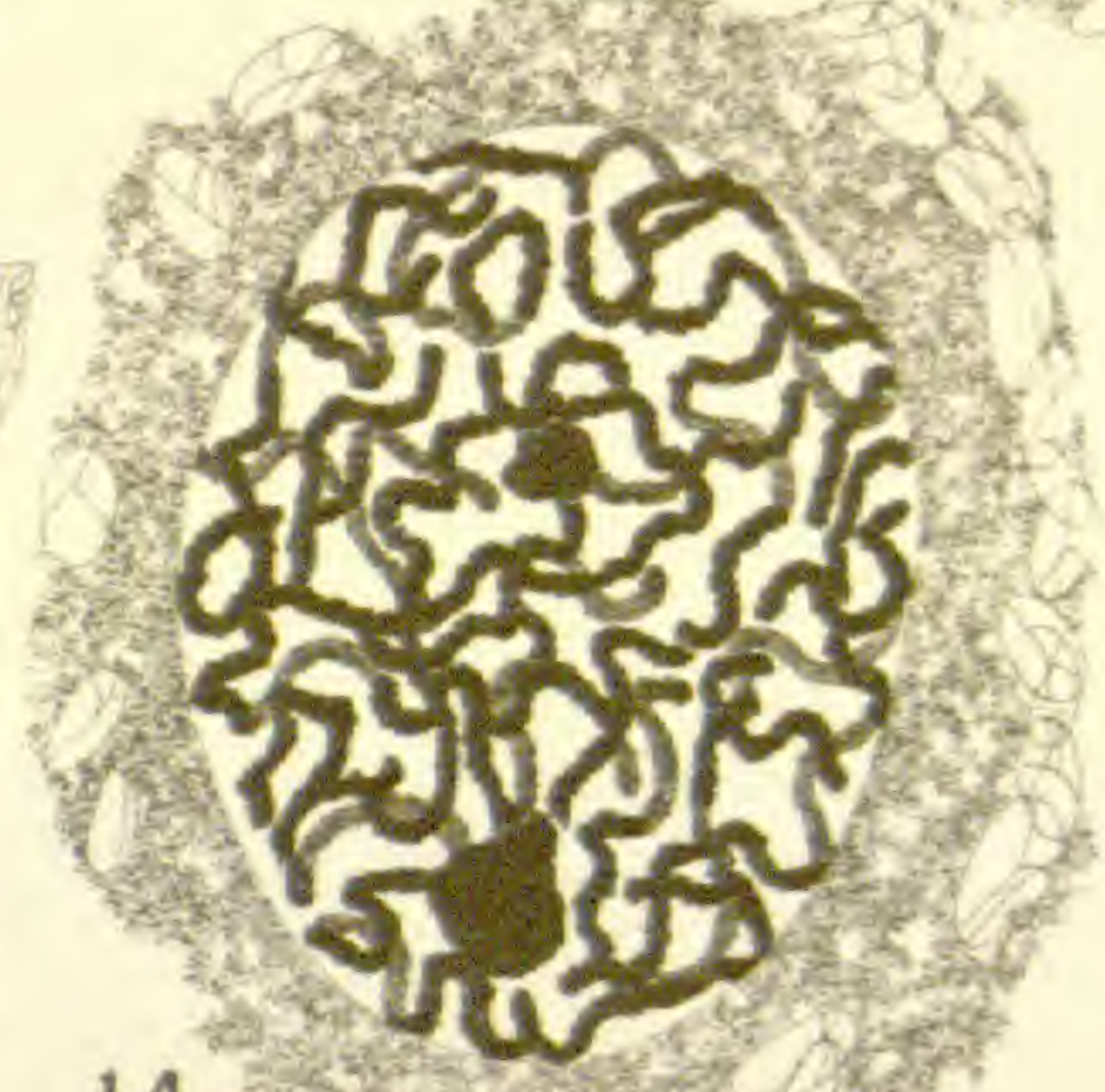
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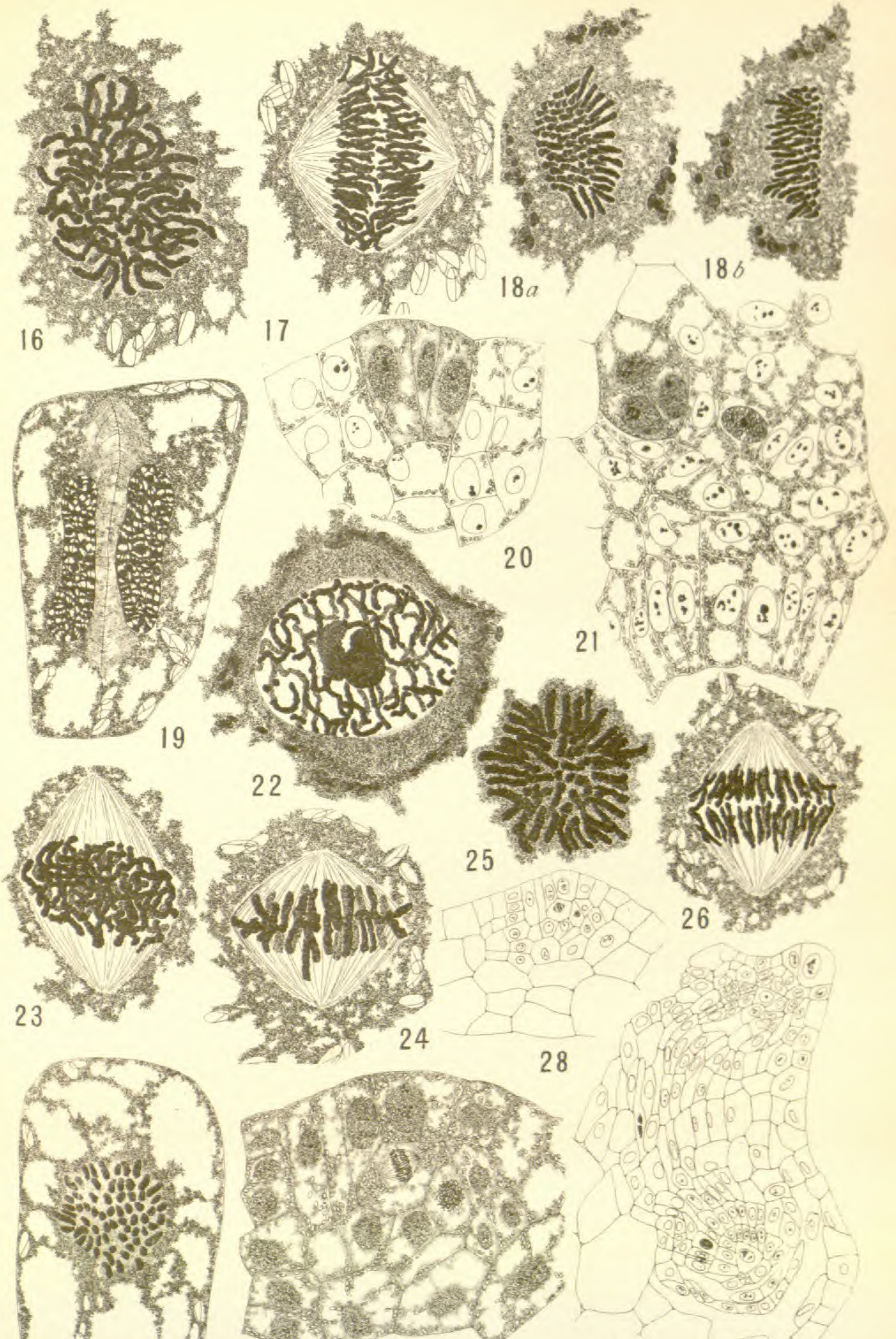
YAMANOUCHI on NEPHRODIUM



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YAMANOUCHI on NEPHRODIUM

FIG. 9.—Section through region where later a cushion arises; narrow cells parallel.

FIG. 9*a*.—Nucleus in metaphase from previous figure, under higher magnification.

FIG. 10.—Section through region forming cushion; next stage after that shown in *fig. 9*.

FIG. 10*a*.—Nucleus in late prophase from previous figure, under higher magnification.

FIG. 11.—Section through region forming cushion; later stage than that shown in *fig. 10*.

FIG. 11*a*.—Telophase of nucleus shown in previous figure, under higher magnification.

FIG. 12.—Section including cushion region; next stage after that given in *fig. 11*.

FIG. 12*a*.—Mitotic figure in telophase, shown in *fig. 12*, under higher magnification; cell plate laid down parallel to surface of prothallium.

FIG. 13.—Section of cushion region; a nucleus within superficial cell in metaphase.

FIG. 13*a*.—Nucleus in metaphase, shown in the previous figure, under higher magnification.

Mitosis in conspicuous superficial cell

FIG. 14.—One of the sections of the cushion region shown in *fig. 3* (text); one superficial cell has increased considerably in size; nucleus in prophase.

FIG. 14*a*.—Nucleus in prophase, shown in *fig. 14*, under higher magnification; several ends of the spirem seen are cut sections; two nucleoli present.

FIG. 15.—Late prophase, showing long slender chromosomes before arrangement in an equatorial plate.

PLATE X

FIG. 16.—Polar view of the late prophase shown in preceding figure.

FIG. 17.—Metaphase; two daughter halves of each chromosome just separated.

FIGS. 18*a*, 18*b*.—Two groups of daughter chromosomes in late metaphase of a nucleus cut obliquely into two sections; 66 chromosomes present.

FIG. 19.—Telophase; cell plate, vertical to surface of prothallium, laid down to cut superficial cell into two daughter cells.

FIG. 20.—Portion of section cut through cushion region; three superficial cells drawn showing contents, middle one of which is cut obliquely as an apical cell.

FIG. 21.—Surface view of a stage similar to that shown in preceding figure; the three superficial cells in question represented by drawing all the contents.

Mitosis in interior cells directly beneath superficial cells

FIG. 22.—Nucleus containing a spirem; visible ends of spirem are cut sections.

FIG. 23.—Prophase; chromosomes irregularly crowded in a mass.

FIG. 24.—Late prophase, showing chromosomes before their arrangement in an equatorial plate.

FIG. 25.—Polar view of metaphase, where L-shaped chromosomes are arranged in an equatorial plate; vertical arms of L's are visible as dots and lateral arms as radiating lines.

FIG. 26.—Late metaphase; two daughter halves of each chromosome have just separated.

FIG. 27.—Early telophase, showing polar view of a group of daughter chromosomes; 66 chromosomes present.

FIG. 28.—Section of cushion region where a sporophytic structure has been worked out.

FIG. 28*a*.—Approximate portion that belongs to the sporophytic structure shown in the preceding figure, under higher magnification; difference of size of cells in prothallial region and sporophytic structure shown.

FIG. 29.—Section through sporophyte apogamously produced, in a considerably later stage; one of the two apical cells seen is a leaf initial, the other a root initial; apical cell of stem not drawn in this section; no structure standing for a foot is present.

ALPINE VEGETATION IN THE VICINITY OF LONG'S PEAK, COLORADO

WILLIAM S. COOPER

(WITH EIGHT FIGURES)

This paper is the result of observations made during the summers of 1904 and 1906. It makes no pretensions to completeness, but is intended to serve as a general survey of an intensely interesting region. It is the intention of the author immediately to begin a more extensive and detailed study.

My thanks are due, first of all, to Dr. E. N. TRANSEAU of the Charleston (Ill.) Normal School, and to Dr. D. S. JOHNSON and Dr. CHARLES K. SWARTZ, of Johns Hopkins University, for many valuable suggestions; also to Dr. AVEN NELSON, of the University of Wyoming, for determination of plants.

I. Location and general topography

The Front Range of the Rocky Mountains, which in the vicinity of Long's Peak constitutes the continental divide, is a rugged mass carved from the Archean granite and gneiss. South of Long's Peak, which is an eastern projecting spur therefrom, its direction is nearly north and south, and it stands about 24^{km} west of the eastern border of the mountain region. North of Long's Peak it turns to the northwest, and continues in this direction for a distance of 24^{km}, until it meets the Medicine Bow and Rabbit Ear Ranges; the continental divide is continued in the latter range. At the eastern base of the Front Range, just north of Long's Peak, lies Estes Park, a mountain valley which here separates the main range from the lower forest-clad mountains toward the east. Estes Park lies at an altitude of 2285^{m}; the average altitude of the divide is 3810^{m}; most of the peaks exceed 3900^{m}, and Long's Peak attains an altitude of 4350^{m}.

The localities studied are on the summit and eastern flank of the Front Range, from Mt. Tyndall, 6.4^{km} south of Long's Peak, to Stone's Peak, 16^{km} northwest. Particular attention was given to two glacial cañons: the Chasm Lake cañon, immediately below the east face of Long's Peak; and Glacier Gorge, west of the same mountain.

II. Physiography

A. PRESENT.—For convenience we may divide the physiographic features of the region into two groups: (1) the flat summit of the divide and slopes similar to it; (2) the glacial cañons.

The summit of the divide is an elevated plateau, in some portions level, more often gently rolling (*figs. 1, 2*). In places it is 1.6^{km} or more wide, in others it narrows to less than 0.4^{km}. A few low peaks rise from it, often near its edge, so that they have a precipitous face on that side. The surface is covered with a rather thin layer of rock fragments split off by frost and temperature changes, of small size in most places, but much larger on the higher summits. Similar in every way to the summit of the divide are the slopes that descend to the region of timberline where glacial erosion has not formed cliffs.

FIG. 1.—Summit of the continental divide from Stone's Peak; Long's Peak at the left; the dry meadow covering the flat surface of the divide is shown.

The glacial cañons head in a series of fine cirques, immediately under the steep cliffs that form the face of the continental divide (*fig. 3*). They are separated from each other by projecting spurs of varying width, sometimes flat-topped, but oftener mere ridges, so close together were the sources of the glaciers. When flat-topped they are essentially similar to the summit of the divide, and are in fact continuous with it, being covered with a thin layer of frost-broken fragments. Some of the ridges bear high rugged peaks, but in most cases they decrease gradually in altitude away from the divide, and frequently end in long interlobate or medial moraines, where the adjacent glacier tongues met or coalesced. The floors of the cañons descend toward the lower altitudes in an alternating series of very gentle slopes and abrupt steps, the latter often 30^{m} or more in height.

B. Historical.—A glance at the physiographic history of the region will make clearer the present conditions and processes. For the purpose of this paper, it is unnecessary to go back farther than the time of Pleistocene glaciation, since during that period practically every trace of vegetation must have been destroyed. The Front Range in the region studied was glaciated during the Pleistocene period over a width of about 25^{km}[1]. The summit of the range was deeply covered with snow, which, accumulating in larger quantities in favorable locations along the flanks of the range, became consolidated at these points into glacier ice and commenced the work of shaping and modifying the cañons.

Fig. 2.—Summit of the divide near Mt. Tyndall; a more detailed view of the dry meadow, showing also the top of cliffs that surround a glacial cirque.

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[1] King, Clarence, Exploration of the Fortieth Parallel.

In due time the climate began to change and the amount of snow to decrease accordingly. Finally, the winter snowfall decreased to such an extent that the summit of the divide and the gentler slopes became bare during the summers. These surfaces had heretofore been protected by their load of snow from the process of weathering. Now, however, began the work of frost and of temperature changes, which is responsible for the condition of the summit today. The thinness of the covering of rock fragments on these surfaces is due to the protection from sudden changes of temperature afforded the solid rock beneath by the broken rock itself, with the layer of vegetation that has become established upon it.

FIG. 3.—View in Glacier Gorge, showing glacial topography and wet meadow areas on ledges; a glacial lake in the foreground, and snow banks below the cliffs.

In the meantime the glaciers themselves steadily shrank, because of the decreasing supply of snow at their sources. The large accumulation of névé in the cirques, however, enabled them to hold out long after the perennial snow mantle of the summit and exposed slopes had disappeared. In fact, there still remain at the heads of several of the cañons remnants of the former ice tongues, which, though small, are true glaciers.

The cañons thus formed exhibit the usual glacial characteristics (*figs. 3–6*). At their lower ends are found series of terminal moraines and for some distance up there are extensive lateral moraines. The floors of the cañons, except for an occasional small terminal moraine, are nearly bare of morainic material, such as is present being an exceedingly sparse scattering of single bowlders. The floors are very smooth, frequently almost polished, and are marked by the usual scratches and striae. *Roches moutonnées* are numerous but not prominent. At intervals are found the great "steps" mentioned above, where were the ice falls of the glaciers. At their bases are nearly always lakelets, occupying basins excavated by the ice, which naturally possessed increased erosional power at these points. Lake basins are not, however, exclusively confined to the bases of the "steps."

FIG. 4.—Smooth glaciated floor of Glacier Gorge, with scattered glacial detritus; the wet meadow patches have formed among this material.

The results of post-glacial weathering in the cañons are seen in the masses of talus piled along the bases of the cliffs. Where the cañons are narrow, the weathered material covers the whole breadth of the floor, and has begun to fill many of the lake basins with large fragments. Usually, however, there is a wide central space which the talus from the side cliffs does not reach. Another effect of weathering, more important ecologically than physiographically, is the enlarging of the cracks or joint planes in the smooth floor of the cañons, by disintegration of their edges and the deposit, in or near the cracks, of the fine disintegrated material. Stream erosion has so far produced practically no effect upon the topography of the upper portions of the cañons.

III. Vegetation

In considering the vegetation of the higher mountain region, we naturally divide it into two great formations: the alpine grassland, and the forest, and place the division between these at timberline. In general this arrangement is a right one for the region, but in studying the vegetation dynamically we are compelled to recognize an exception in the wet meadows of the cañons. Above timberline we find two types of grassland: the dry meadow, occupying the exposed slopes and ridges; and the wet meadow, in the upper portions of the glacial cañons. The former is a true climatic grassland. The latter, however, as will be shown, is probably not permanent grassland brought about by climatic factors, but is a stage in the forest succession, and, conditions remaining the same, will become forest after the lapse of sufficient time. In this paper the term "alpine grassland" will be used to include both dry and wet meadow. We will consider first the development of these two types of alpine meadow; and second, the forest formation in its successional relation to them.

A. THE ALPINE GRASSLAND

1. *The dry meadow*

The societies which form the stages in this succession (*figs. 1, 2*) cover a large part of the area above timberline—an area which is practically coextensive with the flat summit of the range and the slopes similar to it, which, as has been said, are covered with a layer of broken fragments. While this layer of broken material protects the bed rock from further weathering, the fragments themselves are continually disintegrating under the influence of frost and of lichens. Disintegration by lichens is an important process here as is evidenced by the thick crust of these plants upon every stone.

Every stage in the development of the dry meadow may be seen on the summit of the range. On the more elevated portions, where have been split off blocks of very large size, no plants except lichens have as yet been able to gain a foothold. Where the blocks are smaller and more disintegrated, a layer of fine material has accumulated around and among them—the first step in the formation of a soil; and here a few herbaceous plants of extreme xerophytic nature, such as *Silene acaulis*, *Phlox caespitosa*, *Dryas octopetala*, have established themselves. A further stage is seen where the vegetation has become more luxuriant both in number of species and of individuals, frequently partially concealing the remnants of the disintegrating rock fragments. In the mature dry meadow the rock fragments have mostly broken down to form, with the humus deposit, an even layer of soil which is well covered by a growth of low herbaceous vegetation. The dry meadow of this region resembles quite closely the so-called "alpine tundra" of the summit of Mt. Ktaadn, Maine, as described by HARVEY,[2] and observed by the author of this paper. It differs from that in the practical absence of mosses and fruticose lichens so characteristic of the summit of Ktaadn. At the same time, it does not perfectly correspond with the common idea of meadow land, since the vegetation is low and of a decidedly xerophytic nature. As is the case in the Pike's Peak region[3] the mature dry meadow has two seasonal stages, the earlier characterized by the prominence of *Rydbergia grandiflora* in flower, the later by *Campanula rotundifolia*. It must not be supposed that the soil on these exposed summits and slopes, thin as it is, is everywhere dry. Even along the summit of the divide are frequent depressions receiving all the run-off from the surrounding slopes, which often are gentle and embrace large areas. The solid granite beneath prevents absorption; therefore the water remains stagnant or flows very slowly over the surface. The supply seems to be fully equal to the loss by evaporation, for these areas remain wet all summer long. The depressions are characterized by a more luxuriant vegetation, and therefore deeper deposits of humus, but the species are the same as those found on the drier portions of the meadow. A list of the dominant species of the dry meadow follows:

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[2] HARVEY, LEROY H., A study of the physiographic ecology of Mt. Ktaadn, Maine. University of Maine studies, No. 5.

[3] CLEMENTS, F. E., Research methods in ecology.

PRINCIPAL SPECIES.—Silene acaulis L., Sieversia turbinata (Rydb.) Greene, Dryas octopetala L., Phlox caespitosa Nutt., Campanula rotundifolia L., Rydbergia grandiflora (T. & G.) Greene.

SECONDARY SPECIES.—Selaginella densa Rydb., Deschampsia caespitosa alpina Vasey, Trisetum subspicatum (L.) Beauv., Carex atrata L., Juncoides spicatum (L.) Kuntze, Zygadenus coloradensis Rydb., Lloydia serotina Reichenb., Polygonum viviparum L., Oreobroma Grayi (Britton) Rydb., O. pygmaea (Wats.) Howell, Arenaria sajanensis Willd., Draba crassifolia Graham, Sibbaldia procumbens L., Dasiphora fruticosa (L.) Rydb., Trifolium dasyphyllum T. & G., Oreoxis alpina (Gray) C. & R., Primula angustifolia Torr., Androsace subumbellata (A. Nels.) Small, Douglasia Johnstonii A. Nels., Polemonium confertum Gray, Mertensia viridis Nels., Castilleja puberula Rydb., Pedicularis Parryi Gray, Solidago scopulorum (Gray) A. Nels., Tetraneuris acaulis (Nutt.) Greene, Tonestus pygmaeus (T. & G.) Nels., Erigeron pinnatisectus (Gray) Nels., Artemisia scopulorum Gray.

2. *The wet meadow*

It must be borne in mind that on account of a smaller deposit of snow, due to exposure to the wind and sun, the summit of the range and the unglaciated slopes were first exposed, while the cañons still contained glaciers fed from the névé accumulated in the cirques at their upper ends. Thus it was long after the dry meadows above had begun their development when vegetation, following the retreat of the ice, first gained access to the upper portions of the cañons. At the present day the spruce-fir forest extends up the cañons to an altitude of about 3350^m. Above this is an area supporting a mesophytic meadow vegetation, which, however, only partially covers the ground, being confined to the vicinity of the watercourses. Still higher, where the last remnants of the ice lingered, there is no vegetation save a sparse growth of lichens. In the heads of the cañons, therefore, we may study primary invasion in all its stages, and the next few paragraphs will be devoted to a description of it as observed in these places.

A. THE GLACIAL CAÑON AS A HABITAT.—*Atmospheric conditions.*—As to the atmospheric conditions in the cañons I can give no exact data, but it may be said with certainty that they are comparatively favorable to plant growth—much more so than in the region of the dry meadows. The most noticeable difference between the two habitats, in regard to atmospheric conditions, is the exposure to the full force of the wind on the dry meadows, contrasted with the effective shelter from that agent afforded by the cañon walls. The difference in altitude between the two habitats, averaging about 610^m, also favors the cañons, since atmospheric rarity tends to increase evaporation.

Ground water.—The smooth polished floor of the cañons has already been described. In some parts the surface slopes gently and evenly; in others there are low rounded prominences (*roches moutonnées*) with depressions between. The greater part of this region is dry during all of the growing season, since the surface drainage from the snow banks above is the only source of moisture that need be taken into account. The small amount of rainfall during the growing season may be neglected altogether, since the smooth rock surfaces cannot retain it, and it very soon evaporates or joins the drainage channels from the snows. Snow accumulates at the bases of the upper cliffs in large quantities, and many of the drifts remain through the summer, affording by their melting a constant supply of water to a restricted portion of the area below. The drainage channels from the various permanent drifts form a network over the cañon floor in following the principal depressions among the *roches moutonnées*. Some cases were observed where the water flowed in a broad sheet for long distances over a smooth gently sloping rock surface.

Sources of soil materials.—The soil materials which render possible the establishment of the pioneers of the higher plants are derived principally from two sources: rock decay and wind-blown dust. Rock decay has made very little progress in the cañon heads since glacial times. Since the process is conditioned by the presence of moisture, the larger part of that which has been done and is now being done is within the sphere of influence of the drainage channels. As has been said in describing the physiography of the region, decomposition is going on principally along joint planes, and the fine products of this process are either deposited in the cracks near to where they were produced or are carried down by flowing water and deposited in other situations.

I believe that wind-blown dust is an important addition to the scanty supply of soil material available to the pioneers. Its source doubtless is largely the dry meadows above the cañons, where during the summer large areas of sparsely covered soil become desiccated. Some of the dust from this and other sources is doubtless carried directly to its final destination on the cañon floors. It is likely, however, that a greater amount is brought down by the streams from the snow banks at the base of the upper cliffs, upon which it had fallen, perhaps years before. The wet surface of the drifts would hold all such fine material deposited upon it. This deposit is not so slight as might be imagined, for during a single summer the drifts become so coated with dust that the discoloration is conspicuous at a great distance. The fate of the dust thus deposited is as follows: Most of the run-off from a large snow bank in an arid mountain region comes from the melting of the compact basal portion. The water which results from surface melting nearly all evaporates. Thus the layer of dirty snow upon the surface of the drift is slowly but continually sinking. Each winter a fresh layer of snow is deposited, which, during the following summer, accumulates a layer of dust upon its surface. Frequently a section through a snow bank will reveal a series of these dirt bands, roughly marking off what remains of each winter's snowfall. It is evident that because of the basal melting, these dirt bands will finally sink to the bottom of the drift, and that the matter which the dirty layers contain will be carried down with the stream of water which issues from beneath the snow bank, often with great velocity. This material, converging from numerous sources to a few restricted drainage areas, is by no means to be neglected, and being chiefly organic in nature, it is a valuable contribution to the making of the first soil. Being exceedingly fine material, it may be carried to a considerable distance, and is deposited in basins, or wherever the velocity of the stream slackens. The disintegrated rock material, on the other hand, being comparatively coarse, if carried away at all, is deposited very near to its point of origin.

As soil producers, lichens have accomplished practically nothing in the glacial cañons. The fact that the rock is a hard granite, and the smoothness of its surface, as well as the comparatively short time that the rock has been exposed, are sufficient to explain this.

B. THE INVASION OF THE CAÑONS.—The unimportance of lichens in preparing for the establishment of the higher plants has just been mentioned. The advance of the latter must therefore be conditioned by the other sources of soil material, rather than by any furnished by lichens. And so we find two groups of invaders, one making use principally of the decomposed rock material in the cracks, the other developing later, but usually on new ground, in basins and very wet places, and profiting from the organic material brought down from the snowdrifts.

In cracks and joints of the floor we find a pioneer group consisting of the following species, all but one or two of which are perennials.

1.—Deschampsia alpicola Rydb., Agrostis tenuiculmis Nash, Trisetum subspicatum (L.) Beauv., Carex atrata L., Juncoides spicatum (L.) Kuntze, Sagina Linnaei Presl., Draba crassifolia Graham, Sibbaldia procumbens L., Androsace subumbellata (A. Nels.) Small, Artemisia scopulorum Gray.

2.—Salix petrophila Rydb., Oxyria digyna (L.) Camptd., Saxifraga debilis Engelm., Senecio Fremontii T. & G.

The species in the first group, to which further exploration would doubtless add many more, are commonly found in the dry meadows. Since these meadows are all above the level of the cañon floors, this group of pioneers must have come from seeds blown or washed down from them. There is one other possibility—that these plants have followed the glaciers as they retreated. It seems likely, however, that pioneers in the invasion of the lower portions of the cañons were plants belonging to lower altitudes and more favorable conditions; since they were as well sheltered from the wind as are the pioneers of today, and the water supply was far more abundant. The plants in the second group are commonly found in clefts and among bare talus in the higher altitudes. These species, with those of the first list, constitute a group of invaders of xerophytic character from higher altitudes. They do not occupy a definite zone, but are scattered over a broad area of the cañon floor wherever conditions permit them to exist. In some measure they prepare the way for the group of invaders that comes from the opposite direction, but like the lichens they are relatively unimportant, and, as will be shown, the plants that come in from below to fill the depressions may be entirely independent of any aid from earlier arrivals. Sometimes, however, the cracks containing these plants may become the starting-point for quite large mats of turf. If the water supply is abundant enough to make possible the establishment in a crack of the more mesophytic sedges and grasses, such as grow in the basins soon to be described, these may spread out over the surface of the rock by means of rootstocks, forming a thick and permanent carpet which may be lifted from the rock at its edges, but which is firmly anchored at its point of origin.

FIG. 5.—View in Chasm Lake Cañon; the wet meadow vegetation in the foreground has completely filled a basin; similar patches of vegetation are visible in the vicinity of the cascades.

We now come to the group of invaders that is advancing toward the heads of the cañons from below. The pioneers are few in species, but exceedingly numerous in individuals, covering the areas over which they advance with a thick carpet of vegetation. Their line of attack is along the watercourses. It has been said that deposition of earthy matter from the melting snows, fine rock material, and some organic matter from the plants growing in cracks farther up, is continually going on where the current of the watercourses slackens. The first plants to appear in this sediment are usually mosses, including *Philonotis fontana*, which sometimes becomes established even in shallow water. The mosses are followed by species of Carex, which by means of their creeping stems spread extensively and form thick mats. Grasses now come in, with a scattering of other herbs characteristic of marshy ground and stream banks, nearly all of which are found in marshy situations at considerably lower altitudes. The deposits of humus from these plants hastens the filling-in of the depressions, and the result is a wet swampy meadow with one or more streams meandering through it (*fig. 5*). These streams often run over the rock itself, since the current has so far kept their beds free from sediment. Prominent plants growing in these wet meadows are as follows:

Lycopodium selago L., L. annotinum L., Carex (several species) dominant, Caltha rotundifolia (Huth) Greene, Sedum rhodanthum A. Gray, Sieversia turbinata (Rydb.) Greene, Kalmia microphylla (Hook.) Heller, Primula Parryi A. Gray, Gentiana frigida Haenke, G. plebeja Holmii Wettst., Swertia congesta A. Nels., Elephantella groenlandica (Retz.) Rydb., Crepis alpicola (Rydb.) A. Nels.

Later a few shrubs make their appearance. *Betula glandulosa* is by far the commonest of these, and *Dasiphora fruticosa* and *Salix chlorophylla* are frequent.

Where a stream flows among glacial débris scattered over a smooth gently sloping rock surface, plants themselves are instrumental in forming small basins. Fine material accumulates around the rock fragments, and in this mosses obtain a foothold. From the rock fragments as centers mats of moss spread out, and these coalescing partially dam up the stream, so that more fine material is deposited. The development of the meadow then progresses as in the natural rock basins. Frequently the damming-up of its course deflects a portion or all of the stream, and in its new course it encounters other débris where the process is repeated. These little meadow patches often coalesce to form areas of quite respectable size. The turf mats that start from the cracks also sometimes become joined to these, and thus in various ways considerable areas on the cañon floors obtain a solid covering of herbaceous vegetation (*figs. 4, 6*). Ledges on the cañon walls, where water trickles over them, are covered with a turf mat similar in composition to that upon the cañon floor (*fig. 3*). Where cascades occur, principally at the great "steps," there is naturally an abundant vegetation, consisting of mosses, sedges, and other herbs growing within the influence of the spray (*fig. 5*). The lake basins are so large and deep that comparatively little filling has been accomplished. The shallower portions along the shore are partially converted to meadow, and where a lake occurs at the base of a "step" good-sized alluvial cones extend out into the water at the upper ends. Of course this is not the case with the highest lakes, where very little alluvial matter is available.

The mature wet meadow is thus of a decidedly mesophytic character. Composed as it is of a thick bright-green turf of sedges, grasses, and other flowering plants, it has a truly meadow-like appearance.

It must be borne in mind that only the depressions have been thoroughly taken possession of by vegetation (*fig. 6*). The higher and drier portions of the cañon floor—the *roches moutonnées*—including much more than half of the total area, are still bare, save for a sparse covering of lichens, and a few xerophytic herbs in the crevices. These areas, of course gradually decreasing, but with exceeding slowness, are destined to remain very much the same for a long time. Far down the cañons, in the midst of the forest, there are many places where the bare floor is still exposed, more incrusted with lichens and slightly more decomposed, but nevertheless surprisingly similar to the bare areas at the cañon heads.

FIG. 6.—View looking down Glacier Gorge; the dark areas in the foreground are wet meadow occupying depressions in the cañon floor; scattered over the meadow areas are still darker patches, the Picea *Krummholz;* farther down the cañon may be seen the Picea-Abies forest surrounding two glacial lakes.

B. THE FOREST

For the present purpose we need to consider only the upper forest zone, which in this region is composed of two societies governed by the factor of soil water content. The white pine society consists of an almost pure growth of *Pinus flexilis*. It covers the dry summits and upper slopes of the ridges between the cañons up to the limit of tree growth. The spruce-fir society is confined to situations where there is an abundant soil water content. It follows the cañon bottoms up to where they emerge above timberline, and extends up the sides as far as there is a sufficient amount of ground water available. The two trees that dominate the society are the Engelmann spruce (*Picea Engelmanni*) and the subalpine fir (*Abies lasiocarpa*). The latter is more abundant close to the streams, and seldom or never in this region ascends quite to timberline.

1. *The Krummholz (figs. 7, 8)*

The altitude of the present upper limit of tree growth averages about 3350^{m}, with a range between extremes of perhaps 150 or 180^{m}. Since *Abies lasiocarpa* does not ascend to timberline, the *Krummholz* is composed of two trees only, *Pinus flexilis* and *Picea Engelmanni*, the former on the ridges, the latter in the cañons. Both reach their upper limit at approximately the same altitude, but their appearance and behavior there is so strikingly different that a description of each will be of interest.

PINUS FLEXILIS.—This tree, even when growing in its most congenial habitat, is very irregular and contorted. In its *Krummholz* form it assumes the most fantastic shapes and yet in all of these a general uniformity of behavior may be seen. A very typical growth of the *Krummholz* form is found at timberline on the Long's Peak trail (*figs. 7, 8*). Here there is considerable exposure to the prevalent west wind which sweeps with great force over a saddle on the ridge behind. The trees form an open growth, with the individuals usually growing singly. They are of great age, and often 30^{cm} thick, but few are more than 1.5^{m} in height. The live branches all point to the eastward, those on the windward sides are dry and dead, and the windward portions of the trunks are in nearly every case devoid of bark. Frequently the greater part of the trunk is worn away by the ice particles borne by the winter gales. The degree of exposure to the winds from the west determines the height of the tree. If somewhat sheltered, the trunk may be nearly erect, but in the most exposed places it becomes absolutely prostrate (*fig. 8*), creeping along the ground for 4.5 to 6^m, and sometimes partially or wholly buried in sand and needles. It is a noticeable fact that on the ridges the *Krummholz* ends abruptly—in other words there is a very definite timberline. Beyond the main mass of the forest outlying groups of trees are rare, and are never at any distance from it.

PICEA ENGELMANNI.—In the cañons, on the contrary, it is seldom possible to determine the precise limit of the forest formation. In ascending we find that the trees become more scattered and at the same time smaller, until finally they are mere bushes, growing singly or in groups scattered over the wet meadow areas. They are seldom much contorted, nor do they often show the effects of wind and ice crystals. Unlike the white pine, the Engelmann spruce persists in its upright habit. In its most common *Krummholz* form, as found in most parts of the cañon floors, it has a thick erect trunk a meter or two in height, and a broad flat matted crown composed of branches so tough and intricately woven that where the trees grow close together it is often possible to walk for considerable distances over their tops. Where *Picea Engelmanni* occupies more exposed positions it almost always

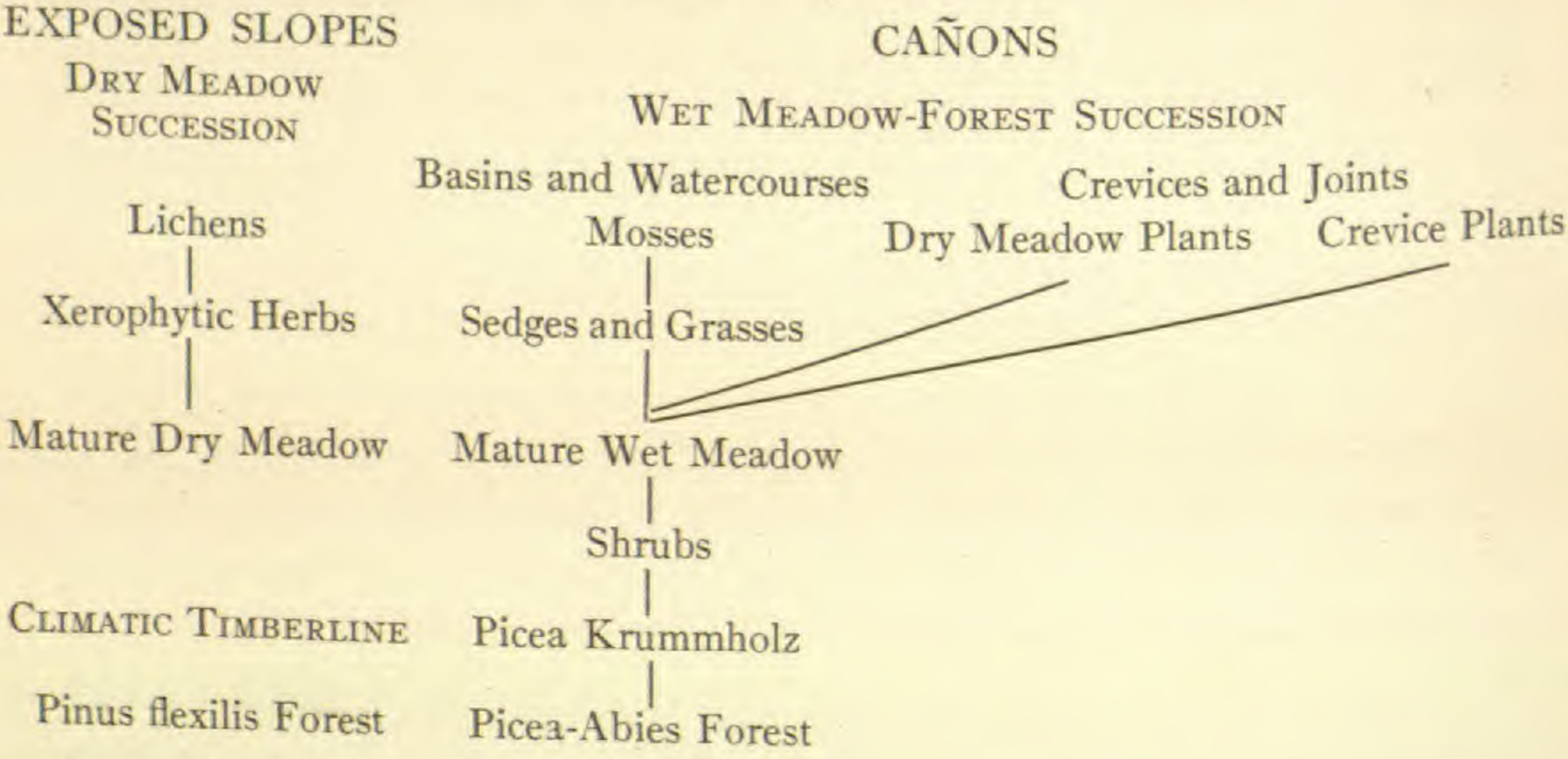
FIG. 7.—*Krummholz* form of *Pinus flexilis* near the Long's Peak trail; the view is from the windward side.

retains its erect habit, but the branches point to leeward of the prevalent wind. Occasionally the trunk is prostrate, but in these cases erect branches are produced, the branchlets of which are turned to leeward. There is frequently a tendency in this species, when growing, as it occasionally does, in much exposed places, to form long narrow groups of individuals, parallel to the direction of the prevailing wind, the height gradually rising from two or three decimeters at the windward end to a meter or more at the leeward end of the group. The explanation of this phenomenon evidently is that some small shelter allows a seedling to live and develop into a small tree; and this gives protection in its lee to others, each of which in turn is able to rise slightly higher than the one in front. The same mode of growth is seen also in such small shrubs as *Betula glandulosa* and the low alpine form of *Populus tremuloides*, which are found in the same habitat with *Picea Engelmanni*.

FIG. 8.—*Pinus flexilis* growing in a very exposed situation near the Long's Peak trail.

Having now a general view of the forest at its upper limit, we will consider the question as to whether these two trees are at present invading the grassland. The evidence here, though it is not as complete as might be wished, points toward the general conclusion that the forest formation is advancing in the cañons, but that on the ridges it has reached its climatic limit. The aged, battered, and twisted white pines, with no young trees among them, indicate a repulse in the effort of the forest to ascend the ridges; while, on the other hand, the gradual decrease in height, the scattering of individuals and groups in advance of the main mass, suggest that in the cañons the forest is still advancing—that it has not yet reached the point where tree growth is climatically excluded. HARVEY[4] has cited a similar condition in evidence that the forest is not climatically excluded from the summit of Mt. Ktaadn. It is an interesting fact in this connection that the Picea *Krummholz* in the cañons of the Long's Peak region bears a striking resemblance, in type of trees represented, in the *Krummholz* form which they take, and in the manner of their seeming advance, to the mesophytic Picea-Abies *Krummholz* of the eastern mountain.

It will be well at this point to recall the fact that the agency most important in preventing tree growth is dry wind during frosty weather, since it accelerates transpiration to a high degree, while the frozen condition of the ground prevents a renewal of the water supply.[5] The conditions in the Long's Peak region—the permanent timberline on the exposed ridges and the advancing forest in the sheltered cañons—agree perfectly with this theory.



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[4] HARVEY, LEROY H., *Op. cit.*

[5] KIHLMAN; referred to by SCHIMPER, Plant geography 78.

C. THE SUCCESSIONS

In this study we have endeavored to trace in a general way the stages through which the vegetation of the alpine region has passed. It remains only to bring into an orderly arrangement the successions and their stages which have been described. This may best be done by means of the foregoing diagram, which in the light of what has gone before will need no further explanation.

IV. Summary

In the retreat of the Pleistocene snow masses the unglaciated slopes and summit of the divide were first uncovered and occupied by vegetation. On these areas the xerophytic "dry meadows" of today have developed.

Owing to the slower shrinkage of the valley glaciers, invasion of new areas is still going on at the heads of the glacial cañons.

The glacial cañon as a habitat is characterized by (1) comparatively favorable atmospheric conditions, (2) restriction of surface water to limited areas, (3) dependence of the production and distribution of soil materials upon surface water.

These conditions result in a vegetation consisting of a lichen society inhabiting the dry rock surfaces for an indefinite length of time, and a wet meadow society confined to the vicinity of the drainage channels. The latter society is preceded by two groups of pioneers: one, the less important, derived from the xerophytic dry meadows, becoming established in cracks and joints of the floor; the second, mesophytic in character, invading from the lower altitudes, occupying depressions and very wet places.

The evidence at hand indicates that the forest, represented at timberline by *Pinus flexilis* on the ridges, and by *Picea Engelmanni* in the cañons, has reached its climatic limit on the ridges, being prevented from further advance by the dry winter winds; but that in the cañons the forest is still invading the wet meadow society.

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BRIEFER ARTICLES

VARIATION IN HELIANTHUS

In a recent number of this journal, Dr. GEORGE H. SHULL[1] calls attention to the dark disk found in the wild sunflower of the prairie region, but shows that certain material received from that region was heterozygous with a yellow disk recessive. It may be worth while to report that this wild plant does actually produce a yellow-disked variety, which I have observed both in Colorado and New Mexico. The western sunflower, according to RYDBERG, is separable as a distinct species, *Helianthus lenticularis* Dougl. This seems to be going too far, and I think it should be called *H. annuus lenticularis*. The color of the disk is considered a specific character in Helianthus; but it certainly varies within specific limits, not only in *H. annuus*, but in *H. petiolaris* also, as I have shown in *Nature*, June 19, 1902, p. 174.—T. D. A. COCKERELL, *Boulder, Colorado.*

ENDOSPERM OF PONTEDERIACEAE

(WITH FOUR FIGURES)

In a recent paper[2] on the seeds of Pontederiaceae, COKER makes some references to my paper published in 1898,[3] to which I wish to make a brief reply. He says: "Oddly enough he completely overlooked the interesting peculiarity in the endosperm of all three genera." The reason for my overlooking such a peculiarity is clear enough, as a very casual reading of the paper will show that I made no attempt to follow endosperm formation. My study was based chiefly on Eichhornia, and in that genus, as grown in Washington Park, Chicago, the endosperm nucleus almost never divides, and the contents of the embryo sac disorganize without development of endosperm or embryo.

Some of my slides of Pontederia, however, extend beyond the fertilization stage, and since COKER's article appeared I have reexamined these, and am able in three cases to confirm his account of a division of the embryo sac into upper and lower chambers. *Fig. 3* shows the condition of the

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[1] Some new cases of Mendelian inheritance. BOT. GAZETTE 45:103–116. 1908.

[2] COKER, W. C., The development of the seed in the Pontederiaceae. BOT. GAZETTE 44:293–301. *pl. 23.* 1907.

[3] SMITH, W. R., A contribution to the life history of the Pontederiaceae. BOT. GAZETTE 25:324–337. *pls. 19, 20.* 1898.

embryo sac just after the division of the endosperm nucleus, and *fig. 4* the lower end of the sac a short time later, with several nuclei in the lower chamber. Of later conditions of the endosperm my slides do not furnish examples.

Again Coker says: "The antipodals never divide, but they are not ephemeral as described by Smith." There may be some difference of opinion as to what degree of persistence my description implies, but after a reexamination of my slides, and even from Coker's own figures, I am still convinced of its correctness. Coker makes the error of regarding the antipodals as a group of nuclei; he cites the persistence of such nuclei after the initiation of endosperm formation as a disproof of my statement; but the antipodals are cells, not nuclei. In those embryo sacs in which they are recognized as typically persistent, such as numerous Ranunculaceae, Sparganium, and others, they become a conspicuous mass of cells; they do not persist as free nuclei. In the case of Eichhornia and Pontederia they are organized cells at the stage shown in *figs. 1* and *2* herewith, and in *fig. 13* of Coker's plate. These cells disorganize immediately after the fusion of the polar nuclei, sometimes even earlier, a behavior which amply justifies my describing them as ephemeral. In later stages of the embryo sac only the nuclei can be distinguished, and these in a partially disorganized condition, as in *figs. 3* and *4*. Compare also Coker's *figs. 13, 15, 17*, in which the disintegration of the antipodal cells and the absorption of their nuclei may be clearly traced.—R. Wilson Smith, *McMaster University, Toronto, Canada*.


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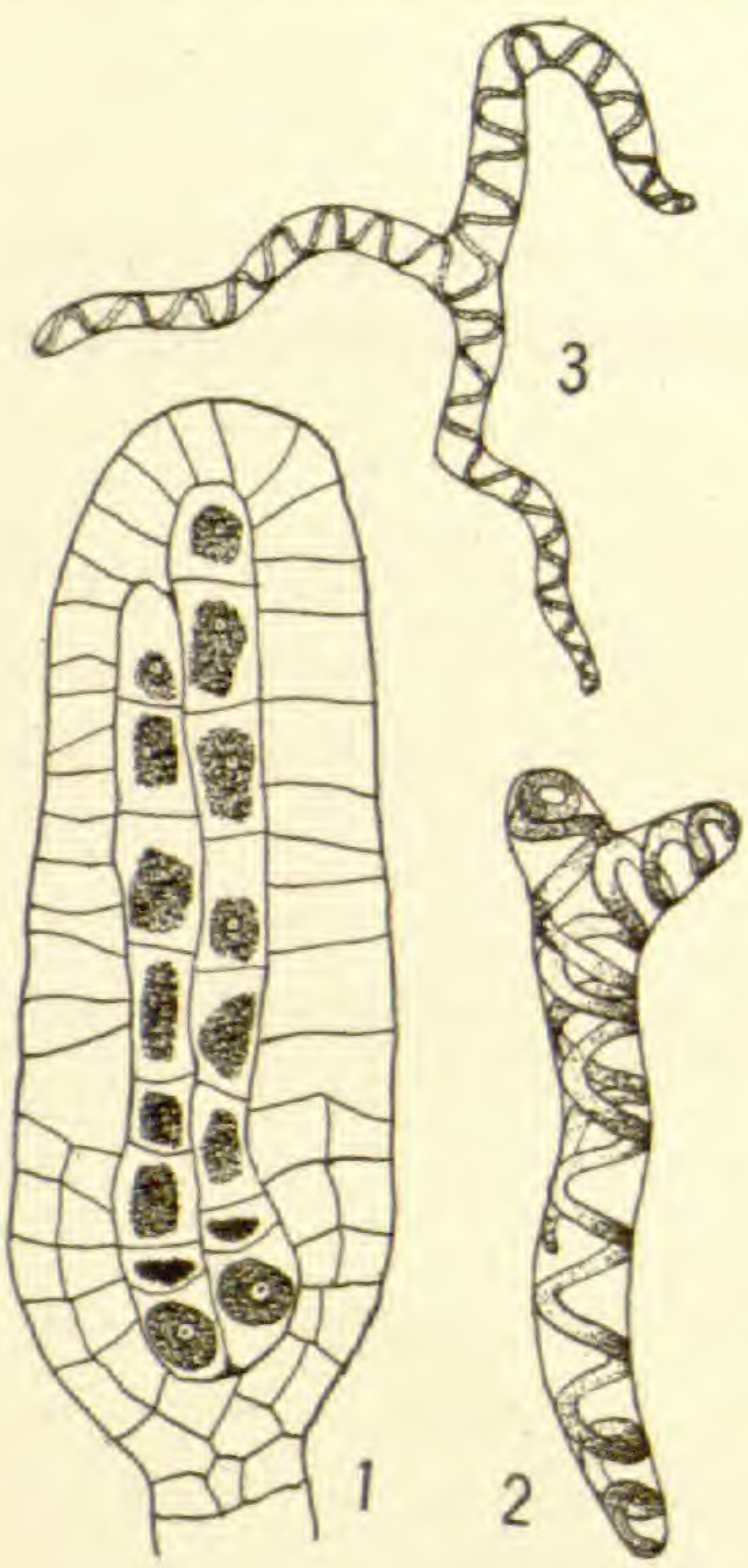
AN ABNORMAL *PORELLA PLATYPHYLLA*

(WITH THREE FIGURES)

In examining some material of *Porella platyphylla* I found one archegonium which differed in internal structure from any thus far observed.[4] This internal difference consisted in the presence of two perfectly formed egg cells, each with a row of canal cells (*fig. 1*). There were also two ventral canal cells. One row of the canal cells, as will be seen from *fig. 1*, was somewhat shorter than the other; the number of cells in each, however, was the same. The egg cells with the canal cells taken together occupied a somewhat larger space than is usual in this plant. The archegonium shown here was larger than those usually found. The two egg cells and canal cells were only clearly seen when lying in the plane shown in figure. If the archegonium was viewed at right angles to this position, they were difficult to see. This specimen was gradually brought into a strong solution of glycerin and allowed to become clear; it was then drawn with a camera lucida.



Porella platyphylla also often shows branched elaters.[5] *Fig. 2* shows one slightly branched at the end; *fig. 3* shows a more pronounced instance of such branching. In some elaters branching of the larger branches was noticed, which formed a rather complicated figure. The spiral thickenings were often very difficult to follow with certainty at the point of branching. *Fig. 2* shows their arrangement in one specimen.—F. M. ANDREWS, *Leipzig.*

[4] See also: COKER, W. C., On the occurrence of two egg cells in the archegonium of Mnium. BOT. GAZETTE **35**:136. 1903.

BLISS, MARY C., On the occurrence of two venters in the archegonium of Polytrichum. BOT. GAZETTE **36**:141. 1903.

LYON, HAROLD L., Polembryony in Sphagnum. BOT. GAZETTE **39**:365. 1905.

GOEBEL, K., Homologien in der Entwicklung männlicher und weiblicher Geschlechtsorgane. Flora **90**:277. 1902.

[5] See also: CAMPBELL, D. H., Mosses and ferns. 2d ed. 1905.

CURRENT LITERATURE

BOOK REVIEWS

Warming's textbook

In 1880 Dr. EUG. WARMING published his *Lehrbuch der allgemeinen Botanik*, which was followed by revised editions until the fourth appeared in 1901. In that edition he associated with himself Dr. W. JOHANNSEN, who elaborated the sections on the cell and on physiology.

This fourth edition has been translated into German by Dr. E. P. MEINECKE,[1] with the collaboration, in two chapters, of Professor JOHANNSEN, who has taken occasion to revise these in part. Other slight changes have been introduced by MEINECKE. But the book cannot be up to date, because, first, it is based on an edition dated 1900–1, and second, the printing of the first part, issued late in 1907, began in 1904. This delay in publication the publishers apologize for, and exonerate themselves, without saying to what it is due. The second part, about one-third the dimensions of the first, was promised before the end of 1907, but at this writing (March 2) has not been received.

The first five sections deal with what we should call the general morphology of plants. Their titles are: 1, The internal and external form of plants; 2, External morphology of higher plants, nutritive organs; 3, The plant cell and its constituents; 4, Histology; 5, Anatomy of the root, stem, and leaf. Sections on reproduction, including discussion of the flower, inflorescence, fruit, seed, relation to habitat, and evolution, are to follow in the second part.

The term morphology is much restricted by these authors, who hold that it concerns itself merely with the origin, development, and form of the external features of the plant body. However, under whatever head they are embraced, these chapters are well written, and the illustrations, mostly not original, are well selected.

The physiology, to which two long sections (180 pp.) are devoted, is in general of the formal type, though with a peculiar distribution of emphasis (as was to be expected), there being a strong tinge of ecology. The section on metabolism begins with a discussion of respiration as an exchange of gases, and makes much of the respiratory quotient; has little to say of the physical processes of exchange between the plant and the external medium, but gives much attention to the relations of plants to each other and to other organisms. The chapter on movement is too brief for anything but an exposition of the fundamental features.

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[1] WARMING-JOHANNSEN, Lehrbuch der allgemeinen Botanik. Translated by E. P. MEINECKE. Imp. 8vo. Part I. pp. vi+480. *figs. 444.* Berlin: Gebrüder Borntraeger. 1907. *M*12.

While the book is a good one, we doubt whether, considering its handicap, there is room for it in the German market in competition with the other excellent texts which are there available.—C. R. B. and C. J. C.

The lighting of plants

For almost the entire period of his half-century of fruitful activity as a physiologist, WIESNER has been occupied with the relations of light to the functions of plants, and for the last fifteen years he has devoted his attention particularly to the relation of light to the plant *as a whole*. This relation is expressed briefly in the word *Lichtgenuss*, a word impossible of translation. On that account he has suggested the less happy *photolepsy* (in English "light-catching"); for "lepsy" lacks the idea of limits set by satisfactoriness that is involved in *Genuss*. WIESNER has now brought together in book form an easily comprehensible synopsis of the results of his long study, carried on in many and diverse regions.[2] It would be a mistake, however, to suppose that the book consists of extracts from earlier works, on which confessedly it is based. In it a considerable number of researches are now first published, especially those on the specific green of the leaves of woody plants as related to their illumination. Also new is the attempt to present a physiological analysis of *Lichtgenuss*.

The *Lichtgenuss* (L) of a plant may be expressed either relatively or absolutely. Relatively it is that part of the total daylight which reaches it. Thus if i be the intensity of light reaching the plant taken as unity, and I the intensity of the total daylight expressed in that unit, then $L=i/I$. Absolutely L may be expressed in any convenient photometric units, and to photometric methods the author devotes the first chapter of the book. These, convenient and adaptable as they are, leave still to be desired a method which will reveal more accurately the photosynthetic value of the light which falls on a plant.

The second chapter analyzes the daylight, consisting as it does of direct and reflected sunlight in varying proportions, directions, and intensities, and shows how total light may be reckoned and graphically represented through the course of a day or a longer period. In the third chapter the illumination of plants is discussed, showing how it is affected by all sorts of conditions, and how plant form is influenced by the lighting. The fourth chapter is devoted to specific observations upon the photolepsy of various types of plants in their habitats; the fifth treats of the constancy or variability of photolepsy in different stages of development, and the optima thereof; the sixth discusses the dependence of photolepsy upon latitude and altitude, and includes particularly the author's observations in this country in 1904; the seventh deals with various sorts of leaf-fall; and the eighth points out the connection of mycorhiza formation with the *Lichtgenuss*.

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[2] WIESNER, J., Der Lichtgenuss der Pflanzen. Photometrische und physiologische Untersuchungen mit besonderer Rucksichtnahme auf Lebensweise, geographische Verbreitung und Kultur der Pflanzen. 8vo. pp. viii+322. *figs. 25*. Leipzig: Wilhelm Engelmann. 1907. *M*9.

In the ninth chapter WIESNER describes the differences in tone that have often been observed in the green of leaves and connects it with the excess, deficiency, or sufficiency of the illumination, much as STAHL and JÖNSSON had done on somewhat different grounds. The tenth chapter attempts a physiological analysis of the *Lichtgenuss*, which inevitably is "up in the air." The last chapter shows how the study of light relations by the photometric methods proposed can be of service in plant culture; and how the results of such study may serve as guides to proper planting in all sorts of conditions and locations. A bibliography (4 pp.) and a double index (topics and plant names) complete the book.

While for him who wishes to carry on investigations along these lines the various memoirs that have come from WIESNER'S hand are indispensable, this book will better serve one who desires merely a general statement of results and principles. At the same time it must be said that the book contains much that is wholly familiar and commonplace, so that it might have been much condensed to advantage; but perhaps the picture would not have been so complete. Furthermore, the book is by no means free from doubtful generalizations and generous assumptions; indeed, it seems that everyone who deals with adaptations must allow his imagination a rather loose rein. Withal there is in the work an important nucleus of no little value, and even an occasional flight of fancy may be permitted, if it stimulate interest.—C. R. B.

MINOR NOTICES

Cryptogamic flora of Brandenburg.[3]—The second part of the volume on Algae, by LEMMERMANN, has now appeared. It concludes the Oscillatoriaceae; includes Nostocaceae, Microchaetaceae, Scytonemataceae, Stigonemataceae, Rivulariaceae, and Camptotrichiaceae; and begins the Flagellatae.—J. M. C.

List of British plants.—DRUCE[4] has published a list of British plants, including the "Spermophytes, Pteridophytes, and Charads," found either as natives or growing in a wild state in Britain, Ireland, and the Channel Isles. The introduction contains a protest against the *nomina conservanda* of the Vienna Congress, and these are "deliberately ignored." The list is especially for "working botanists and members of the exchange clubs." The census of species shows 1390 native species, 144 alien species now well established, and 940 more or less fugitive aliens, the total enumeration including 2964 numbers.—J. M. C.

Trees and shrubs.—A preliminary announcement of SARGENT'S *Trees and shrubs* appeared in this journal in 1902, and there followed notices[5] of the four parts which completed the first volume in 1905. The first part of the second

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[3] LEMMERMAN, E., Kryptogamenflora der Mark Brandenburg, Band 3, Heft 2. Algen. pp. 129–304. Leipzig: Gebrüder Borntraeger. 1907. Algen (Band 3, Heft 1 u. 2). *M*9.50.

[4] DRUCE, GEORGE CLARIDGE, List of British plants. pp. xv+104. Oxford: Clarendon Press. 1908. 2*s*. 6*d*.

[5] BOT. GAZETTE **34**:388. 1902; **35**:62. 1903; **36**:68. 1903; **37**:155. 1904; **39**: 372. 1905.

volume has now appeared.[6] The twenty-five species illustrated include six new species of Crataegus from Missouri, and new species from China or Japan under Ulmus, Berberis, and Viburnum (3). Four new species of Lonicera from China are described without illustration by REHDER, who also describes and illustrates a new hybrid under Malus. The tropical American (Florida and Mexico to Central America) species illustrated are *Alvaradoa amorphoides* Liebm., *Pinus Greggii* Engelm., and *P. Lumholtzii* Robinson and Fernald. The ten remaining species are from China or Japan, and belong to Berberis, Acer, Rhododendron, Viburnum (5), and Lonicera (2).—J. M. C.

Plant phyla.—Professor BESSEY[7] has been working for many years upon a natural (evolutionary) classification of plants, and the result has just appeared in published form. He recognizes fifteen great "phyla," and presents a diagram to illustrate their relationship. It is impossible to give any adequate conception of the scheme, for it is very compactly presented and includes an enormous mass of details. A glimpse of the point of view may be obtained from the following list of the "phyla," the number following each name indicating the number of families included: Myxophyceae (9), Protophyceae (17), Zygophyceae (21), Siphonophyceae (18), Phaeophyceae (23), Carpophyceae (26), Carpomyceteae (145), Bryophyta (54), Pteridophyta (13), Calamophyta (4), Lepidophyta (7), Cycadophyta (9), Gnetales (1), Strobilophyta (9), Anthophyta (280). The labor involved in organizing and defining these 636 families must have been enormous.—J. M. C.

American Breeders' Association.—The literature of breeding which is now growing with great rapidity is necessarily much scattered. The third annual report of the American Breeders' Association[8] contains a large number of papers covering a wide range of subjects relating to both plant and animal breeding. The papers which are of most interest to scientific breeders and students of heredity are "Inheritance in pedigree breeding of poultry" and "Recent advances in the theory of breeding," by C. B. DAVENPORT; "The production and fixation of new breeds," by W. E. CASTLE; "Some results in selecting red clover for disease resistance," by S. M. BAIN; "Heredity in carnation seedlings," by J. B. NORTON; "Report of the committee on theoretic research in heredity," by CHARLES W. WARD; "The chromosome in the transmission of hereditary characters," by W. J. SPILLMAN. There are also a number of excellent papers and reports which must be of the greatest value to breeders of the economic crops. One of the best of these

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[6] SARGENT, C. S., Trees and shrubs. Illustrations of new or little known ligneous plants, prepared chiefly from material at the Arnold Arboretum of Harvard University. Vol. II. Part I. pp. 1–55. *pls. 101–125.* Boston and New York: Houghton, Mifflin & Company. 1907. $5.00.

[7] BESSEY, CHARLES E., A synopsis of plant phyla. Univ. Nebraska Studies 7:no. 4. pp. 100. 1907. Lincoln: University Publishing Company. 50 cents.

[8] Annual report of the American Breeders' Association, Vol. 3. 8vo. pp. 305. Washington, D. C. 1907.

is a scheme for corn breeding worked out in very clear detail by C. G. WILLIAMS, by which it is hoped to secure the greatest possible improvement without encountering the injurious effects of inbreeding. In the rapid development of all breeding problems, due to the universal interest which is being taken both in scientific and economic aspects of the subject, the annual reports of the American Breeders' Association must continue to be a most valuable source of information as to the present state of knowledge, and no one who is interested in these problems from either point of view can afford to be without the annual volumes.—GEORGE H. SHULL.

NOTES FOR STUDENTS

Ascent of water.—EWART becomes a stronger and stronger opponent of the DIXON-JOLY theory of the ascent of sap, and in his last paper[9] adduces some powerful arguments, backed by observations, against it and in favor of the theory of vital maintenance of suitable conditions for conduction and some sort of pumping action. The latter may be by surface tension, but while theoretical ways abound in which this might be applied, no practical proof of the existence of any such action in wood is forthcoming. EWART's experiments all tend to show, he thinks, that the continuous ascent of water is possible only in living wood, and that the power of conduction is rapidly lost at death. Experiments on the suction and exudation of trees at different levels showed no continuous water columns or high internal tensions in the tracheae during active transpiration, and this fact, coupled with the high total resistance to flow, indicates that this resistance is overcome locally from point to point, and not by enormous tension from above, which leads to blocking by gas bubbles, nor by pressure from below, which leads to great loss by lateral exudation from the vessels.

EWART makes several corrections of his own and others' previous observations. He finds no vessels open the whole length of the tree, as STRASBURGER thought to be the case in oak. Wistaria furnished him the longest—5.64^{m}. In the oak they are seldom over 1^{m}. Nor does EWART sustain STRASBURGER's results on the conduction of water through dead wood, for if these experiments are sound, vital action is out of the question. His own earlier observations on the osmotic pressures in leaves at different levels are also disowned, for he now finds as great differences in leaves at the same level. Incidentally he looked up the evidence as to the height of the tallest trees in Australia, and concludes that none appreciably exceed 300 feet, instead of being 472 as reported. He thinks that the height of some of our American big trees may likewise shrink on investigation.

In the course of the discussion of exudation he makes the very good point that fully turgid cells, for example, those of the root cortex, may act simply as a membrane between the water in the vessels and the outside water, their own osmotic pressure, however high, having no influence on the transfer of water from soil to vessel so long as they remain fully turgid. This also explains how cells of unlike

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[9] EWART, A. J., The ascent of water in trees (second paper). Phil. Trans. Roy. Soc. London B 199:341–392. 1908.

osmotic pressure can exist side by side without those of higher pressure draining those of lower, which, however, they would do as soon as their turgor was relaxed ever so slightly, thus releasing some of the osmotic energy that had previously been expended against the wall. This point has been often overlooked.

URSPRUNG[10] makes what he himself properly calls "a small contribution" (which nevertheless fills sixty-odd pages!) to a more extended study of the relation of live cells to the ascent of water in woody plants. His data will be more useful than his interpretation. The experiments, carried on in the forests, embraced five species of gymnosperms and fifteen of angiosperms. The axes were killed for greater or less distances at various levels by steam, and usually others were girdled at the same time. Microscopic examination was made of both dead portions and neighboring parts. URSPRUNG explains the dissimilar behavior of different species on the assumption (and therefore begs the question) that the experimental interference eliminates the vital component of the lifting forces, but does not affect the physical; so as the vital factor is greater or less the wilting of the leaves occurs in a few days in some experiments or is delayed to 100 in others. His "results" he summarizes thus: "In all the plants investigated a participation of the living cells in the production of the lifting force is to be assumed." (Is this a *result?*) "Water conduction occurs chiefly in the younger layers of the wood." (This has been long known.) "In all observed plants the cortex must be present in order to make possible the continuance of a sufficient water movement; its removal acts injuriously, though not everywhere equally. The significance of the cortex for the ascent of sap lies probably in its protective function for the periphery of the wood." (This has scarcely been doubted.) "For a sufficient water-movement a small fraction of the conducting tissues suffices, if in the part remaining the wood cells be living." (The *if* is not a condition proved but assumed.) "The force component arising from the living cells attains great significance in comparison with the purely physical." (*Quod est demonstrandum.*) —C. R. B.

Items of taxonomic interest.—C. B. CLARKE (Contrib. U. S. Nat. Herb. **10**:443–471. 1908), before his death, had prepared a synopsis (in Latin) of the Cyperaceae of Costa Rica. This has now been published, with such changes as were necessary, such as translation into English (by E. L. GREENE), rearrangement of synonymy, completion of citations, elimination of *nomina nuda*, etc. A synoptical key to the 19 genera precedes the presentation of the 105 species. The large genera are Rynchospora (16), Cyperus (15), Eleocharis (13), and Scleria (11), and a single new species is described in each of the following genera: Cyperus, Rynchospora, and Carex.—J. J. SMITH (Bull. Dept. Agric. Ind. Néerland. no. 13. pp. 78. *pls. 2.* 1907), in the first of a series of papers on the orchids of Java, has described 15 new species and 2 new genera (*Silvorchis* and *Lectandra*).—E. ULE (Notizblatt **5**: no. 41*a*. pp. 52. *pls. 4.* 1908), in connection with an account of the

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[10] URSPRUNG, A., Abtötungs- und Ringelungsversuche an einigen Holzpflanze. Jahrb. Wiss. Bot. **44**:287–349. *pl. 4.* 1907.

caoutchouc industry of Bahia, has described 3 new species of Manihot.—P. B. Kennedy (Muhlenbergia **4**:1–4. 1908) has described a new species of Dicoria from Nevada.—S. Sommier (Giorn. Bot. Ital. N. S. **14**:496–505. *pl. 14.* 1907) has described a new genus (*Melitella*) of Compositae (Cichoriaceae) from Malta.—R. Pampanini (*idem* 591–606), in describing a fascicle of new species, includes a Codonanthe from Brazil and a Sesbania from Central America (Mexico ?).—A. B. Rendle, E. G. Baker, and S. LeM. Moore (Jour. Linn. Soc. Bot. **38**:228–279. *pls. 16–19.* 1908), in an account of the Wollaston collection of plants from Mt. Ruwenzori (Africa), describe 34 new species, among which there is a new genus (*Choananthus*) of Amaryllidaceae.—A. D. E. Elmer (Leaflets Philipp. B **1**:212–219. 1907) has described 8 new species of Freycinetia from Lucban; also (*idem* 220–232) 11 new species of Leguminosae, including the new genus *Luzonia* (nearest Canavalia); and (*idem* 236–261) has presented 41 species and varieties of Ficus collected within a radius of three miles of Lucban (Luzon), including 6 new species.—E. B. Copeland (*idem* 233–235) has described 3 new species of ferns from the Philippines.—J. H. Barnhart (Bull. Torr. Bot. Club **34**:579–582. *pl. 34.* 1907) has described a new Utricularia from Long Isand.—J. C. Arthur (*idem* 583–592), in his sixth fascicle of new Uredineae, describes 15 new species.—K. K. Mackenzie (*idem* 603–607), has described 4 new species of Carex.—H. Rehm (Annales Mycol. **5**:516–546. 1907), in describing 95 new species of Ascomycetes from various parts of the world, includes 18 from North America and 38 from South America.—In publishing the third fascicle of Weberbauer's new plants from the Andes (Bot. Jahrb. **40**:225–395. 1908), 264 new species are described, including the following new genera: *Urbanodoxa* and *Englerocharis* (Cruciferae), by R. Muscher; *Urbanosciadium* (Umbelliferae), by H. Wolff; and *Acrobotrys* (Rubiaceae), by K. Schumann and K. Krause.—R. Pilger (*idem* 396, 397) has described a new genus (*Glischrothamnus*) of Aizoaceae from Brazil.—R. Schlechter (Bot. Jahrb. Beibl. **40**^{3}:1–19. 1908), in describing 36 new Asclepiadaceae from the East Indies, includes the new genera *Oistonema* and *Anatropanthus;* the same author (*idem* 20–45) describes 54 new species from New Caledonia, and includes four new genera (*Dolichanthera, Rhopalobrachium, Atractocarpus, Bonatia*) of Rubiaceae.—C. C. Hosseus (Bot. Jahrb. **41**:55–61. *pls. 1, 2.* 1908) has described a new genus (*Richthofenia*) of Rafflesiaceae from Siam; he has also published (*idem* 62–73) a list of 55 species of Acanthaceae from Siam, including 12 new species.—E. Koehne (*idem* 74–110) has supplemented his account of Lythraceae in Engler's *Pflanzenreich* (1903) by intercalating species published since that date, many of them American (chiefly Mexican), and by adding 16 new species published here for the first time.—N. L. Britton and J. N. Rose (Smithson. Misc. Coll. **50**:503–539. 1908) have published a preliminary account of the Opuntioideae of North America, recognizing 143 species of Opuntia (15 of which are new), 6 of Nopalea, and 11 of Pereskiopsis, a genus recently published in the same serial (331–333).—J. M. C.

The individuality of the chromosome.—By subjecting root tips of *Pisum sativum* to a 0.75 per cent. solution of chloral hydrate at 20° C. for one hour, then washing away the chloral hydrate in water and growing the tips in sawdust, NEMEC found that after 20 hours many cells have become binucleate. After 27 hours few cells are binucleate, but many contain large nuclei which contain 28 chromosomes, twice the $2x$ number. After 40 hours the mitoses show regularly 14 chromosomes, the $2x$ number. NEMEC believed the quadruple number to be brought back automatically to the $2x$ number by a heterotypic mitosis.

STRASBURGER[11] has traversed the same ground, treating material of *Pisum sativum* in the same way and making an exhaustive study of all the cytological phenomena. He found cells with two nuclei and observed that such nuclei fuse to form large nuclei with 28 chromosomes, the quadruple number; but such mitoses, aside from the large number of chromosomes, present no striking peculiarities. There are abnormalities, due to the chloralization, but there are no characteristics of the heterotypic mitosis or anything else to indicate a reduction to the $2x$ number; but rather there is evidence that the $4x$ number, once established, persists.

The investigation of chloralized nuclei was made particularly exhaustive, that it might form a basis for comparison with nuclear phenomena in graft hybrids. An investigation of typical and chloralized nuclei of *Laburnum vulgare*, *Cytisus purpureus*, and *Laburnum Adami* presented rather constantly 48 chromosomes, the $2x$ number, and showed no evidence of any fusion, reduction, or autoregulation. These plants gave no response to such chloralizing as that described for *Pisum sativum*, and after greater stimulation nuclear divisions were not resumed. The study of nuclear phenomena threw no light upon the problem of graft hybrids. There were no heterotypic mitoses and the author doubts whether heterotypic mitoses are to be found in vegetative or somatic tissues. Such mitoses as those described by English writers for malignant tumors STRASBURGER believes to be incorrectly interpreted.

The noted Mespilus at Bronvaux, recently studied by NOLL, is discussed, and here again there is no evidence of fusion or reduction. A study of the Bizzaria orange compared with "Agrumi," which is not a hybrid, showed no cytological differences; both have 16 chromosomes. The evidence showed that Bizzaria is a hybrid of sexual origin and that there is nothing to support any theory of autoregulation.

The paper gives a full account of the cytological details of graft hybrids, together with theoretical discussions which cannot be treated adequately in a brief review.—CHARLES J. CHAMBERLAIN.

Phototropism and perception of light.—FITTING has undertaken[12] to solve the question whether a localized tropic or nastic sensitiveness must be considered as a sign of localization of the *perceptive faculty* for the stimulus, and whether it

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[11] STRASBURGER, EDUARD, Ueber die Individualität der Chromosomen und die Pfropfhybriden-Frage. Jahrb. Wiss. Bot. **44**:482–555. *pls. 5–7.* 1907.

[12] FITTING, H., Lichtperception und phototropische Empfindlichkeit. Jahrb. Wiss. Bot. **45**:83–136. 1907.

is justifiable to speak of a localized phototropic irritability as merely a localization of sensitiveness to light. For this purpose he chose seedlings of *Panicum miliaceum*, in which the tip of the cotyledon alone is phototropically sensitive, and determined how the growth in length of the part not thus sensitive was affected by direct illumination and by illumination of the cotyledon tip, preliminary investigations having shown that the growth, which is restricted almost entirely to the uppermost part of the hypocotyl, is greatly retarded, and that the amount of retardation is closely dependent upon the intensity of the light. In light of moderate intensity the growth of the hypocotyl is checked almost equally, whether the cotyledon alone or the hypocotyl alone is illuminated; but only half so much as when the whole seedling is illuminated. At higher intensities, on the contrary, the illumination of the cotyledon retards the growth more; from which it is evident that the effect of the light is transmitted downward to the hypocotyl. But if one illuminates all but the uppermost $2-4^{mm}$ of the hypocotyl, the growth of the parts above is not retarded, and the light effect is clearly not transmitted upward. The retarding effect of illumination of the cotyledon does not proceed from its tip, which is phototropically very sensitive, for illumination of this tip alone produces far less retardation of the growth of the hypocotyl than does the illumination of a greater part of the hypocotyl. Clearly the light acts as a stimulus and both cotyledon and hypocotyl are sensitive to it. Although, as ROTHERT has shown, the hypocotyl is in general not phototropically sensitive, FITTING found that 17–25 per cent. of the seedlings whose cotyledons were darkened curved phototropically after one-sided illumination. These facts show that the distribution of *phototropic* sensitiveness does not indicate which part of the seedling perceives light. Similar results were obtained with *Sorghum Dora*, *S. vulgare*, *Zea Mays*, and *Tinantia fugax* (Commelinaceae).

FITTING found nothing to indicate that phototropic sensitiveness proceeds from any other process of light perception than that which expresses itself in retardation of growth. He suggests "that the localization of phototropic sensitiveness may perhaps be referable to the fact that the polar condition characteristic of induction of phototropism can be produced only in the phototropically sensitive zone."

He discusses also the problem of etiolation, but adds nothing new, merely emphasizing the existing view that the normal form of leaves in dicotyls depends upon the direct action of light as a stimulus without the correlations which are usually considered.—C. R. B.

Javanese Anthocerotales.—As one of the results of his recent visit to the oriental tropics, CAMPBELL[13] has published an account of certain Javanese Anthocerotales. Among a number of species of Anthoceros collected at the base of Mt. Salak and also near Tjibodas, he found two forms which he believes should constitute a new genus, *Megaceros*, into which he would throw all the non-

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[13] CAMPBELL, D. H., Studies on some Javanese Anthocerotaceae. Annals of Botany **21**:467–486. *pls. 44–46.* 1907; **22**:91–102. *pls. 9,10.* 1908.

stomatiferous species with spiral elaters. The important differences between Megaceros and Anthoceros are that the former has multiple chromatophores, no stomata, a solitary antheridium, spiral elaters, and green spores. In one species (*M. salakensis*) the sporogonium dehisces along one side. The thallus closely resembles that of Anthoceros. The reviewer has frequently observed single antheridia in *A. laevis* and the same is reported for *A. Pearsoni*. The axial row is cut out of the archegonium initial by three intersecting walls, as is true for all Hepaticae. The neck-canal cells are four, rarely five. The early development of the embryo and details of the sporogonium closely resemble Dendroceros, but the sporogenous tissue is more extensive than in either Anthoceros or Dendroceros. One figure suggests that perhaps a portion of the tip of the endothecium is sporogenous, as has been found in Notothylas by LANG. The sterile cells form an irregular network inclosing the spore mother cells, which are slightly lobed. The elaters are branched. No germ tube is produced.

Two Javanese species of Dendroceros, designated *A* and *B*, were studied. Species *A* had a better-developed columella than *B*. In a Jamaican species the reviewer finds that the tip of the sporogonium is occasionally sterile. Spores of *A* and *B*, as well as other Javanese species of Dendroceros, germinate before being shed.

Notothylas javanicus closely resembles the cosmopolitan *N. orbicularis*. The archegonia are broader than in Anthoceros, but the neck-canal cells are reduced to three. The first division of the embryo is longitudinal, and not transverse as MOTTIER thinks is true in *N. orbicularis*. It is suggested that possibly the endothecium contributes something toward sporogenous tissue, as has been recently shown in another species of Notothylas by LANG, although CAMPBELL makes no mention of LANG's work.

CAMPBELL thinks that perhaps the affinities of the Anthocerotales are with the Marchantiales rather than with the Jungermanniales; that perhaps the sporogonium of Notothylas is best compared with that of Cyathodium. He thinks that the relationships of the group are so remote from other Hepaticae that it should form a special class, "Anthocerotes."—W. J. G. LAND.

Ever-sporting varieties and variegation.—Ever-sporting varieties have received illuminating treatment by BAUR,[14] who places the sport condition among the normal fluctuating reactions of the particular variety in question. When there is a complete parallelism between the modification of a character and the variation of external stimuli which cause the modification, a chance distribution of varieties results; but when this parallelism is only partial, as is frequently the case, a "half-Galton" or otherwise modified curve will appear, and the occurrence of such modified curves may serve as a sign of ever-sporting varieties.

The sport condition appears only when a certain intensity of the combi-

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[14] BAUR, E., Untersuchungen über die Erblichkeitsverhältnisse einer nur in Bastardform lebensfähigen Sippe von *Antirrhinum majus*. Ber. Deutsch. Bot. Gesells. 25:442–454. 1907.

nations of external modifying stimuli and nutritive conditions, in the widest sense, is exceeded. In each ever-sporting variety there is a critical period at which the "normal" or the "sport" condition is determined. If this period occurs late in the ontogeny, the sport appears as a partial variation, as in the five-leafed clovers; and if it is very early in the ontogeny, the variation appears to be individual, as in the case of double stocks.

A sharp distinction is drawn between these modifications and mutations, and Klebs is criticized for failing to keep them separate. This criticism would apply equally well to many other writers.

With ever-sporting varieties, many variegate-leaved varieties have a great but purely superficial resemblance. The author recognizes two types of golden modification, one due to disease, the other hereditary. Pedigree cultures with the latter type present striking results in the case of *Antirrhinum majus pumilum fol. aureis* and *Pelargonium zonale*. In Antirrhinum the cross between any two variegated specimens gives variegated and green in ratio 2:1, of which the green breed true. The conclusion was reached that variegation is in this case a Mendelian character, in which the union of two "variegated" gametes does not give rise to a successful zygote. In proof of this view, the cross between variegated and green (DR × R) gave in every case the expected ratio 1:1. Every variegated individual is thus a heterozygote, and the problem of the "fixation" of the variegated race is the same as that of the fixation of the blue Andalusian race of fowls.

In *Pelargonium zonale* a somewhat similar situation seems to exist, but here the extracted dominants, instead of being entirely incapable of development, appear as pure-white individuals which perish very early, leaving again the ratio of variegated to green, 2DR:1R.—Geo. H. Shull.

Injury by smoke.—Continuing the studies embodied in the volume published a few years ago on the injury of vegetation by smoke,[15] which treated chiefly of gases, Haselhoff, with the assistance of several colleagues, has been investigating the effect of the solid constituents of smoke and other factory emanations, which fall as dust, and may injure plants either directly by contact with their leaves or indirectly through their roots by way of the soil.[16] Such investigations are particularly important in continental countries, where huge factories are often closely surrounded by cultivated fields. They are of less value in this country, where the cultivation is less close and a strong tendency exists to mass factories in industrial centers. Yet at any time such data as are here set forth may be called for as a guide for legislation, or in civil cases.

Haselhoff finds that the dust varies much in composition even from similar fuels and the same sort of factories, so that it becomes necessary in each case to determine its composition. Chlorids ($NaCl$), sulfids ($NaSO_2$ and $Ca(SO_2)_2$), and perhaps also sulfates ($NaSO_4$) are particularly injurious. The sulfids are

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[15] See Bot. Gazette 38:148. 1904.

[16] Haselhoff, E., Versuche über die Einwirkung von Flugstaub auf Boden und Pflanzen. Landw. Versuchs-Stat. 67:157–205. *pls. 1, 2.* 1907.

injurious through their action upon the soil, especially if its unfavorable condition facilitates the formation of H_2S. Analysis of plants often shows them to contain an increased quantity of the constituents of the dust. Microscopic investigation of the leaves shows clearly the injurious action of various dusts, which is substantiated by tests with the component salts; but there are no typical anatomical marks by which the injury wrought by one kind of dust may be distinguished from another.—C. R. B.

Polystelic roots.—Polystely in stems, as described by VAN TIEGHEM, has been disproved, but it seems clear that the phenomenon occurs in roots. CORMACK and later DRABBLE[17] described a multistelic condition in the roots of palms, and a recent research by WHITE[18] proves its occurrence in both the lateral and the tuberous roots of certain orchids belonging to Ophrydinae. In *Habenaria orbiculata* the stele of the lateral root is at its base monostelic, farther out it flattens, becomes horseshoe-shaped, then divides into two steles which at the tip merge into a common plerome cyclinder. In *H. blephariglottis* a protostele acquires a pith and internal endodermis, then opens out to a horseshoe-shape from the free ends of which steles are constricted; as in the former case these steles merge into a common plerome at the root tip. In *H. hyberborea* the steles are separate from the start, but increase in number as they proceed farther from their origin. In the tubers the steles have a similar diffuse origin, and it was upon a study of these that VAN TIEGHEM founded his theory of "concrescence" to account for the conditions seen in orchid roots. The present research strongly supports the view advanced by JEFFREY as to the extrastelar nature of the pith, for in *H. blephariglottis* the pith of the proximal part of the central cylinder merges into the fundamental tissue of the polystele. Moreover, the plerome initials are seen to give rise to both fundamental and vascular tissues, hence HANSTEIN's theory as to the correspondence of plerome and periblem to stele and cortex respectively cannot be maintained.—M. A. CHRYSLER.

Salt marshes of New England.—The construction of an electric railroad across a salt marsh at York, Maine, recently afforded an opportunity of examining a vertical section through the deposits beneath the turf of the marsh. A study of such a section by PENHALLOW[19] shows that there was an abrupt conversion of a fresh-water bog into a salt marsh by the sudden intrusion of sea water, and that this phenomenon was connected with the gradual subsidence of the general area in which the marsh is situated. It would appear that at some former time (Pliocene) shallow basins existed between islands along the Atlantic coast, and some of these basins, being cut off by the formation of barrier reefs, were converted into

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[17] Reviewed in this journal 39:382. 1905.

[18] WHITE, J. H., On polystely in roots of Orchidaceae. Univ. of Toronto Studies, Biological Series no. 6. pp. 20. *pls. 1, 2.* 1907.

[19] PENHALLOW, D. P., A contribution to our knowledge of the origin and development of certain marsh lands on the coast of New England. Trans. Roy. Soc. Canada III. 1:13–56. 1907.

fresh-water ponds by the drainage from the adjacent areas. The ponds subsequently became sphagnum bogs, with the usual succession of vegetation culminating in a white pine swamp. The weight of this forest submerged it in the quaking bog upon which it rested, killing the trees, and establishing more hydrophytic conditions. The white pines then reestablished themselves and dominated the area for a century, when the forest development was abruptly terminated by the influx of salt water caused by the subsidence of the region and the consequent breaking-through of the barrier reef. The duration of the marsh from the establishment of bog plants on the sphagnum to the present time is estimated at 420 years. —GEO. D. FULLER.

Studies in the rusts.—OLIVE has published abstracts[20] of two recent studies among the rusts. One deals with the conflicting statements of BLACKMAN and CHRISTMAN in reference to the sexual performance in the caeoma type of rusts. As a result of the study of several species of caeoma rusts, OLIVE confirms CHRISTMAN as to the fusion of two similar gametes by the development of a conjugation pore; but he also finds that the process may begin through a very small pore, so that the nucleus of the migrating protoplast may become stretched out or constricted, thus giving the appearance of BLACKMAN's "nuclear migration." OLIVE also finds that the two gametes differ somewhat in time of development, and presents the observations on which this important conclusion is based.

The other study is concerned with the origin and relationships of the more compact, "cluster-cup" type of structure. It seems that large, irregular, multinucleate cells arise after the sexual fusion, and that the basal cells of the aecidiospore rows arise as the ultimate branches of these cells. Hence the cup structure is derived from a limited and deep-seated group of cells, and the peridium arises in consequence. OLIVE also suggests the method by which the aecidium-cup type may have originated from the simpler caeoma type, and concludes that the former type is the last member of the evolutionary series in this group.—J. M. C.

Endosperm of caprifigs.—LECLERC DU SABLON[21] has discovered that in those pistillate flowers of the caprifig in which Blastophaga has deposited eggs the endosperm develops, although fertilization has not occurred. Such endosperm he speaks of as parthenogenetic, and observes that it is digested by the larva in the same way that the normal endosperm is digested by the plant embryo, the destruction of the endosperm in both cases being complete. The parthenogenetic endosperm differs strikingly from the ordinary kind, in the absence of cellulose walls, dense cytoplasm, and very large, often irregular nuclei, which are variable in number. In the rare cases in which fertilization has occurred in a pistillate flower of the caprifig, endosperm identical with that of the "Smyrna fig" is formed, that

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[20] OLIVE, E. W., The relation of "conjugation" and "nuclear migration" in the rusts. Science N. S. 27:213. 1908.

———, The relationships of the aecidium-cup type of rust. *Idem* 214.

[21] SABLON, LECLERC DU, Structure et développement de l'albumen du caprifiguier. Rev. Gén. Botanique 20:14–24. *pl. 6.* 1908.

is, with cellulose walls, relatively scanty cytoplasm, and small, solitary nuclei. The pistillate flowers of the caprifig in which there has been neither egg-deposition nor fertilization usually do not develop farther and become atrophied. The author thinks the conclusion to be inevitable that the "stimulus" produced by the presence of the egg and larva of Blastophaga does just what is usually accomplished by fertilization, in determining not only the development of endosperm but also the further growth of the ovule and of the pericarp, and the general form of the fig.—J. M. C.

Spore formation in Derbesia.—DAVIS[22] has published a paper treating of spore formation in Derbesia, one of the Siphonales. He studied the development of sporangia and the formation and germination of zoospores. The most interesting points of the paper are the results concerning the behavior of nuclei in the sporangium previous to the formation of zoospores. Many nuclei which do not take part in the formation of zoospores undergo degeneration. Plastids become arranged radially about the nucleus which survives the act of degeneration. Including the nucleus as a center, segmentation of protoplasm takes place to form the beginning of the zoospore; then the formation of the blepharoplast begins. Previous to the formation of the blepharoplast, the nucleus moves from near the center of the young zoospore toward the periphery. Granules around the nucleus seem to move toward the periphery, where they fuse with one another to form a ring which becomes the blepharoplast. DAVIS believes that the belepharoplast of Derbesia is not a development from the plasma membrane, but from the granules closely associated with the protoplasm investing the nucleus. The paper closes with a discussion of the cytology of the blepharoplast and the value of zoospores and gametes as taxonomic characters in Siphonales.—SHIGÉO YAMANOUCHI.

Gummosis.—RUHLAND, who had been working with the late Dr. R. ADERHOLD in the Imperial Biological Station at Dahlem upon the problem of the formation of gum, has published a preliminary paper upon the physiology of gum formation,[23] promising the full account later, with developmental and anatomical details, in the *Arbeiten* of the station. A part of the paper is devoted to a criticism of the latest theory, that of BEIJERINCK and RANT,[24] who hold that cytolytic substances issuing from the dead cells in the neighborhood of a wound cause the liquefaction of embryonal wood tissues, anomalously developed through the wound stimulus. RUHLAND, however, holds that, whenever such tissues are produced by wounding, it is the influence of the oxygen of the admitted air that determines a cessation of further division in these cells, because of the transfor-

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[22] DAVIS, B. M., Spore formation in Derbesia. Annals of Botany 22:1–20. *pls. 1, 2.* 1908.

[23] RUHLAND, W., Zur Physiologie der Gummibildung bei den Amygdaleen. Ber. Deutsch. Bot. Gesells. 25:302–315. *figs. 3.* 1907.

[24] Wundreiz, Parasitismus, und Gummifluss bei den Amygdaleen. Centralbl. Bakt. II. 15:366 ff.

mation of certain carbohydrates, suitable for forming the partition walls, into gums rich in oxygen. Parasites and saprophytes affect the formation of gum only by creating or enlarging rifts or wounds, hindering their recovering and recovery, and so making possible the access of oxygen. Experimental proofs are adduced for this view, whose probability is increased by many chemical considerations.—C. R. B.

Fixation of free nitrogen.—Dr. CHARLOTTE TERNETZ, baffled in an attempt to identify the pycnidial fungi which belong to the endotrophic mycorhiza of the Ericaceae, turned to a study of the ability of these fungi to fix free nitrogen when grown in N-free cultures.[25] The fungi doubtless belonged to the genus Phoma, and five species are described, derived from the roots of as many genera. These all fix atmospheric nitrogen, though in very different amounts. The three from Vaccinium, Oxycoccus, and Andromeda, while they work far less energetically in this respect than most N-fixing bacteria, nevertheless gain the largest amount of nitrogen of any known organisms in comparison with the amount of carbohydrate consumed. *Aspergillus niger* and *Penicillium glaucum* likewise are capable of fixing free N in small amounts, comparable with those yielded by the other two Phomas from the roots of Tetralix and Erica.

It may be that this action, relatively small when the fungi are grown in culture media, is much more pronounced when the mycelium grows in the cortex, and that we have here proof of the advantages which the Ericaceae derive from this association. The author's cultures show that the infection may be transmitted through the seeds and does not come necessarily from the soil.—C. R. B.

Development of leaves.—LEWIS[26] has published a second study of leaf development, in which he shows "that the basipetal and basifugal directions of growth may both occur in a single leaf; and that, although one becomes predominant, evidences of the other are apparent." In a previous paper[27] he discussed the question whether certain forms of adult leaves could be due to arrested development, "so that by comparing the mature leaves of a given plant something of their embryological history could be learned." The general conclusion is that leaves of very diverse species show a common method of leaf development, in which the basipetal and basifugal directions of growth are combined; and by the predominance of the basipetal or the basifugal elements, palmate or pinnate leaves are produced respectively. The author thinks that there is shown a determinate evolution of leaf forms, whereby diverse species tend to produce similar shapes. Plants with simple leaves constantly show tendencies toward compounding. "The persistent production of the similar forms of compound leaves which have been described is evidence in favor of determinate or orthogenetic evolution."—J. M. C.

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[25] TERNETZ, CHARLOTTE, Ueber die Assimilation des atmosphärischen Stickstoffes durch Pilze. Jahrb. Wiss. Bot. **44**:353–408. 1907.

[26] LEWIS, FREDERIC T., A further study of leaf development. Amer. Nat. **41**: 701–709. 1907.

[27] Amer. Nat. **41**:431–441. 1907.

Inflorescence of Euphorbia.—Another student, SCHMIDT, has undertaken to interpret the morphology of the cyathium of Euphorbia,[28] regarding which there are nearly as many views as investigators. He considers the cyathium as an inflorescence, of unequal development in different species, in which the primary staminate flowers arise first in a spiral of $\frac{2}{5}$ divergence sometimes so low as to bring the flowers almost into a whorl. Next arise the involucral leaves (except in *E. meloformis*). The shoot which forms the primary staminate flowers branches at first dichasially; next to form *Wickeln* (perhaps also *Schraubeln*) so that the whole in the axil of an involucral leaf is a *Doppelwickel* (*Doppelschraubel*). The scales arise mostly at the base of the second and third staminate flowers of each group and are to be looked upon as their bracts. They are unequally developed, being generally larger the more open is the spiral of the involucral leaves, whose insertion is sometimes enlarged on the inside, and concrescent with the scales. The nectaries are to be considered as emergences. *E. capitulata* Rchb. is separated from the genus to form a new one, *Diplocyathium*, on account of its aberrant inflorescence.—C. R. B.

Spectrum of chlorophyll.—It is well known that the absorption bands in the spectrum of a live leaf do not correspond in position to those of a solution of chlorophyll. To account for this "displacement" two theories have been current: that the chlorophyll exists as solid particles like a precipitate in the chloroplast (HAGENBACH, LOMMELL, REINKE); or that the chlorophyll in the chloroplast is dissolved and its solvent modifies the spectrum because it has a higher dispersive power (KUNTH, TSCHIRCH). IWANOWSKI, after spectrophotometric studies, agrees with neither of these views.[29] He succeeded in producing a spectrum almost exactly like that of a live leaf by precipitating chlorophyll in strong alcoholic solution by diluting with water and adding a few drops of $MgSO_4$. On this and other grounds he concludes that the spectrum due to the light reflected from the chloroplasts themselves superposed on the true absorption spectrum produces the displacement, which increases with the size of the granules. This resembles in fundamentals TIMIRIAZEFF's hypothesis of 1872.—C. R. B.

Morphology of Aspergillus.—FRASER and CHAMBERS,[30] in a study of *Aspergillus herbariorum*, reach the following conclusions. The conidiophores are multinucleate, and each conidium contains about four nuclei. The female organ consists of a septate stalk, a one-celled ascogonium, and a one-celled trichogyne, all of which are multinucleate. The antheridium is a small, long-stalked, multinucleate cell which either fuses with the tip of the trichogyne or degenerates before reaching this stage. Normal fertilization probably occurs in some cases, and

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[28] SCHMIDT, H., Ueber die Entwicklung der Blüten und Blütenstande von *Euphorbia* L. und *Diplocyathium* n. g. Beih. Bot. Centralbl. **22**^{1}:21–69. *pls. 2–5.* 1907.

[29] IWANOWSKI, D., Ueber die Ursache der Verschiebung der Absorptionsbänder in Blatt. Ber. Deutsch. Bot. Gesells. **25**:416. 1907.

[30] FRASER, H. C. I., and CHAMBERS, H. S., The morphology of *Aspergillus herbariorum*. Annales Mycol. **5**:419–431. *pls. 11, 12.* 1907.

in others it is replaced by a fusion of ascogonial nuclei in pairs. After either process the ascogonium becomes septate, and each of its cells gives rise to ascogenous hyphae. In the ascus two nuclei fuse, and three successive divisions result in eight spores, which subsequently become multinucleate. The authors regard Aspergillus as a primitive type of Ascomycetes, from which most of the others can be derived; and suggest that the Ascomycetes are related to the Basidiomycetes and the Florideae.—J. M. C.

Adventitious buds in leaves of Gnetum.—In plants of *Gnetum Gnemon* L., grown in a hothouse of the Botanic Garden at Utrecht, the tips of the leaves regularly produced adventitious buds. VAN BEUSEKOM[31] finds that these buds are formed as a result of the attacks of a scale insect, *Aspidirtus dictyospermi* Morg., a species with a world-wide distribution in the tropics and in hothouses. The punctures of this insect result in yellow vesicles at various points on the leaf, and one or more near the apex stimulate the development of endogenous callus buds. The author "explains" the appearance of the buds in the apical part alone of the leaf, by assuming that "the small wound causes an afflux of nutrient matter in an apical direction," and that this necessarily stops just beyond the apical wounds. Of course this is a mere assumption, the like of which is often made, but it would puzzle any of those who use it to show how "an afflux of nutrient matter" could occur before growth actually begins.—C. R. B.

Conjugation and germination in Spirogyra.—An examination by TRÖNDLE[32] of several thousand zygospores, some sectioned and some observed entire, confirmed the current account that the two nuclei remain separate for some time after the zygospore is formed. In *Spirogyra communis* the sexual nuclei fuse two or three weeks after the formation of the zygospore. Two successive mitoses giving rise to four nuclei, and a subsequent fusion of two of these nuclei as described by CHMIELEWSKI, do not occur. The male chromatophores in the zygote disorganize in about fourteen days after conjugation, leaving only the chromatophores of the female gamete. The writer also talks about a reduction of the hereditary mass, referring to the nuclear material, but nothing in the text or figures indicates any counting of chromosomes or any study of the mechanism of reduction. The paper contains a detailed account of the chemical changes occurring during the development of the zygospore.—CHARLES J. CHAMBERLAIN.

Plantae Lindheimerianae.—To students of the Texan flora ENGELMANN and GRAY'S *Plantae Lindheimerianae*, in two parts, is a classic. It now appears that all of the collections of this pioneer botanist were not published and distributed, and the rich remnant came into the possession of the Missouri Botanical Garden

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[31] VAN BEUSEKOM, JAN, On the influence of wound stimuli on the formation of adventitious buds on the leaves of *Gnetum Gnemon* L. Recueil Trav. Bot. Néerl. 4:pp. 27. *pls. 3.* 1907.

[32] TRÖNDLE, A., Ueber die Kopulation und Keimung von Spirogyra. Bot. Zeit. 65:188–216. *pls. 5.* 1907.

with the ENGELMANN herbarium. This unpublished and undistributed material, containing about 650 numbers, represents the collections of 1849–1851, and proves to be very valuable. It has now been published by BLANKINSHIP,[33] who has still further added to the value of the contribution by including also the numbers of the earlier fascicles not previously enumerated (*Plantae Lindheimerianae* having been left unfinished at the end of the Compositae); a bibliography of Texan botany; a complete index of all three parts, with modern equivalents and corrections, the nomenclature comforming to the Vienna code; and a most interesting sketch, with portrait, of "LINDHEIMER, the botanist-editor," from data largely supplied by his son and daughter.—J. M. C.

Mutation and geographic distribution.—WILLIS[34] has continued his arguments in favor of mutation by analyzing the geographic distribution of the Dilleniaceae, stating that this family is chosen simply because it is the first family in HOOKER'S *Flora of British India* "with other than world-wide distribution." The details of the analysis cannot be given here, but the results are intended to show that the theory of mutation greatly simplifies the problems of geographic distribution.

In another short paper[35] WILLIS suggests what seems to be an important consideration in the origin of species of flowering plants, namely, that "while the characters that distinguish species and genera are largely characters of the floral organs, the struggle for existence is almost entirely among the seedlings and young plants, in which these organs are not yet present."—J. M. C.

Fertilization in Polytrichum.—The VAN LEEUWEN-REYNVAANS[36] have published the first account of the details of fertilization in mosses and describe most remarkable behavior by the chromatin. In the next to the last division of the spermatogenous cells each daughter nucleus receives six chromosomes, but in the final mitosis only three, so that the sperm contains only three chromosomes. The mitosis which forms the egg and ventral canal cell shows only three chromosomes for each nucleus. The egg and ventral canal cell become pressed together and their nuclei fuse, forming a nucleus with six chromosomes. Two sperms then unite with this egg, thus restoring the sporophytic number of chromosomes, which was found to be twelve. The full paper with the plates will be awaited with interest.—CHARLES J. CHAMBERLAIN.

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[33] BLANKINSHIP, J. W., Plantae Lindheimerianae, Part III. Ann. Rep. Mo. Bot. Garden **18**:123–223. 1907.

[34] WILLIS, J. C., The geographical distribution of the Dilleniaceae, as illustrating the treatment of this subject on the theory of mutation. Annals Bot. Gard. Peradeniya **4**:69–76. 1907.

[35] Further evidence against the origin of species by infinitesimal variations. *Idem* 17–19.

[36] VAN LEEUWEN-REYNVAAN, Mr. and Mrs. Doctors, On a double reduction of the number of chromosomes during the formation of the sexual cells and on a subsequent double fertilization in some species of Polytrichum. Koninklijke Akad. Wetenschappen **1907**:359–365.

Nutrition of aquatics.—A new investigation of this subject has been published by SNELL.[37] So far as the author's experiments overlap those of the reviewer, there is perfect agreement in the conclusion that aquatic plants rooted in the soil take nourishment therefrom, and that the roots function as regular organs for the entrance of food substances. In the case of *Pistia Stratiotes* the roots, though not attached to the soil, still function as roots in allowing food substance in the water to enter. The roots of Lemna, however, serve only to balance the plant. Some results were obtained which do not agree with those obtained by the reviewer, the value of which he is not willing to concede; but since the author does not regard the differences of enough importance to state them formally in the final conclusions, further discussion is not necessary.—RAYMOND H. POND.

A new pteridosperm.—ARBER[38] has described a new Pteridosperm (*Carpolithus Nathorsti*) from the coal fields of Germany, from a specimen preserved in the collections of the Swedish Academy at Stockholm. The seeds are extraordinarily small, the average length being about 1^{mm}, and the greatest width 0.75^{mm}. They are borne upon an undoubted sphenopterid frond, and were probably inclosed in cupules. It is not certain that this seed is a true Lagenostoma, but it confirms strongly the habit of the fertile fronds inferred for the species of Lagenostoma.—J. M. C.

Wound tissues.—In a somewhat lengthy discussion of wound tissues resulting from removing rings of cortex from twigs, KRIEG[39] finds that in plants growing in the sun greater masses of wound tissue are produced than in plants growing in the shade; and that in forms with larger leaves the callus above the ring is much larger than that produced by small-leaved forms.—W. J. G. LAND.

Economy of chlorophyll proper.—STAHL suggests[40] that the earlier disappearance of the blue-green component of chlorophyll, leading to the yellowing of leaves in autumn (which is checked by preventing migration of materials from the leaf or any part of it), as well as its later appearance in seedlings of etiolated plants, is a matter of economy.—C. R. B.

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[37] SNELL, KARL, Untersuchungen über die Nahrungsaufnahme der Wasserpflanzen. Flora **98**:213–249. 1907.

[38] ARBER, E. A. NEWELL, On a new Pteridosperm possessing the Sphenopteris type of foliage. Annals of Botany **22**:57–62. *pl. 6.* 1908.

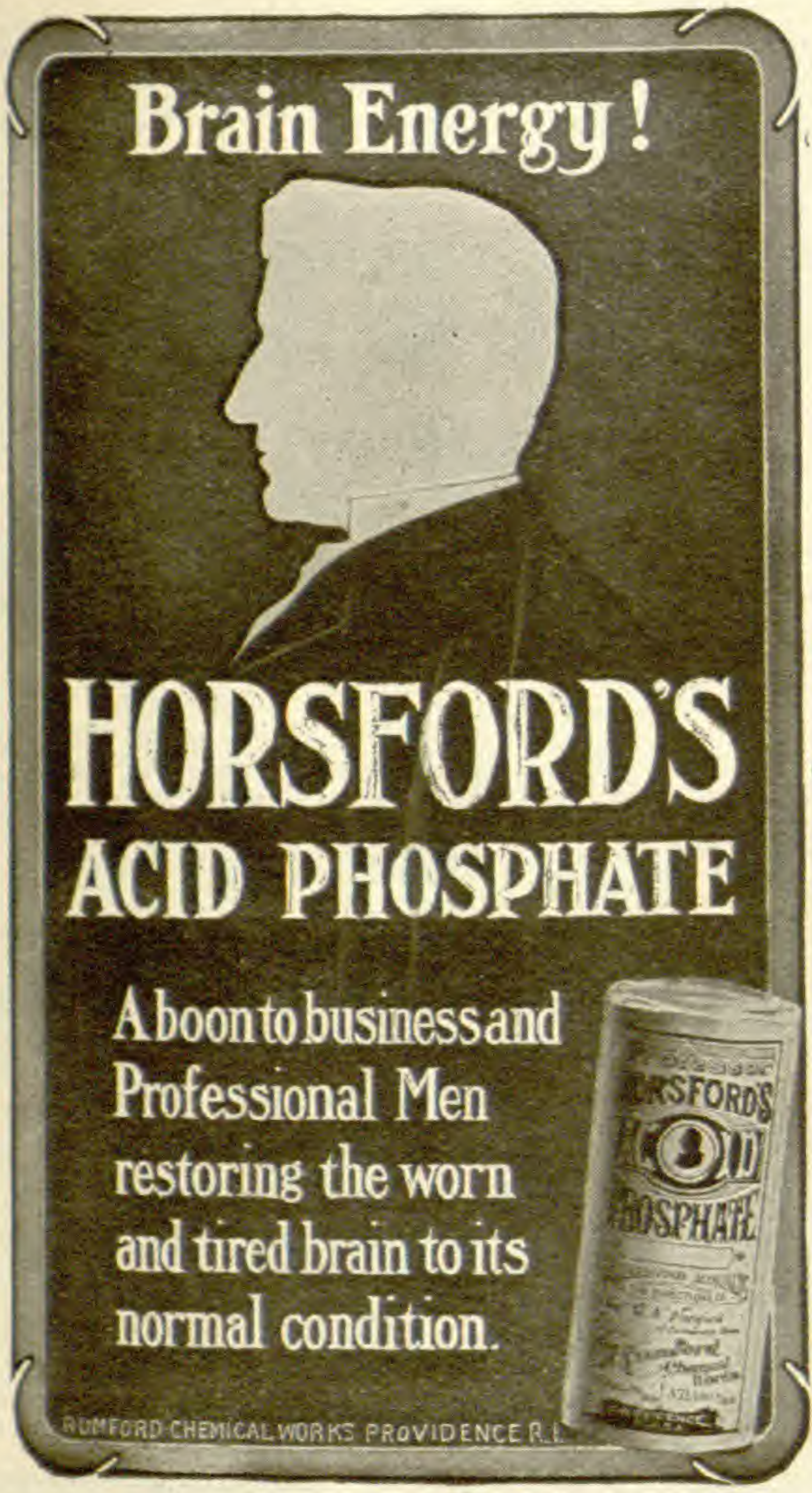
[39] KRIEG, AUGUST, Beiträge zur Kenntniss der Kallus- und Wundholzbildung geringelter Zweige und deren histologischen Veränderungen. pp. 68. *pls. 25.* Würzburg: A. Stuber. 1908.

[40] STAHL, E., Ueber das Vergilben des Laubes. Ber. Deutsch. Bot. Gesells. **25**:530–534. 1907.

NEWS

The University of Washington will open its marine station at Friday Harbor on June 22 for a session of six weeks. Dr. R. B. Wylie, University of Iowa, will be in charge of the botany. The station is in the heart of an evergreen forest in the winter rainbelt, and the marine plants are extraordinarily abundant. The university has also announced a botanical field trip to Alaska, under the direction of Dr. T. C. Frye, to leave Seattle about July 1, to go as far north as Skagway, and to continue for six weeks.

A French *Dictionary of botany* is in preparation by Gaston Bonnier, professor of botany at the Sorbonne, with the assistance of Edouard Griffon, professor of botany in the National College of Agriculture at Grignon and adjunct-director of the Paris Station for plant pathology, Henri Coupin, director of the botanical laboratory at the Sorbonne, and René Viguier, preparator at the Museum of Natural History. It is to include taxonomy, geographical distribution, morphology, physiology, ecology, pathology, paleobotany, agriculture, horticulture, industrial and commercial applications of botany, mythology, etymology of names and terms, and historical, biographical, and bibliographical information, all in two volumes of about 2000 pages, illustrated by more than 10,000 figures. How so comprehensive a scheme can be realized within these limits remains to be seen, but the French—publishers and authors alike—are master-hands at encyclopedic work. Publication will not be begun until the manuscript is complete, insuring regular issue of the parts.

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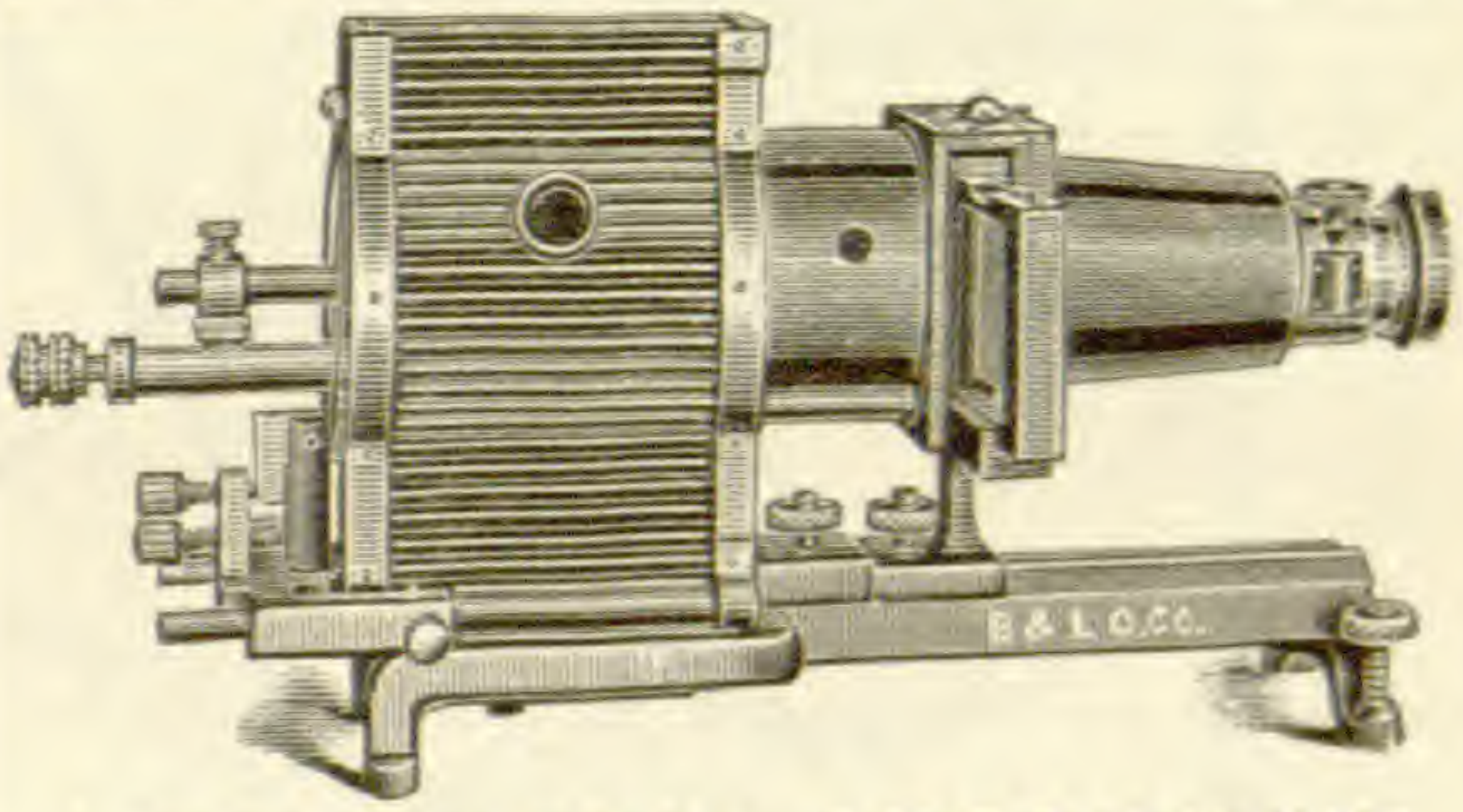

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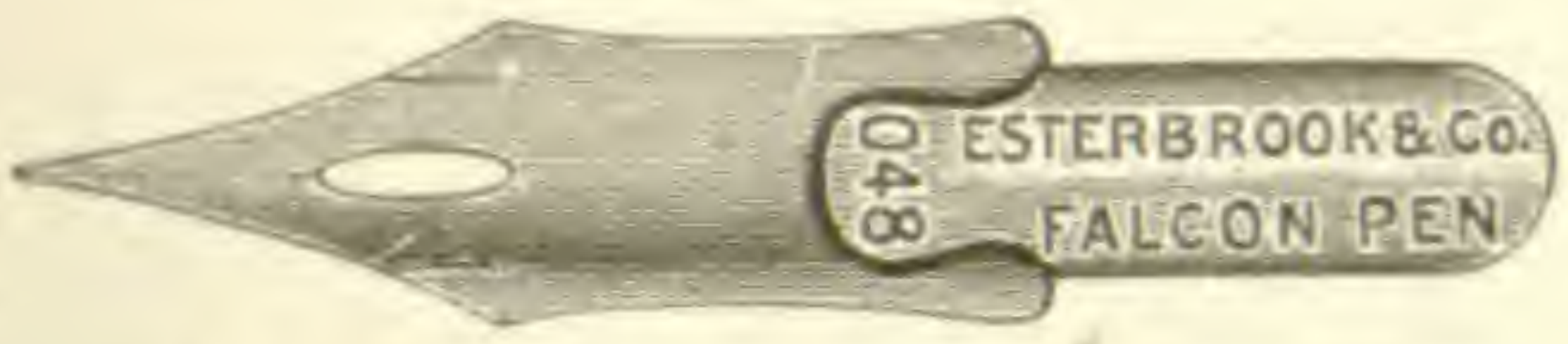
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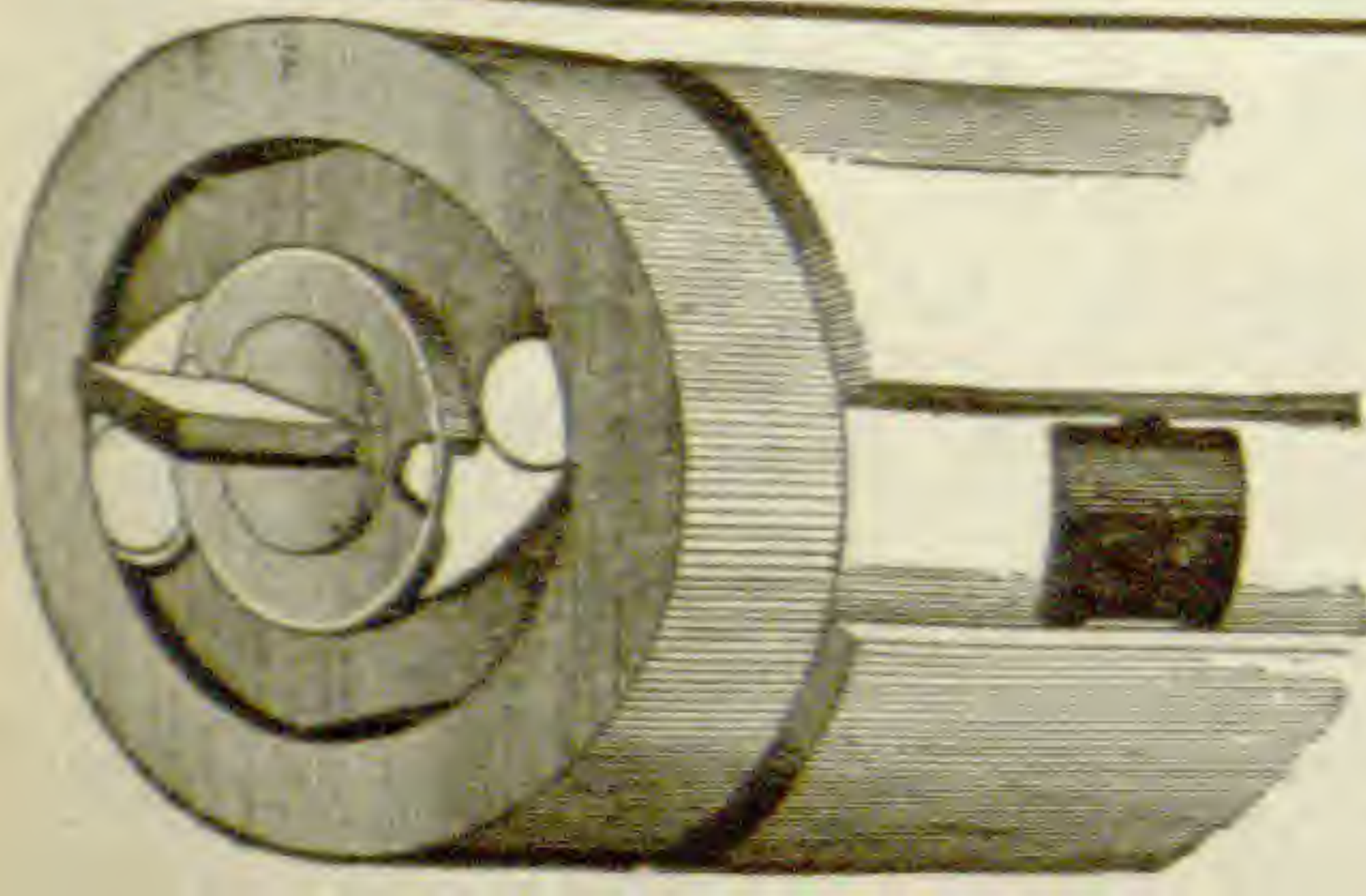

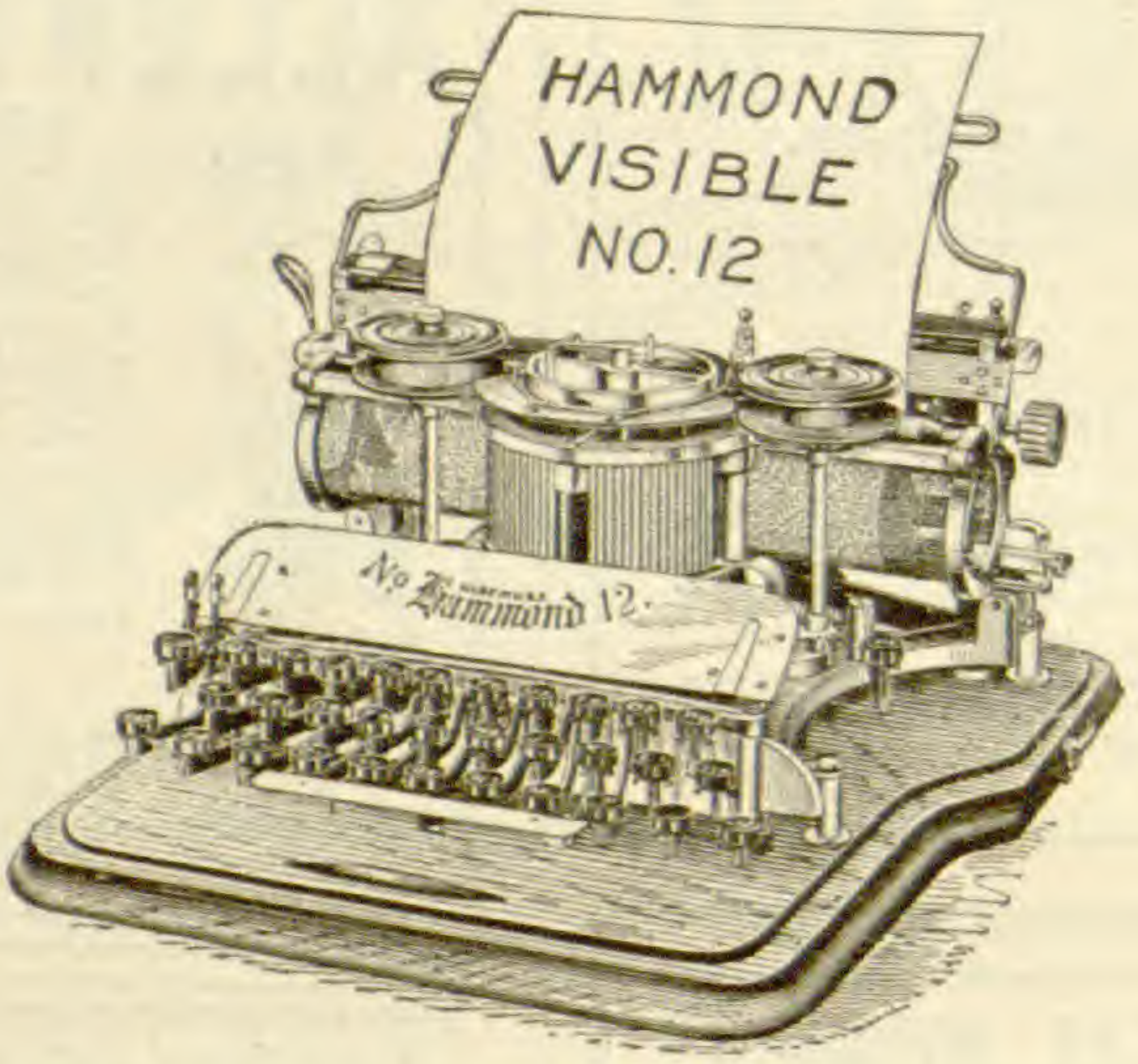
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THE BOTANICAL GAZETTE

June 1908

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CONTENTS FOR JUNE 1908

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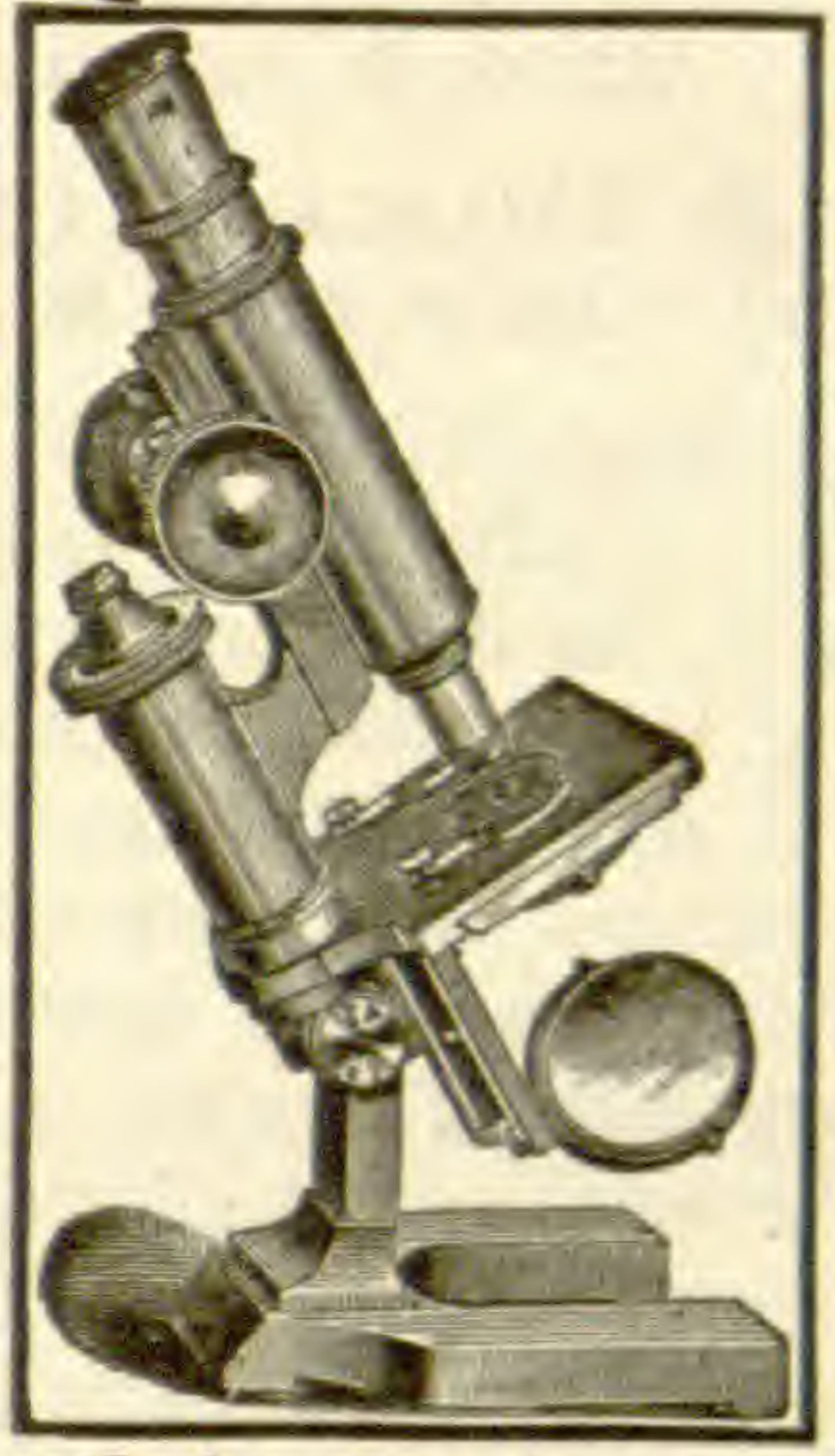

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BOTANICAL GAZETTE

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RELATION OF MEGASPORES TO EMBRYO SACS IN ANGIOSPERMS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 110

JOHN M. COULTER

The recorded cases of unusual conditions in the angiosperm embryo sac are multiplying. Especially is this true in reference to the occurrence of more than eight nuclei in the ante-fertilization development of the gametophyte. There is a tendency to regard the latter cases as representing a more primitive condition of the gametophyte, and to exclude from consideration a general view of the genesis of the nuclei. It seems to be a fitting time to call attention to certain facts that may interpret this situation, may connect it with other "irregularities," may check the tendency to ill-considered generalization, and may indicate certain things that must be included in any investigation of the embryo sac.

In the genesis of the ordinary angiosperm embryo sac from the megaspore mother cell five successive nuclear divisions are involved, the first two being the reduction divisions (usually resulting in four megaspores with walls), and the other three resulting in the production of eight nuclei from the nucleus of one of the megaspores. The functions of these eight nuclei of the female gametophyte do not enter into the present discussion. Attention is called to the following facts: this genesis begins with the division of the mother cell; the essential part of the process is found in the first two divisions, which cannot be omitted, so far as we know, when fertilization is to occur; the result of these first two divisions is the organization of megaspore nuclei, definitely recognized by their cytological history and structure.

The most familiar illustration of departure from this ordinary sequence is the case of Lilium and its allies. In current description the mother cell of Lilium is said to "function directly as a megaspore," and the resulting eight-nucleate sac seems to be quite normal. The inference is that the formation of megaspores has been omitted, and that the nucleus of the mother cell holds the same relation to the eight nuclei of the embryo sac that the megaspore nucleus does in ordinary cases. Analysis of the situation, however, shows that this is not true, for megaspores, at least their nuclei, cannot be omitted. The first two divisions from the mother cell are reduction divisions, and therefore the first four nuclei of the embryo sac of Lilium are megaspore nuclei, to be recognized as such by their cytological history and structure. This means that the nuclei of four megaspores have entered into the structure of the female gametophyte of Lilium; and that to complete its ante-fertilization development each of these nuclei divides once. Therefore, in the case of Lilium, there is only one division after the reduction divisions, instead of the usual three; and the total number of divisions is reduced from the customary five to three. In this case the history of an embryo sac containing the usual structures shows a very unusual reduction in the number of successive divisions, and this reduction seems to have been attained by many more monocotyledons than dicotyledons.

The case of Cypripedium[1] is an illustration of a similar reduction; but with the old methods of interpretation it would stand as remarkably different from Lilium and most angiosperms, for the completed embryo sac, ready for fertilization, contains only four nuclei. But in this case the mother cell divides, and one of the daughter cells forms the embryo sac. The first division of the nucleus of this daughter cell is the second reduction division, resulting in two megaspore nuclei. It is these two megaspore nuclei that are involved in the development of the female gametophyte, each dividing once as in the case of Lilium. Therefore, in Cypripedium also the usual five successive divisions are reduced to three; the only difference being that in Lilium four megaspore nuclei are involved, while in Cypripedium only two are used. In her paper Miss PACE calls attention to this comparison, and also to the fact that the elimination of one more division would result in an oogenesis similar to that of animals.

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[1] PACE, LULU, Fertilization in Cypripedium. BOT. GAZETTE 44:353–374. *pls. 24–27.* 1907.

In the older papers many records of "three megaspores," "two megaspores," and "no megaspores" appear, which either must be due to incomplete investigation or represent such an elimination of divisions as is shown by Lilium and Cypripedium.

What has been regarded as the most striking exception to the usual structure of the angiosperm embryo sac is the case of *Peperomia pellucida*, described by CAMPBELL[2] and JOHNSON.[3] In this sac sixteen nuclei appear before fertilization; that is, there appears to be one more division than usual. Lately JOHNSON[4] has described a similar condition in the Jamaican *Peperomia hispidula*, in which there are also eight-nucleate sacs.

Fortunately, in the case of Peperomia we know that the mother cell does not divide to form walled megaspores, so that the probable interpretation of the situation is evident. Four megaspore nuclei are formed, all of which are probably involved in forming the female gametophyte. By two successive divisions each of these gives rise to four nuclei, and the result is sixteen nuclei. It follows that the ordinary number of successive divisions has been reduced from five to four; and that if Peperomia had suffered no reduction, there would have been thirty-two nuclei in the embryo sac. In number of divisions, therefore, Peperomia lies between the ordinary angiosperms on the one side, and Lilium and Cypripedium on the other. This condition should be regarded as advanced in the direction of the elimination of divisions, rather than primitive. If an embryo sac derived from a single megaspore should contain sixteen nuclei, it could be regarded as relatively primitive; but the sac of Peperomia could contain thirty-two nuclei and only be normal. *Peperomia hispidula* is interesting in developing eight-nucleate sacs in addition to the sixteen-nucleate ones, which perhaps puts it upon a level of reduction with Lilium. It may have some bearing on the situation to note also that JOHNSON in his account of this species described the first four nuclei of the sac as "arranged in a perfect tetrad," which would be quite natural for

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[2] CAMPBELL, DOUGLAS H., Die Entwicklung des Embryosackes von *Peperomia pellucida* Kunth. Ber. Deutsch. Bot. Gesells. **17**:452–456. *pl. 31.* 1899.

[3] JOHNSON, D. S., On the endosperm and embryo of *Peperomia pellucida.* BOT. GAZETTE **30**:1–11. *pls. 28, 29.* 1900.

[4] JOHNSON, D. S., A new type of embryo sac in Peperomia. Preliminary notice. Johns Hopkins Univ. Circ. **1907**:19–21. *pls. 5, 6.*

megaspores. In fact, it may not be out of place to state that JOHNSON has expressed the opinion privately that these four nuclei are those of megaspores.

Recently CAMPBELL[5] has examined *Pandanus odoratissimus* and *P. Artocarpus*, and found usually fourteen nuclei in the embryo sac. At the four-nucleate stage there is the usual arrangement of two micropylar and two antipodal nuclei; but the former do not divide, and the latter give rise to twelve nuclei. If this record is confirmed by tracing the history of the nuclei, there is introduced in this case an irregularity in the succession of divisions. It may be assumed, subject to subsequent observation, that four megaspore nuclei are involved in the formation of these fourteen nuclei; and the irregularity would consist in the fact that two of them do not divide; that one of the others added two successive divisions (the elimination of one division); and that the remaining one added three successive divisions (the usual number). There is thus no greater number of divisions than usual, and even a certain amount of elimination. It is interesting to note that if the two micropylar nuclei do not divide and are really megaspore nuclei, the egg is removed from the mother cell by only two divisions, which is the condition of the animal egg. A careful cytological investigation of these divisions would seem to be most desirable.

The introduction of a certain amount of irregularity in the succession of divisions is suggested by the records of several species. For example, in Gunnera,[6] while eight nuclei are common, any number up to sixteen may be found; and in *Trillium grandiflorum*[7] ten sometimes occur. In neither of these species, however, do we have any information as to the behavior of the mother cell in forming megaspores.

In *Ulmus americana*[8] we know that the "mother cell functions

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[5] CAMPBELL, DOUGLAS H., The embryo sac of Pandanus. Preliminary note. Annals of Botany **22**:330. 1908.

[6] SCHNEGG, H., Beiträge zur Kenntniss der Gattung Gunnera. Flora **90**:161–208. *figs. 28.* 1902.

[7] ERNST, A., Chromosomereduction, Entwicklung des Embryosackes, und Befruchtung bei *Paris quadrifolia* L. und *Trillium grandiflorum* Salisb. Flora **91**: 1–46. *pls. 1–6.* 1902.

[8] SHATTUCK, CHARLES H., A morphological study of *Ulmus americana*. BOT. GAZETTE **40**:209–223. *pls. 7–9.* 1905.

directly as the megaspore," as in Peperomia, and in the majority of embryo sacs there are eight nuclei (the normal number of nuclei, but an elimination of two divisions); but sometimes there are "twelve or more nuclei" (meaning another division, but still one less than the usual number).

The situation among the Araceae deserves attention, for great irregularities in the embryo sac have been observed.[9] In Aglaonema the nuclei are four to twelve; while in Nephthytis they are reported as ranging in number from two to twelve or thirteen. Unfortunately, in these cases we are not sure of the behavior of the mother cell; but so far as the records for Araceae go, the mother cell divides once or not at all. It is needless to draw any conclusions as to the amount of reduction involved in the four nuclei of Aglaonema or the two nuclei of Nephthytis until we know something of their history. At all events, there is a reduction of the ordinary number of divisions in these forms; and this is probably true even in the cases where twelve or thirteen nuclei appear.

The only case reported, in which there remains some evidence that an additional free nuclear division may occur, is that of the Penaeaceae, a small African group of shrubby xerophytes restricted to southwestern Cape Colony. In a preliminary note, STEPHENS[10] has announced his results with five species, representing three genera (Sarcocolla, Penaea, and Brachysiphon), and his account applies to all of them. There are sixteen free nuclei formed in the sac before fertilization, which are said to be derived from the innermost one of a row of three megaspores. In such a case three "megaspores" are as good as four, except that the outermost cell is not a megaspore. The testimony breaks down, however, at the critical stage, for the investigator is not certain as to the "row of three." Of course if it should turn out to be a "row of two," the megaspore condition would be that of Cypripedium, followed by the usual number of divisions. The behavior of the nuclei, however, suggests strongly

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[9] CAMPBELL, DOUGLAS H., Studies on the Araceae. The embryo sac and embryo of Aglaonema and Spathicarpa. Annals of Botany 17:665–687. *pls. 30–32.* 1903.

———Studies in the Araceae. III. Annals of Botany 19:329–349. *pls. 14–17.* 1905.

[10] STEPHENS, E. L., A preliminary note on the embryo sac of certain Penaeaceae. Annals of Botany 22:329, 330. 1908.

that four megaspore nuclei are concerned, and that there is no "row" developed by the mother cell at all. It is a very interesting performance as described; for the original nucleus of the sac produces four nuclei in tetrahedral arrangement; these four separate and become parietally placed (one micropylar, one antipodal, two equatorial); each of the four produces a group of four nuclei; three nuclei of each group organize after the fashion of an egg apparatus; and the four free nuclei pass to the center of the sac and fuse to form the primary endosperm nucleus. We have here at least the assurance of simultaneous successive divisions, which we do not have in the case of all 16-nucleate sacs. If four megaspore nuclei are involved in this organization, the reduction in the number of successive divisions is just that which probably occurs in Peperomia.

Some evident conclusions may be drawn from the above statement, and they should be of use in investigations of the angiosperm embryo sac. The nuclear divisions within the embryo sac vary in character; they may include both reduction divisions, the second one, or neither of them (the usual case). The appearance of more than eight nuclei in an embryo sac is no evidence that there has been any more than the usual number of successive divisions. As many as thirty-two nuclei may appear in an embryo sac without increasing the usual number of divisions. It is absolutely necessary to trace the nuclear succession from mother cell to completed embryo sac before any safe conclusions can be drawn as to the significance of the conditions observed within the sac. The cases of an increased number of nuclei, as in Peperomia, which have sometimes been cited as showing a primitive condition of the sac, have actually turned out to be cases of reduction from the ordinary condition. There is a tendency among many groups to eliminate the divisions that follow the two reduction divisions, but the tendency is not general among angiosperms, since among the Sympetalae it does not seem to exist; hence the forms which exhibit it may be regarded as more or less specialized in this feature.

If an angiosperm embryo sac should be found containing sixteen or more nuclei derived from a single megaspore nucleus, it may be regarded as relatively primitive; but such a sac has not yet been demonstrated.

THE UNIVERSITY OF CHICAGO

THE PHYSIOLOGY AND DEVELOPMENT OF SOME ANTHRACNOSES[1]

CLAUDE WILBUR EDGERTON

(WITH SEVENTEEN FIGURES AND PLATE XI)

The group of fungi commonly known as the anthracnoses, including the genera Gloeosporium and Colletotrichum, has received much attention from botanists during the past few years. It has been worked from the economic and scientific standpoints, the former being a very important aspect. Many of our most important diseases of orchard, farm, and garden crops are due to some member of these genera, the annual loss to the country reaching into millions of dollars. The bitter rot fungus alone in 1900 cost the apple growers of this country $10,000,000 or more (46). The bean anthracnose, due to *Colletotrichum lindemuthianum*, sometimes causes almost a total loss of the bean crop, and other members of the group cause losses in proportion. In some sections certain crops have ceased to be raised on account of the ravages of these fungi.

From the scientific standpoint the group is interesting on account of the peculiar polymorphic life-history of the different members. Until fifteen or twenty years ago, the anthracnoses were known only in the conidial stage. Previous workers were mostly systematists who did little more than describe in a few lines the conidial fructifications; but about fifteen years ago the perfect stage of one of the species was found, and this gave an impetus to the study of the group. Since then the perfect stage of several species has been found.

The history of previous work is familiar to most mycologists through recent papers, and references to this will only be made in connection with the discussion of the different forms.

Some would limit the use of the word anthracnose to those fungi having an ascigeral stage, as in the genus Glomerella; but I prefer to use it, as commonly used, for all fungi having a Gloeosporium-like conidial stage.

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[1] Contribution from the Department of Botany, Cornell University, No. 125.

I have had three points in view in studying this group: (1) To work out the perfect stage of as many forms as possible and to make a careful study of it. All the perfect stages of Glomerella that have been found are very similar, and some writers have gone so far as to say that there is only one species. There also seems to be a difference of opinion as to the structure of the perfect stage. Glomerella was described as aparaphysate, but recently SHELDON (39) has reported the presence of paraphyses. Several forms have been studied to obtain some evidence on this point. (2) To determine if the forms of Gloeosporium found on apple represent a single species or more than one. The true bitter rot fungus occurring on apples in the south spreads rapidly and causes a great deal of damage, while the form occasionally met with in the north spreads slowly and causes but little damage. The form from the south produces perithecia in abundance on various culture media; the northern form was studied carefully by Miss STONEMAN, but no perithecia were found. CLINTON expressed surprise that she was unable to find them. (3) Can species of Gloeosporium be distinguished by culture methods? Miss STONEMAN has attempted to find characters in this way that would help to differentiate them. It is impossible to distinguish all the species by the characters found on the hosts. The spores in many of them are alike, the acervuli vary but little, and the cross-inoculation experiments that have been tried by various authors have proved but little.

The following paper is based on work carried on with anthracnoses from about thirty different hosts and from fifty or more sources. The forms have been studied in the usual manner. They have been obtained in pure cultures and studied in various artificial media as well as upon the host. The structure of the acervuli, and of the perithecia where found, has also been studied in thin, carefully stained microtome sections. Most of the material was fixed in Gilson's fixing solution.[2] As is well known, the spores of Gloeosporium are imbedded in a gelatinous substance which is very soluble in water. If pieces of the material are put in an aqueous fixative, as Flemming's or chromacetic, the spores are almost entirely washed away. The gelatinous

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[2] Gilson's fixing solution: 95 per cent. alcohol 42^{cc}, water 60^{cc}, glacial acetic acid 18^{cc}, nitric acid (conc.) 2^{cc}, corrosive sublimate (sat. sol.) 11^{cc}. The material is left in the fixer 6–24 hours and washed directly in 70 per cent. alcohol.

substance is not soluble in Gilson's fixer and hence the spores are held in place (see *fig. 7*). While this fixer has not proved very good for nuclear phenomena, it has been very satisfactory for the results desired in this work.

The culture media that have been most used are bean agar, made from an infusion of fresh bean pods; potato agar with 10 per cent. of glucose added; Elfving's nutrient solution (a synthetic medium); and bean pods in tubes. Various other media have been used to some extent, as beef extract gelatin, potato agar plus various organic and mineral substances, sterilized cornmeal, cassava plugs, etc., but these seem to have little value. Some writers have been advising the use of cornmeal for obtaining perithecial stages, but I have had no better results with that than with various other media. Bean agar has proved as satisfactory as any used, several of the forms producing perithecia on it, although not all of them.

Characters of the group

The anthracnoses are recognized by the characters of the conidial stage. The genera mentioned above are very similar; in all the spores are borne in the same way in the same sort of pustule. Colletotrichum is supposed to be separated from Gloeosporium by the presence of setae in the conidial pustule, but as this distinction is a poor one, in the following discussion the name Gloeosporium will be used for all species, except perhaps occasionally where another name is the one in common use.

The spores are borne in pustules underneath the cuticle, the epidermis, or in a few cases underneath several layers of cells. The spore-bearing stroma appears first as a weft of thickly woven mycelium, which increases in size and finally breaks the tissue above, bearing spores in abundance on short conidiophores. The spores are two to several times as long as broad, straight or fusoid, and hyaline. There seems to be some confusion in the literature as to the color of the spores. For some time it went without question that they were hyaline, but in 1894 ALWOOD (**1**) described the spores of the bitter rot fungus as slightly greenish. Later SPAULDING and VON SCHRENK (**33**) detected the same color, and since then other writers have almost invariably seen the same tint. SPAULDING and VON SCHRENK say:

"When highly magnified they have a very delicate light green color. This color is quite distinct, and it seems strange that of many observers ALWOOD (1894) seems to be the only one to recognize this greenish color." Looked at with the microscopes commonly used, corrected for two colors alone, the spores do have a greenish tint. Spores were examined with all the different types of microscopes in the Cornell University laboratory, and with one exception, the Zeiss apochromatic lenses, they showed a greenish tint; but with these lenses, which are corrected for three colors, the spores are absolutely hyaline; so we must return to the original conception of the color.

The spores as a rule have one nucleus, though I have occasionally seen two in very large spores. The nucleus appears as a rather large clear area near the center of the spore and generally close to one wall. It is generally clearly seen, though in old spores, or in those beginning to germinate, or in poorly developed spores, it is sometimes not visible. This structure has been noticed by nearly every investigator, but so far as I have found, it has not been recognized as a nucleus. It is spoken of as a "clear hyaline area," a "round spot not granular as the rest," a "vacuole," etc. Studied in stained sections, however, its true character appears. It takes the nuclear stains readily, though it is difficult to distinguish the structure. In some of the related forms, as *Myxosporium corticolum*,[3] large oil drops are sometimes present in the spores, which may be confused with the nucleus, but the latter is distinguished by its lighter color. Oil drops are highly refractive, presenting a slightly greenish tint with the ordinary microscope, while the nuclei do not show it. The nucleus in the conidium is considerably larger than the one directly below in the conidiophore.

As yet the perfect stage of many of the anthracnoses is unknown, but the ascospore form of several has been found recently. In some it has been found several times; but in others it has been found one, two, or at least only a few times. This may be due to the fact that the ascospore forms are rarely developed, or that the investigators have overlooked them, perhaps on account of their rarity or inconspicuous nature.

While the conidial stage of all the forms is very similar, yet even in the few forms worked out they are connected with at least three

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[3] *Myxosporium corticolum* Edgerton. Ann. Mycol. **6**: 48–52. 1908.

genera of ascomycetes. According to our present classification of ascomycetes, no two of these genera are in the same family, while some are in widely separated orders. The genera that are at present known to be connected with the Gloeosporium-like conidial stage are Gnomonia, Glomerella, and Pseudopeziza. In the following pages, these different types will be taken up separately.

Gnomonia type

KLEBAHN (**25**) seems to have been the first to connect a Gloeosporium with Gnomonia. As a result of his work on the sycamore anthracnose, extending over several years, he gave a most interesting discussion of the peculiar polymorphism of the fungus commonly known as *Gloeosporium nervisequum.* He studied the fungus both in the field and in the laboratory on artificial media, finding the perfect stage in the late winter and early spring on old diseased leaves that had lain under the trees over winter. Later he was able to obtain it in abundance by cutting out the anthracnosed spots on the leaves and putting them out in wire netting to winter. According to him, the perfect stage begins to develop in the fall and is mature about Christmas or a little later. By careful examination of his material and by comparison with other herbarium material, he was able to identify it with *Laestadia veneta* Sacc. & Speg., which he showed to be a species of Gnomonia, and named it *Gnomonia veneta* (Sacc. & Speg.) Klebahn.

KLEBAHN was also able to show that the conidial stage is polymorphic. While the conidia are in nearly all cases very similar, the manner in which they are borne is different. The types that he found are as follows:

1. The conidia may be borne in acervuli under the cuticle on short basidia. This is the common stage and has long been known as *Gloeosporium nervisequum* (Fuckel) Sacc.

2. The conidia may be borne in acervuli under the epidermis on long basidia. This stage has been known as *Gloeosporium platani* (Mont.) Oud. Before KLEBAHN, both LECLERC DU SABLON (**29**) and J. BEAUVERIE (**5**) had shown that this was but a form of *G. nervisequum.*

3. The conidia may be borne on twigs in pustules, being known

as *Myxosporium valsoideum* (Sacc.) All. and *Discula platani* (Peck) Sacc. VON TAVEL (44) previously had connected *D. platani* with *G. nervisequum*, and BEAUVERIE (5) had shown that both of these names are but synonyms of *G. nervisequum*.

4. The pycnospores may be borne in cleistocarpous pycnidia on old leaves on the ground. This stage had been found before and, as was shown by KLEBAHN, had been named *Sporonema platani* Bäumler and *Fusicoccum veronense* C. Massalongo.

I took up the study of this fungus in order to confirm KLEBAHN'S work and to find whether the perithecia normally develop in this country. In nearly all cases I was able to confirm KLEBAHN'S results completely. In a few cases, however, there seemed to be some discrepancies. These will be brought out in the following discussion of the life-history.

The disease due to this fungus, as it is commonly seen, appears on the veins of the sycamore leaf, killing a strip on each side of the vein; later it often spreads to other parts of the leaf. The diseased portions die and become brown, generally accompanied by a distortion of the leaf. On the under side of the diseased spots, and occasionally on the upper side the acervuli develop in abundance, being about 100–300 μ in diameter. The conidia develop in abundance on short conidiophores. In moist weather, or when the leaves are placed in a moist chamber, the spores ooze out in creamy white masses or in white strings. They are usually about $10\text{–}14 \times 4\text{–}6\ \mu$, hyaline, slightly granular, generally somewhat pointed at one end and more or less rounded at the other.

An examination of the leaves in late summer and autumn shows that the petioles have been attacked also. KLEBAHN does not mention this effect of the disease. Diseased patches may be formed by the fungus growing down the petiole from the leaf blade, or they may be entirely distinct from the leaf blade. Quite often, especially in the autumn, these diseased spots are present at the very base of the petiole, where it is attached to the twig. Whether the fungus passes from the petiole into the twig was not determined, though many leaves were examined; but this seems at least possible. Conidial pustules form in these diseased petioles just as on the leaves. The presence of the disease on the petioles often causes a premature fall of the leaves.

When the leaves fall to the ground, the fungus takes on a saprophytic mode of life, continuing to develop on the dead leaves, spreading much more rapidly on them than it did on the living leaves. It often covers considerable areas, sometimes entirely covering the leaf, and on these areas acervuli and conidia are produced in abundance. KLEBAHN in developing the perfect stage cut out merely the affected areas, in order to save time in looking over the leaves in the spring. He does not speak of the saprophytic growth of the fungus on the dead leaf, and perhaps, did not observe it; otherwise he would not have taken the trouble to cut out the spots. The conidia develop in these pustules throughout the winter; they were examined often and each time spores were found that were viable, and they were particularly abundant after a fairly warm period of a day or two. The pustules on the dead leaves do not seem to differ from those developed normally on the leaves in the summer.

During February or March, a different sort of conidial fruit body begins to form in favored positions. In places where the leaves were kept moist, where they were piled up and sheltered from drying, or where they were placed close together between pieces of wire netting, a sort of pycnidium was formed. This is what KLEBAHN (*l. c.*, p. 548) described as the Sporonema or Fusicoccum stage on dead leaves. The conidia are the same in shape and size as those in the acervuli, but the stroma bearing them has been favored by the moisture and has continued to grow until it has completely surrounded the developing spores. These pycnidia-like bodies (*fig. 3*) are grayish to black, not imbedded in the host tissue, and generally covered by a rather hairy growth of hyphae; they vary in size, some being over 1^{mm} in diameter. While this structure is a closed one and perhaps would be classed as a pycnidium, it hardly seems to be a true one. It is always more or less irregular and nearly always there are trabeculae consisting of strips or masses of the stroma imbedded with the spores (*fig. 3*). This shows that the spores and the pycnidial wall must have been developing at the same time; while in a true pycnidium, the wall is formed before the spores begin to develop.

An examination of the diseased petioles at this time shows the same formation. The pycnidia are developed in the cankered spots so that they appear to be imbedded in the host tissue. In the petioles the larger ones were obtained, the pycnidium shown in *fig. 3* being from a cankered petiole. Nearly all the diseased petioles contained the covered pustules in abundance.

In favored situations the perithecia develop also on the dead leaves. KLEBAHN was able to see the little perithecia on the leaves in the fall and found them mature about Christmas. These dates differ considerably from those I observed at Ithaca. Leaves on the ground were examined once or twice a week from autumn until the perithecia were mature; and the first appearance of young perithecia was on January 6. They form on the inside of the leaf and are not visible until they rupture the epidermis. Generally a very small three-cornered or irregular piece of the epidermis, perhaps 1^{mm} in diameter, is slightly raised; and this is the first visible evidence of the perithecium. Perhaps the perithecia may have been developing since early autumn, but they could not be seen. At this time they are nearly globose, about 150–200 μ in diameter, and generally reach nearly from epidermis to epidermis. Usually the leaf bulges out on the lower side of the perithecium, so that it is really thicker than the ordinary leaf. At this time the beak of the perithecium is just beginning to form, and the wall consists of four or five layers of small, black, somewhat elongated, thick-walled cells. The asci have not formed yet, but the ascogenous tissue takes a different stain from the surrounding contents of the perithecium. The perithecium seems to lie free in the leaf, and does not appear to be connected with it by hyphae.

From this time the development is very slow. Although in January the perithecia looked as if they were about ready to develop asci and spores, mature spores were found first on April 21, and they were on leaves that were covered and had been kept moist. This difference in the time of maturing the asci as observed by KLEBAHN and myself must be due to the different weather conditions.

While many perithecia were found occurring normally on the fallen undisturbed leaves, the best success was obtained by placing a large number of the anthracnosed leaves in wire netting and putting them in a moist shady place. A large number of leaves packed close together prevented drying out, which seems to be the greatest hindrance to perithecial development. Also keeping the leaves close together and undisturbed prevents the perithecia from falling out of the leaves. Quite often they grow so large that they push themselves out of the leaf.

The mature perithecium fits exactly KLEBAHN'S description of it. It is subglobose or slightly flattened on the upper and lower sides, and is about 150–200 μ in diameter. At the upper side it is elongated into a beak, though this is short compared with other forms of Gnomonia. In most cases it is not more than one-fourth to one-third as long as the perithecium (*fig. 4*).

The asci (*figs. 18, 19*) are long clavate, 48–60×12–15 μ, generally bent at right angles near the base. Near the apex of the ascus the wall is much thickened and the pore is surrounded by a very refractive ring, appearing under the microscope as two white glistening spots, one on each side of the pore. The ascus is eight-spored, the spores (*fig. 20*) being hyaline, 14–19×4–5 μ, straight or slightly arcuate, unevenly two-celled, the upper cell as it is borne in the ascus being several times as long as the lower one.

Still another stage of the fungus is found on the small twigs. When the leaves fall, all the twigs seem to be perfectly healthy, even to the formation of buds. But toward the last of December and until spring, they begin to show the presence of the disease. The diseased portion, which sometimes extends back several inches from the tip, is covered with the Myxosporium stage. The fungus may live in these diseased twigs for more than a year, producing spores when weather conditions are favorable. Most of the twigs remain alive until spring and start to put out leaves; but when the leaves are about one-third grown, they quite suddenly wither and die, presenting the appearance of blight. On badly affected trees a greater part of the leaves die, some large trees being observed which had only a few green tufts remaining.[4] The Myxosporium pustules (*fig. 2*) are scattered

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[4] Since this paper was written, papers by VON SCHRENK (Rep. Mo. Bot. Gard. 1907:81–83) and CLINTON (Rep. Conn. Exp. Sta. 1907:360. 1908) have come to notice, in which it is claimed that the injury to the sycamore in 1907 was due to frost and not to anthracnose. From an almost daily observation of the trees at Ithaca, I am convinced that frost had practically nothing to do with the injury. The frosts came on May 11 and 21, while the injury developed during the first three weeks in June. Furthermore, the blighted twigs were covered with the perfectly mature Myxosporium pustules, while twigs that were free from the pustules had no blighted leaves.

thickly over the terminal portions of the twigs, forming under the cork layer of the wood and raising it until it is ruptured (*fig. 5*). The pustules are 500–900 μ in diameter and are entirely filled with the ordinary spores of *Gloeosporium nervisequum.*

FIGS. 1, 2.—*Gnomonia veneta.* 1, Young trees badly affected with the Myxosporium stage; 2, the same, natural size, shows Myxosporium pustules.

This stage is extremely fatal to young sycamore trees. A careful search was made for young trees that could be transplanted for inoculation purposes, but they were found to be very scarce. When some were found, however, the reason for their scarcity was evident; they were being killed by the anthracnose. The young trees had the same appearance as the terminal twigs on the larger trees; the leaves were nearly all killed when they were about one-quarter grown, and the young shoots were covered with Myxosporium pustules (*fig. 1*). The tree on the left in the figure was in the condition of most of the seedlings. Only after careful search was a young tree found that was nearly healthy and could be photographed for comparison; and an examination of the photograph shows that a few leaves are dead on this one. If the time ever comes when this tree is planted for commercial purposes, this disease may develop into a very serious pest in the nursery.

How the fungus enters the twigs was not satisfactorily determined. There are two possibilities; it may pass down the diseased petioles into the twigs, or the twigs may become infected directly from spores. The twigs are infected very close to the apical bud. The presence of the disease on the petioles and the lack of any wounds of any kind on the twigs make it seem possible at least that the former indicates the real method of infection.

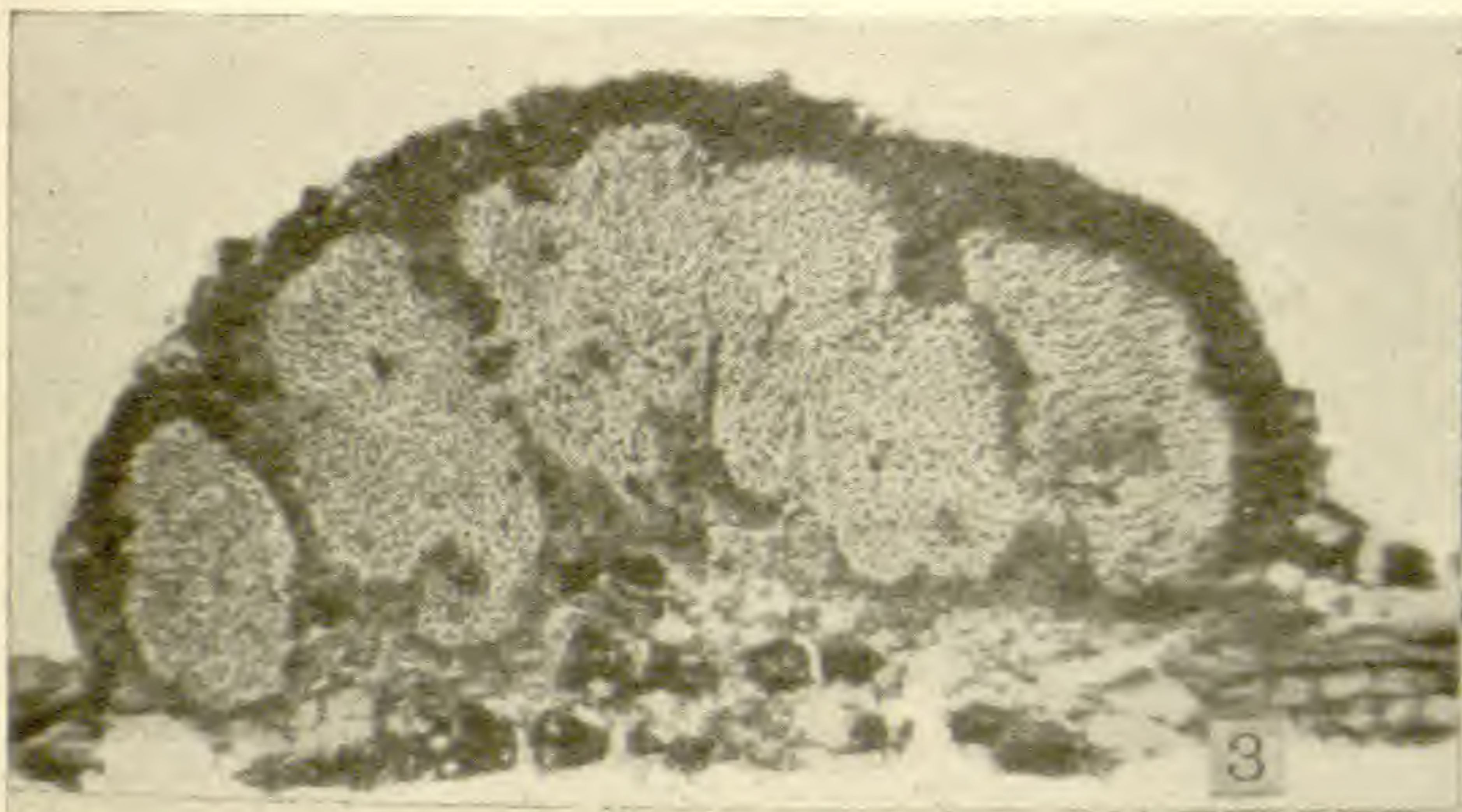
Also the question of the fresh infection of the leaves in the spring is not entirely settled. Inoculation experiments tried on leaves in the laboratory were without success. Perhaps the period of incubation is too long to attempt inoculations on branches cut from the tree and kept fresh by standing in water. However, it was useless to attempt inoculations out of doors where nearly every tree was already infected. Several investigators have attempted to infect the leaves artificially, but mostly without success. KLEBAHN after making a large number of inoculations obtained a few successful ones; but one of his checks also took the disease.

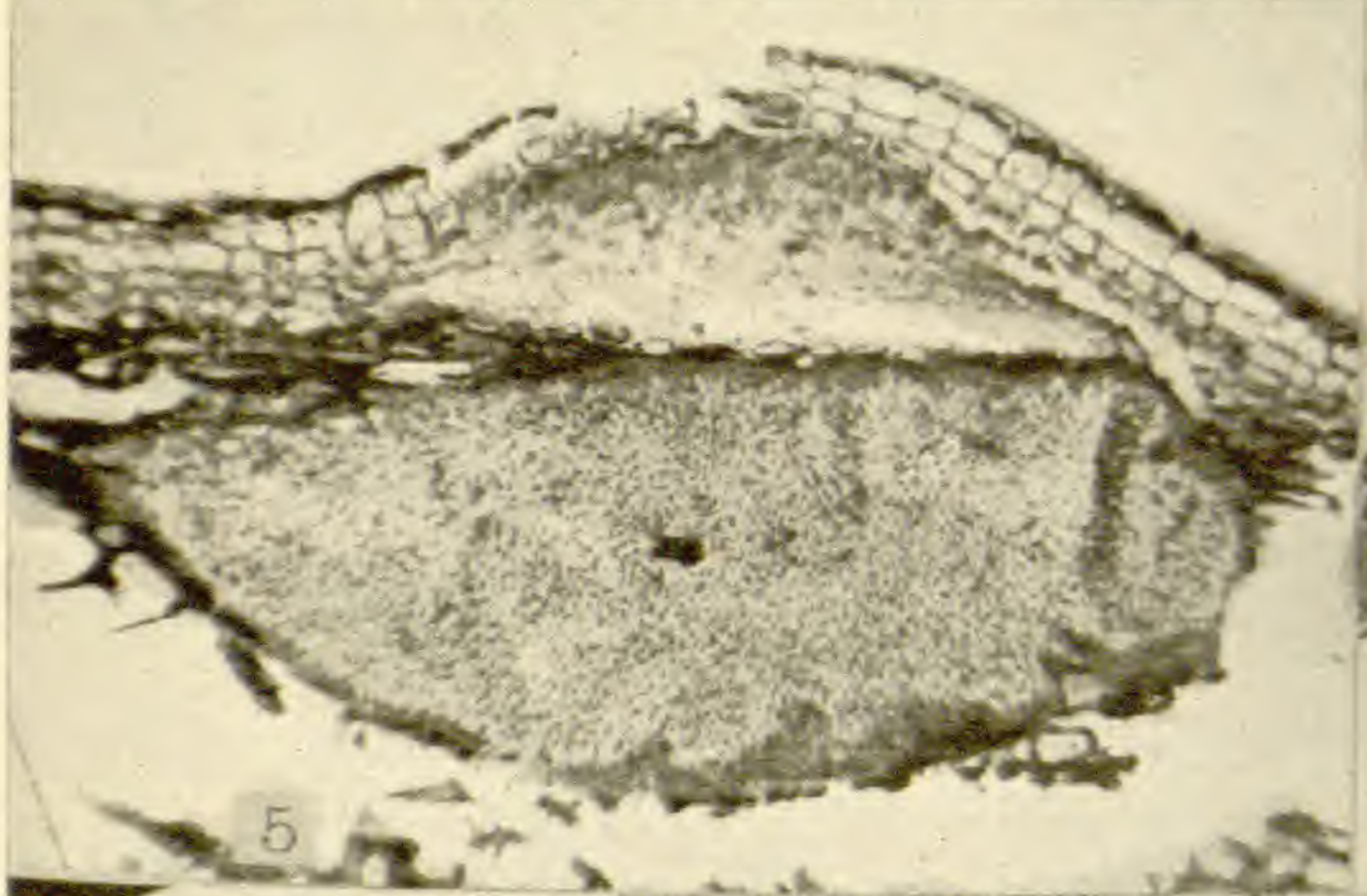
The Myxosporium stage undoubtedly plays the larger part in causing the spring infection of the disease. While the spores, both conidial and ascigeral, are borne in abundance when the leaves are on the ground, they are held together by a mucilaginous substance and are not carried by the wind. The Myxosporium spores on the twigs may be easily washed by rains to the neighboring leaves and cause infection.

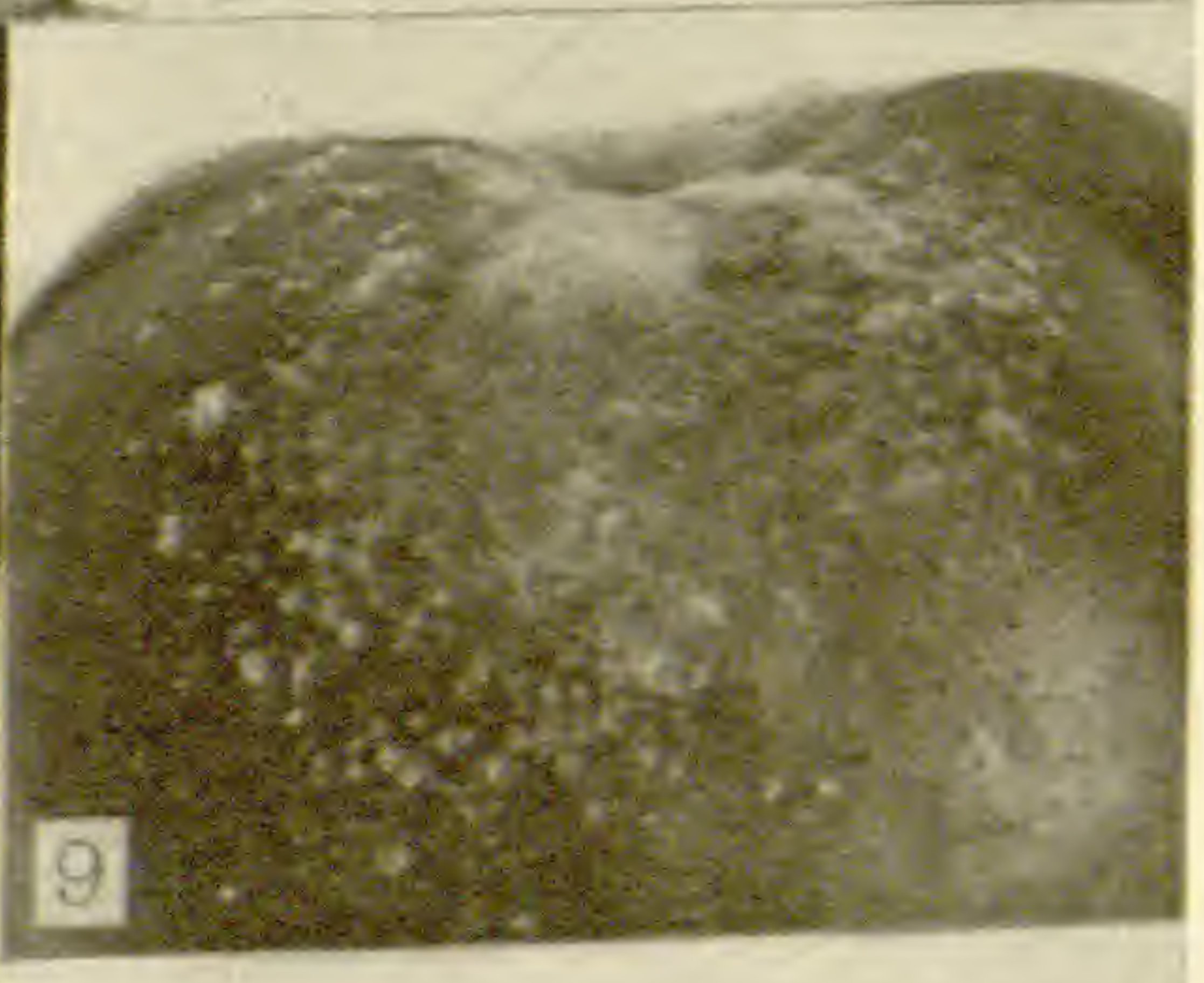
Pure cultures on artificial media were made from all of these different stages and the resulting colonies compared. In all cases the same mycelium and the same colony were produced. The mycelial threads are 1–10 μ in diameter, the small ones being nearly continuous, while the larger ones are closely septate. The colony characters agree perfectly with those given by KLEBAHN. Both conidia and ascospores germinate readily, the latter, however, only from the large cell. The small cell was never seen to send out a germ tube.

This fungus (*Gnomonia veneta*) also occurs on three other hosts at least; *Quercus alba*, *Q. velutina*, and *Q. coccinea*. In 1890 HALSTED (**17**) described the disease occurring upon white oak as due to *Gloeosporium nervisequum*, but there has always seemed to be some question of it. The anthracnose of white oak generally is said to be due to *G. canadense* E. & E. Dead white spots from a few millimeters to a centimeter in diameter are formed, generally scattered over the leaf, though sometimes the tip of the leaf is killed, and sometimes the spots follow down the veins. The conidial pustules and conidia (*fig. 7*) do not differ from those on the sycamore. The two were studied in pure cultures, and the germination and the colony characters seemed to be the same. To make sure, however, that the fungus was the same as the one on sycamore, diseased leaves were put between wire netting and placed out of doors to winter in order to obtain the ascigeral stage. As on the sycamore, the fungus spread over the dead leaf and in late winter produced perithecia in abundance. The characters of perithecia, asci, and spores are the same as in those produced on the sycamore, with the exception of the length of the necks of the perithecia. Outline drawings of the asci and spores are shown in *figs. 21* and *22*, so that they may be compared with those from the sycamore.

The necks of the perithecia on the oak averaged much longer than those on the sycamore, some being as long as the body or even longer. Those on the sycamore have very short necks, or in some cases necks were almost wanting. This single difference does not seem to me to be sufficient to separate the forms as distinct species. The length







FIGS. 3–8.—*Gnomonia veneta*. 3, Sporonema pustule on petiole of fallen sycamore leaf; 4, perithecium in sycamore leaf; 5, Myxosporium stage on sycamore twig; 6, perithecium in *Quercus alba* leaf; 7, acervulus from *Quercus alba* leaf; 8, *Quercus alba* leaf, showing the perithecia, ×2.5.

FIGS. 9, 10.—Apple, inoculated with the Gloeosporium (*Colletotrichum gloeosporioides*); 9, from the orange, showing hairy pustules; 10, from raspberry canes.

of the neck in this genus seems to be quite variable. While KLEBAHN found that the necks of *Gnomonia veneta* from sycamore leaves were very short, on sterilized leaves in the laboratory, on which he also produced the perithecia, the necks were very long. I have also found in my study of *Gnomonia rubi* Rehm., a form found on blackberries, that the same variation occurs. The necks in pure culture were twice as long as those growing normally out of doors. Since this is the only difference observed between the forms on sycamore and white oak, it seems best at present to consider them the same.

The perithecia on the oak leaves also very often push themselves entirely out of the leaf (*fig. 6*). There is often no leaf tissue below the perithecium, but its beak passes entirely through the leaf and projects on the opposite side. The large perithecia are easily seen on the leaf with a hand lens, when they appear as black globose bodies apparently lying on the surface (*fig. 8*).

The spores are unevenly 2-septate, as in the form on sycamore. In germination, the spore swells considerably and then sends out tubes from any place on the wall of the larger cell (*fig. 26*).

The Sporonema stage was not observed on the white oak, though leaves were examined repeatedly for it; but it developed on oak leaves that had been put in tubes, sterilized, and inoculated with a pure culture from the conidial stage on the leaves. The tubes were kept in a cool place during winter to see if the perithecia would develop. On examination in the spring, no perithecia were found, but there were many pycnidia-like pustules, perfectly homologous with those that developed on the sycamore leaves normally.

The Myxosporium stage was found on the ends of twigs on a few trees about July 1, the twigs being killed back in the same manner as the sycamore twigs. However, on the white oak the young leaves did not start to develop and then die, as on the sycamore; but the twigs were killed before the leaves started. Anthracnosed spots on the leaves were numerous in close proximity to the diseased twigs, while they were fewer where no diseased twigs were found. It seems very evident from this that the Myxosporium stage is very instrumental in the early infection of the leaves. The spores in the pustules were not different from those on the leaves, and furthermore, they produced the same colony in agar.

On leaves of *Quercus velutina* also there was found a fungus which does not seem to differ materially from the true *Gloeosporium nervisequum*. The disease, which was very common around Ithaca in the summer of 1906, appeared on the leaves in late summer, dead spots being formed 1–2^{cm} in diameter. The spots were not white, as on the leaves of *Q. alba*, but brown. Sometimes the disease killed a large portion of the leaf, but generally it appeared merely in spots.

The acervuli were scattered on the under-surface of the dead spots. The spores are somewhat larger than those on the sycamore, being about $15–20 \times 6–9\,\mu$. This difference seemed at first to separate it from the form on sycamore; but when the two were grown side by side on plates, the difference was not observable. The spores produced in pure cultures at first seemed to be slightly larger than those from the sycamore, but very soon they became the same size (about $9–14 \times 4–6\,\mu$). Only one difference was noticed between the two forms on artificial media. Both forms on bean pods covered the substratum with a grayish to blackish growth and then grew over the liquid in the bottom of the tubes. On this growth, from the liquid especially, in the culture from *Q. velutina* there oozed out large drops of golden yellow liquid, which were so abundant that they almost covered the surface. As the culture became older, these drops dried to yellow, waxy crusts. On the form from sycamore no such golden drops were produced. In other ways the two forms were indistinguishable on artificial media.

In the spring on a few twigs there was also found the Myxosporium stage. The young twigs were killed before the leaves started, and were killed back 15–20^{cm} from the end. They were covered with pustules similar to those from Platanus and *Q. alba*. A culture made from the spores produced a colony identical with the others.

Diseased leaves of *Q. velutina* were also put out in wire netting to see if the perfect stage would develop. In the spring near the anthracnosed spots were found small scattered perithecia, which were identical with those found on the white oak leaves. The asci and spores are illustrated in *figs. 23* and *24*, so they may be compared with the same from the other hosts. Cultures from the ascospores again gave the same mycelium and conidia as had been obtained from the imperfect stage.

While there are some slight differences between this form and the one from sycamore in the size of the conidia and effect on culture media, these do not seem to be sufficient to make a new species, especially since the large conidia do not appear on artificial media. Perhaps the slight difference found on culture media was due to the previous growth for some time on different hosts. The one from white oak, after being grown for several months on artificial media, lost some of the characters it originally had. The zonate growth of the colony became less marked, and the aerial tufts containing pustules were more feebly developed. The difference between the forms on *Quercus velutina* and *Platanus occidentalis* was, perhaps, no more marked than the difference between cultures of the same fungus after growing for different periods on artificial media.

Search on other oaks has also resulted in finding the Myxosporium stage on twigs of *Quercus coccinea.* The disease was not different in appearance from that on the other hosts, and a culture of the spores also gave a colony similar to the colonies from other hosts. Anthracnosed leaves of *Q. coccinea* have not been found, but they undoubtedly can be found at the right season.

While no satisfactory inoculation experiments were carried on, it seems almost certain that these three forms are the same. They are at least no more than biological species. Attempts were made to inoculate young oaks in the greenhouse, but they were unsuccessful, even when the conidia were placed on the same species of oak from which they were developed.

Whether *Gnomonia veneta* occurs on any other host is a question; there appears no reason why it should not be found on other oaks. HALSTED (16) has described the disease as occurring on red maple (*Acer rubrum*). While this is very possible, proof of it will only appear when the perfect stage is developed and the forms are compared on culture media. No Gloeosporium has been found on maple near Ithaca since this investigation was begun, so there has been no chance to find the perfect stage. However, if it does occur on maple, it seems strange that it has not been found, as the fungus is so common on the other hosts.

In adopting a name for the sycamore fungus, KLEBAHN used the specific name first given to the perfect stage (*veneta*). Adopting the first name applied to the perfect stage seems to be the general rule among European mycologists. In America, however, there are many who believe that the first name given to any stage should be the one used. If the latter rule is adopted, then the name *veneta* cannot hold. The first name given to any stage was *Fusarium platani* Mont., and hence the fungus would have to be called *Gnomonia platani* (Mont.). This will only be settled, however, when botanists come to some conclusion as to the nomenclature of polymorphic fungi. Also if we wish to be consistent in our use of names of the imperfect stage, we should use *Gloeosporium platani* instead of *G. nerviscquum*. However, it is of little importance what the imperfect stage is called, it is at best only a synonym.

The synonymy of this fungus as given by KLEBAHN includes sixteen names. To this may be added the following: *Gloeosporium canadense* E. & E. Jour. Myc. **5**:153; *Myxosporium platanicolum* E. & E. Proc. Acad. Philad. **1894**:572 (distributed in N. Am. Fungi as 3180). The form on *Quercus velutina* does not seem to have been described.

Anyone wishing more of the details of the life-history of this fungus must consult KLEBAHN'S work, where every stage is described in fullest detail.

Pseudopeziza type

The genus Pseudopeziza was first connected with a Gloeosporium by KLEBAHN (**26**) in 1905, who found the perfect stage of *G. ribis*, a form common on currant leaves, to be *P. ribis* Klebahn. He developed the perfect stage by putting the leaves out of doors in a protected place to winter; and in the spring he found it in abundance.

My investigation of this form has been very limited. Only a small amount of material was obtainable and little more was accomplished than to study the form in pure culture. An attempt was made to produce the perfect stage, but it was without success.

Glomerella type

Although a great deal of work has been done on this group of anthracnoses, the results obtained have been less satisfactory than those obtained from the other types. The great majority of the anthracnoses that have been described belong in this group. Nearly all those of fruits—and a glance at the literature will show that there are scores—and a large percentage of the forms on herbaceous stems seem to belong to this type. The problems concerned and the difficulties that confront the investigator become more evident the longer the group is studied. Little more can be done in this paper than to add to our knowledge of the biology and physiology of the forms studied. This type is sharply separated from the others, not only in the characters of the perfect stage, but also in those of the mycelial and conidial stages. Nearly always one can recognize this type at a glance.

Some of the evident characters are as follows: (1) The spores ooze out of the acervuli in pink masses or strings; so far as has been observed, the spore masses in the Gnomonia and Pseudopeziza types are white, cream-colored, or yellow. This may not be a sure criterion, but it seems to hold in nearly every case. (2) In artificial cultures, especially where nutrition is lacking, and sometimes on the original host, the dark-colored secondary spores or appressoria are developed; in no instance have these been observed in the other types, although they have been carefully searched for. (3) The mycelium in pure culture varies but little in diameter; this separates these forms at least from those of the Gnomonia type. Other minor points that help to place the forms in this group are the rapidity of growth, the presence of small black wefts or crusts of mycelium in the culture medium, and the presence of a dark-greenish pigment in old mycelium, especially on sugar media. The last two characters are not always present, but their frequency makes it advisable to mention them here.

Most of the work that has been done on species of Gloeosporium, especially by American botanists, has been done on this group. The papers of Miss SOUTHWORTH, Miss STONEMAN, CLINTON, SPAULDING and VON SCHRENK, SHELDON, ATKINSON, and SHEAR have dealt principally with these forms.

GENERAL DEVELOPMENT OF THE FORMS

As to the life-history and development of these forms, little needs to be added here. Each goes through the same stages in development, and these have been described during the past few years for a number of forms, by a number of investigators. Only a few things which do not seem to have been made clear need to be mentioned.

The conidia germinate very readily in water or in nutrient solution, generally within three or four hours. The germ tube may be sent out from any place on the spore, but in most cases the first tube originates near one end. It rarely comes exactly from the end, but very close to it, so that it appears to extend at an angle to the main axis of the spore (*figs. 30, 36, 37*). If it came exactly from the end, the germ tube and the spore would lie in a straight line. In some cases the tube does appear to come from the end, but this seems to be uncommon as compared with the other method. After the first germ tube, several more may develop apparently from any place on the spore.

Germination differs somewhat in different media. Rarely in nutrient solutions does the spore become septate in germination. In water, however, the germ tube is much smaller and generally the spore becomes septate. This variation in germination in different media seems to be characteristic of all members of this type. It is interesting at this point to note a recent paper by DELACROIX (12) on *Gloeosporium musarum*. In studying the germination of its spores, he found that they nearly always became septate. Miss STONEMAN (43) some years previously, in working on the same form, had figured the spores as continuous. DELACROIX was undecided whether to call the form he was working with a new species or not, as this was the only point of difference. If he had germinated his spores on different media, he undoubtedly would have found both types of germination.

If germination takes place in a medium lacking nutrient material, as water, the germ tubes grow only a short distance and then form at their tips rather large brown cells more or less variable in shape (*figs. 32, 33*). These have been spoken of variously by different investigators as secondary spores, appressoria (HASSELBRING 24), etc. They germinate readily under the right conditions, although some seem to have had difficulty in obtaining germination. If nutrition is still lacking after the brown cell germinates, another similar one may be formed at the end of this germ tube (*fig. 32*).

Quite often, however, when a conidium germinates in water, there may be formed at the end of the spore or at the end of a short germ tube a small hyaline conidium perfectly normal except as to size. This was observed by ATKINSON (3) from the form on cotton, and by HALSTED (22) from a form on *Podophyllum peltatum*, but it does not seem to be peculiar to these forms, for it is rather common in all members of this type.

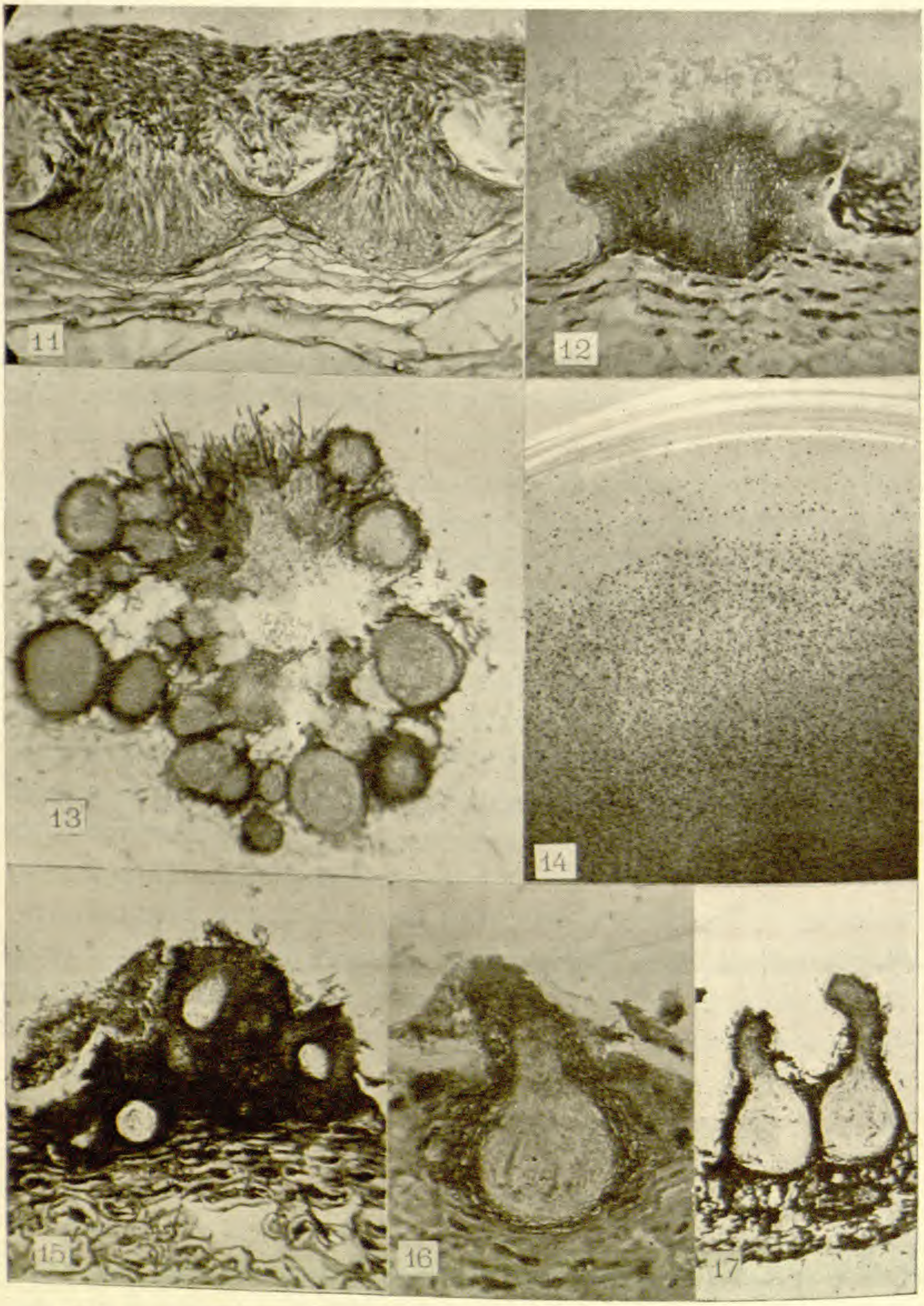
Under proper conditions some forms, either on artificial media or on the original host, develop the perfect stage abundantly. During the past few years this stage has been found from a number of different hosts: from privet, orchid, pepper, red raspberry, and vanilla (?) by Miss STONEMAN (43); from Dracaena (38, 39) and guava (37) by SHELDON; from apple by CLINTON (10); from grape, cranberry, Ginkgo, cotton, rubber plant, honey locust, and bean by SHEAR and WOOD (40); from Artocarpus by DELACROIX (13); from Cattleya by MAUBLANC and LASNIER (27), described as a Physalospora. Several other forms described as Physalospora, Laestadia, and Phomatospora undoubtedly should be placed here. During the past two years I have worked with the ascigeral stage from the following hosts: apples from Missouri and Illinois; Dracaena from West Virginia; rubber plant from West Virginia; cotton from Georgia; *Coelogyne cristata*, *Sarracenia purpurea*, *Coffea arabica*, and *Anthurium waroc-queanum* from the Cornell University conservatory; quince from an orchard in Ithaca, N. Y.; and *Asclepias syriaca* from Ithaca. The last named has been known in the conidial stage as *Gloeosporium fusarioides* E. & K. This is the first time that the perfect stage has been reported from the last five hosts. The perithecia, asci, and spores are very much alike from the different hosts. The following description, based on the perfect stage as it appears on the apple, would fit in nearly all cases the other forms as well.

On apples from the south-central states affected with the bitter rot, the perfect stage seems to develop readily. Apples were received from Illinois and Missouri, and in both cases perithecia were found on the surface accompanying the acervuli. There seems to be considerable variation in the manner in which the perithecia are borne on the apple; *figs. 15* and *16* illustrate perhaps the extremes, the latter having been made from perithecia on apples from southern Illinois.

The perithecia from the Illinois collection were single or collected in twos or threes partially or nearly imbedded in the tissue of the host, and the necks were surrounded by a thin layer of pseudo-parenchymatous fungus tissue. They were generally about 70–125×175–225 μ, the necks being sometimes 50–60 μ in length. The perithecial wall was composed of three or four layers of narrow, long, black, thick-walled cells. Sometimes two perithecia fused, so that there was one internal cavity with two ostioles. At the base of the perithecia there were generally large, dark-colored hyphae made up of nearly globose cells 6–8 μ in diameter (these show plainly in the photomicrograph). *Fig. 15* was made from apples received from Missouri. The perithecia here were not imbedded in the host tissue, but were at the base of the conidial stroma; and the perithecial wall gradually blends into the pseudo-parenchymatous fungus tissue. Necks were almost entirely lacking on these perithecia; in other points they were identical with those described above.

The perithecia develop readily on bean agar, the best success having been obtained with that medium. They appear in raised masses or nodules scattered over the surface of the plates. These masses are black, and by the time the asci are mature they may be 2 to 3^{mm} in diameter. The perithecia (*fig. 13*) form around a small stroma of pseudo-parenchymatous tissue, and are quite variable in shape and size, being 80–140 μ thick×120–225 μ long. The apex may be slightly prolonged into a neck or not, though in no case is it very pronounced, and there seems to be no definite direction for the neck; some of them opened directly into the underlying agar. In a plate kept in the incubator at a temperature of 26–30° C., the nodules of perithecia were generally visible in four to six days, and the asci were generally mature in sixteen to twenty-one days.

The asci are 50–80×8–10 μ, clavate, almost entirely filled by the eight spores; the wall is hyaline and sometimes difficult to see except with fresh material. The spores are hyaline, slightly curved, rounded at the ends, granular, and possess a large clear nucleus near the center and on the concave side of the spore. The spores are irregularly biseriate in the ascus, and are very quickly shed after maturity. The asci go to pieces and the spores ooze out of the neck of the perithecia. What causes the spores to leave the perithecium was not determined.



FIGS. 11–17.—*Glomerella type.* 11, acervuli on tomato; 12, acervulus on apple from Missouri; 13, nodule of perithecia of *Glomerella fructigena*, perithecia in plate culture; 14, variation from *G. fructigena*, perithecia scattered, ×2.5; 15, *Glomerella fructigena*, perithecia on apple from Missouri; 16, *Glomerella fructigena*, perithecium on apple from Illinois; 17, *Glomerella cincta*, perithecia on *Coffea arabica* leaf, showing the long perithecial necks.

Sections of perithecia a little past maturity showed the spores held together in a mass just outside and at the apex of the neck. The whole inside of the perithecium seemed to be empty, with the exception of a few scattered threads. It seems probable from the sections, although this was not determined absolutely, that the ascospores are held together by a mucilaginous substance as well as the conidia.

The question of paraphyses is a much discussed one at present. The genus Glomerella was described by SPAULDING and VON SCHRENK as without paraphyses, but quite recently SHELDON (39) has reported their presence in a number of forms, especially in the young perithecia. SHEAR (40) has taken exception to SHELDON'S observations, finding sterile threads in the perithecia but believing they were entirely outside of the asci, so they would be periphyses and not paraphyses; later (41), however, he calls them evanescent paraphyses. The point is an extremely difficult one to decide. Forms from several hosts have been studied to determine this point. In crushing a fresh perithecium, sterile threads can be easily seen; *fig. 42*, drawn from the form on Dracaena, shows them plainly. These threads are very irregular, many of them much longer than the asci, sometimes branched and sometimes septate. They are very highly granular, in fact the granules are the most prominent part, the wall itself being almost invisible. The difficult question is to determine whether they are between the asci or outside of them. Scores of stained sections have been prepared and carefully examined; some slides have looked a little suspicious, yet never have the threads been seen between the asci with certainty. While the threads take the stain poorly, they can be seen, and appear to form a layer around the asci near the base of the perithecium, but in the upper part of the perithecium above the asci they branch out over them. As the asci and spores break loose from the bottom of the perithecium, they can be seen mixed in with threads. They could hardly be considered evanescent paraphyses, because they remain in the perithecia as long or longer than the asci. It seems best, therefore, to regard the genus as aparaphysate.

The perithecial stages from all of the forms, so far as known, seem to be quite similar. The most variable characters are the size of the spores and the length of the neck of the perithecium. The spores vary in length from 12 to 32 μ; SHEAR and WOOD (40) place the extreme length at 24 μ. While most of the spores are under the latter figure, from perithecia from *Sarracenia purpurea* many spores were seen 30 to 32 μ long; but here all the asci were not eight-spored, some containing two spores (*fig. 45*), while four spores were common. Here, as in other forms where this condition exists, the spores in the few-spored asci are larger than in the eight-spored ones. The length of the neck of the perithecium is also quite variable, and as a rule it is generally longer than those previously described on apples. Miss STONEMAN (43) shows some of them to be very long, and SHEAR was rarely able to find any so long. An examination of her slides, however, which are still in the Cornell University laboratory, leaves no doubt that the perithecia were as she illustrated them. Furthermore, perithecia with necks just as long (*fig. 17*) have been seen many times. In the forms from rubber plant, Dracaena, Coffea, and Sarracenia, which produced their perithecia on leaves, the long necks were the rule rather than the exception. However, these same forms in pure culture, with the exception of the form from Sarracenia which did not produce perithecia in pure culture, formed perithecia with short necks. The length of the neck seems to be dependent to some extent upon the substratum. However, the depth to which the perithecia are imbedded in the substratum does not seem to be the controlling factor. The perithecia may be nearly or entirely superficial, or may be nearly or entirely imbedded in the substratum; many of those which were entirely superficial had the longest necks (*fig. 17*).

Just what the factors are that cause the development of the perfect stage is unknown. I obtained this stage from the different forms as follows: (1) By placing portions of the diseased host in a moist chamber and leaving it for two to three weeks. If the perithecia did not develop in this time, it was useless to continue the experiment. The following forms produced perithecia in this manner: apple, orchid, *Sarracenia purpurea*, *Coffea arabica*, *Anthurium warocqueanum*, and *Asclepias syriaca*. (2) From inoculations on plates of bean agar from a fresh culture from the host (apple, Dracaena, rubber plant, and *Coffea arabica*). (3) On bean plugs (cotton). (4) On plates of bean agar from a culture that had been kept alive in the laboratory for over a year (quince).

The composition or density of the substratum or the humidity seems to have but little influence in the production of this stage. However, the interesting fact was noted that, with but few exceptions, all the forms known to have perfect stages are southern or greenhouse forms. Almost everything affected with anthracnose when brought from the greenhouse and placed in a moist chamber produced perithecia. Apples from Missouri and southern Illinois produced this stage; but apples, tomatoes, fruit of *Podophyllum peltatum*, and various other fruits and stems found out of doors in New York and brought into the laboratory, produced conidia in abundance but no perithecia. The form from quince from Ithaca, N. Y., did produce perithecia, but only after it had been grown in the laboratory for more than a year. Perithecia were also developed on *Asclepias syriaca* from Ithaca, but this form was found late in the fall, after a long growth through the summer. It may be that a long-continued production of the conidial stage or a long-continued growth at a rather high temperature are factors in perithecial development. However this may be, it is certain that the ability to produce perithecia may be acquired or lost by the fungus. Sub-cultures have been made from single conidia from a culture in which perithecia were produced in abundance. Some of these would produce the perfect stage, while by no treatment known could others be made to produce it. However, as a general rule, it is an easy matter to obtain perithecia in sub-cultures from a culture that has already produced them. To me it appears as if in this group the ability to develop perithecia is one of the characters that may be acquired if conditions are right, one that is inherited for some time but will be lost eventually if the environment is not suitable. When it is known that these forms vary in other ways, as described later, this seems all the more plausible.

CULTURAL CHARACTERS

There has always been great difficulty in determining the species in this type. As a result many species have been determined by the host. Recently botanists have been questioning the validity of the method, especially since the perfect stages of several have been found to be very much alike. At present SHEAR and WOOD (40) take the extreme ground that all whose perfect stage has been found repre sent one species, or at best varieties of one species. They base their belief chiefly on the fact that the perfect stages are not morphologically distinct; but because two forms have similar perfect stages it does not necessarily follow that they are the same species. In higher plants there are many instances of the flowers of different species being very similar, if not identical, yet no one would hesitate to call them distinct; differences in the vegetative stage, as the shape or lobing of the leaves, may be marked and constant and hence sufficient to separate them in distinct species. The sexual stage is the least subject to modification, and we must expect very closely allied species to be very similar in this stage. This must be especially true in forms in which the perfect stage is only occasionally produced, as seems to be the case with Gloeosporium.

Theoretically, if we should grow two forms side by side on the same medium and should watch their development, we ought to be able to tell whether or not they are distinct. If the growth characters were different, if the colonies formed were distinct, or the effect on the medium were not the same, we should doubt the advisability of calling them the same thing. Miss STONEMAN carried several forms through on different media and was able to separate some of the different forms by means of these characters. This method has long been in use in bacteriology for determining species; and recently the method has been advocated by THOM (45) for determining species of Penicillium. He has used it in his work on this genus and has found it satisfactory, claiming that the characters of a species on a standard medium are always the same. No matter how long a species may be grown on some other medium, when it is brought back to the standard medium it immediately returns to its original characters.

A thorough attempt was made to test this method with the different members of the Glomerella type; all of these have not developed the ascigeral stage, but they undoubtedly belong to this type. Cultures from over twenty different hosts were used. Petri dishes, filled with a certain medium, were inoculated at the center with a pure culture. Cultures were made from all the similar forms at the same time, generally three plates of each form. The medium for the set of cultures was made in one batch, so that there was no chance for variation in this factor. Many sets of cultures were made employing the following media: potato agar, bean agar, potato agar +10 per cent. of glucose, potato agar+malic acid, and Elfving's nutrient solution. A number of sets of these cultures were carried on during the year 1906–7.

VARIABILITY OF FORMS

A most remarkable fact was made evident by the study of the cultures of the various forms. Of the more than thirty collections studied from over twenty hosts, with less than a half-dozen exceptions, all gave at least slightly different characters. Even the two collections on apples from Missouri and Illinois did not give exactly the same characters, but the differences were slight. The two collections from apples in the north, however, gave entirely distinct characters from the more southern forms on the same host. The southern form, especially on sugar medium, was characterized by very rapid growth and a very dark greenish-black color of the substratum and aerial hyphae; while the northern form grew more slowly and had very little dark color. Generally in the latter the aerial hyphae were colored pink from the profuse development of conidia. Even the form on quince collected in New York did not give the same characters as the northern form on apple. The forms on orchid, Coffea, and Sarracenia, collected in the same greenhouse at the same time, were not exactly alike in culture media. They were similar in most respects, but there were slight differences which would appear after many transfers.

What do all of these differences mean? Miss STONEMAN used these in her description of species. THOM declares that in the genus Penicillium all of the forms which show differences are distinct species. However, he found that a Penicillium would always give the same characters on the same medium no matter how long it had been grown on some other medium. A careful study was made of the forms of Gloeosporium to see whether the same species, or rather the same collection, would give the same characters generation after generation. Some of the forms were studied for nearly two years. Instead of the cultural characters coming true each time, a number of interesting variations appeared, some of which are as follows.

A Gloeosporium was collected on apples at Mexico, New York.

At first the colony on sugar media was covered with an erect growth of mycelium which became pink colored with the spores. But after it had been grown for several months on artificial media, the aerial mycelium lost to some extent its erect character, becoming denser and more floccose. Furthermore, the aerial mycelium lost entirely its pink character due to the formation of spores. The same variation also occurred with the collection from tomato. But it is interesting to note that two collections from raspberry stems[5] and another one from apple, which were identical at first with the two forms above, held their characters as long as they were cultivated—in some cases over a year.

The forms from quince and red raspberry, after they had been under culture for some time, underwent slight changes in the color of the agar and the aerial mycelium. In fact, nearly every culture changed its character in time.

An attempt was made to change the characters of a form collected on apples in the north, which had appeared to remain constant, by growing on different media for several months. The following media were used: potato agar, potato agar + 10 per cent. of glucose, potato agar + copper sulfate (1:100,000), potato agar with a drop of lactic acid in each tube, and Elfving's nutrient solution. Transfers were made every ten days to three weeks into fresh tubes of the same medium; so that at the end of the experiment the fungus in each tube had grown for several months, with many generations on the same medium. At the end of three months these cultures were all brought back on plates of potato and glucose agar, and at first the results were striking. For about a week the growth in this medium from the different tubes was quite distinct; however, after two weeks or more these differences gradually disappeared, and the old cultures looked more alike.

After the fungus had been growing for about four months on the different media, it was again brought back to the same potato and glucose agar, and the results were more striking than in the first case. Differences appeared which did not disappear with the age of the

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[5] The references to Gloeosporium on raspberry stems under the discussion of this type do not refer to the common raspberry anthracnose, *Gloeosporium venetum*. The latter does not appear to belong to the Glomerella type.

colony. The medium on which the fungus had been growing did not seem to be the cause of the variations, for cultures on the same medium would vary in entirely opposite directions, or cultures from very unlike media would vary in the same way. The characters in which there was considerable variation were as follows: amount of aerial mycelium, some very matted, some entirely strict; color of substratum, from pure white to a dark greenish black; presence or absence of pustules; presence or absence of pink color in the aerial mycelium.

However, the most striking variation that was obtained was with the true bitter rot fungus, the culture being obtained from apples sent from Missouri. The fungus had been grown in the incubator for two or three months, having produced perithecia several times on bean agar, and nothing unusual had been noticed; but very suddenly remarkable variation was seen. A dilution culture was made to obtain single colonies for transplanting, but instead of having one kind of colony in the plate, there were two, and they were very distinct. At first the plate looked as if it had been contaminated, but a study of the two forms showed that it had not. Both forms produced typical bitter rot conidia. Cultures were made of the new form and it was studied carefully with the following results:

On bean agar, for the first day or two, the colony characters were identical with the typical form on apple. At that time the perithecia began to develop, and from that time the differences were remarkable. The typical form produced perithecia in nodules scattered over the media as was mentioned above; but the new form produced them singly, or occasionally in twos or threes scattered over the plate. They were produced in such great abundance that the whole surface of the plate was black with them. They began to form as small tufts of pseudo-parenchymatous tissue on mycelium two to three days old, and developed in such great abundance that all of the nutrient material was used up before they could mature. Mature perithecia were not seen, although they were looked for carefully; it is doubtful whether any of them came to full maturity. The perithecia, however, so far as they could be studied, were identical with the typical form. Conidia formed abundantly, scattered over the surface of the agar, but they did not form in pustules. On apple it took but slowly, though finally a few small pink pustules of spores were formed. On sterilized bean pods the growth was abundant, in a few days becoming black from the crust of perithecia formed over the surface. But here, as on the bean agar, the perithecia did not seem to develop asci and spores.

Such a large variation may seem incredible, and it may be attributed to a contamination, for which there is always a chance. If this was a contamination and not a variation, either the two forms were present on the original apple and were transferred together unnoticed from the dilution plate to the tube, or the second form must have entered the tube after the transfer was made. As the apples were obtained in the early autumn and this variation did not occur until February, it does not seem possible that the two forms could have been on the apple together. If they had been, they would have been noticed in the cultures, as they were constantly worked with during the fall and winter. The second possibility seems no more likely. The variation came in the middle of the winter, when spores of Gloeosporium would not be liable to be free in the laboratory; and as the new form was totally unlike any of the other species of Gloeosporium in culture in the laboratory, it could not have come from them. The only explanation of the phenomenon is that one or more individuals of the original form changed quite suddenly their course of development under cultural conditions; but whether that is admitted or not, we have at present this new form. It is undoubtedly a Gloeosporium of the Glomerella type, with the development of the perithecia considerably different from other known forms.

Mutations, so far as is known by the writer, have not previously been recorded among fungi, but the form just described seems to be one without question. Whether all of the variations that have appeared in the study of this group should be classed as such is questionable; the minor variations which appeared gradually perhaps should not be so considered. No two individuals of any species are alike, and the variations are perhaps no greater than we could expect among individuals of almost any group. The effect of variation on our ideas of species in this group will be discussed later.

INOCULATION EXPERIMENTS

As is well known, the forms occurring on fruits have been frequently cross-inoculated by different investigators. HALSTED (**20–23**), COBB (**11**), and many others have shown that the forms are easily transferred. HALSTED used fifteen or more fruits and vegetables, and with a few exceptions he was able to transfer the forms at will. This has been repeated by others; and during the past two years I have made many similar transfers. Also the forms on raspberry stems have been grown just as readily on apples as the original culture from apple (*fig. 10*). The forms which HALSTED worked with must have been mostly northern forms.

HALSTED (**20**) also believed that the forms on watermelon and bean were the same, having inoculated both forms on a citron and obtained identical spots. Other workers, as SHELDON (**35**), have failed to get infection by inoculating spores from the melon fungus on the bean; however, the young bean plant is not readily infected from spores from the bean itself.

Little has been done in transferring the forms that are known to produce the perfect stage. Several investigators have transferred the Gloeosporium from the apple to the grape and *vice versa;* and from these experiments it is generally recognized at present that these two forms are the same. Recently SHELDON has also transferred the form from apple to the sweet pea and obtained successful infection; this I have also confirmed. Leaves of young plants in the greenhouse were inoculated from a pure culture from apple; inoculation took readily and dead spots were formed. In one instance the disease followed the petiole back to the stem, and after it had entered the latter the whole plant wilted and died. It is interesting to note that the inoculations of the form found on apples in the north to the sweet pea did not take so readily; only after the plants began to die for want of water did they become infected.

Another experiment was tried with leaves of rubber plant placed in a moist chamber. The forms from Dracaena, *Ficus elastica,* Coffea, and Sarracenia were then inoculated on the leaves. In all cases the fungi took readily, soon producing conidia in abundance; later the forms from Dracaena and Ficus produced perithecia on the leaf.

Inoculations from Dracaena and Coffea to the apple were entirely successful; those from watermelon and bean to the apple were failures. Inoculations from Asclepias to the apple caused infection, but the resulting diseased area looked different from the true bitter rot; no pustules were formed. Inoculations from orange to the apple were successful, but the spot formed was very different from that formed by the bitter rot fungus. The pustules were steel gray, hairy, and raised from the surface (*fig. 9*), and seemed to be identical with the pustules formed by the guava anthracnose on apple, as described by SHELDON (**37**).

While these inoculation experiments are not yet so complete as they should be, they show that we have a number of forms that will grow on different hosts. Whether these different forms occur normally on the different hosts is another question. It is certain also that there are some forms which cannot be transferred to some of the other plants that are normally infected with anthracnose; for example, the bean anthracnose on apple.

ADAPTATION

After one has studied many of these forms for some time and observed the slight difference between them, and after he has watched the variation in single forms, the question of their origin is sure to arise. It is difficult after such a study as the above, where variations take place in a few months, to believe in the fixity of species. Among higher plants some forms are constantly changing, and breeders can now build up a new variety in a few years. If such things are possible in a few generations with higher plants, how much more so must it be with the lower forms, that pass through a generation in a week and may have many generations in a season. These lower forms are excellent organisms with which to study variation, and considerable work of this kind has been done with the bacterial and yeast organisms. HANSEN (**28**) in working with yeasts was able to build up asporogenous varieties by cultivating them under conditions where spore formation was impossible; the varieties became fixed and would not again under any conditions produce spores. He has also been able to build up fixed varieties differing from the parent form in shape of cells and power of fermentation.

Among the bacteria it is a common thing to speak of attenuated forms; that is, forms which have lost some of their characters, due to growth on a different substratum or host from the customary one. Many of the pathogenic forms have been cultivated until varieties have been produced which are unable to produce the poisons characteristic of the parent form.

It is doubtful whether we can say for any form that it is absolutely stable. THOM claims this for the species of Penicillium; and doubtless some forms are more fixed than others. But, as PFEFFER (**28**) suggests, the fact that a form has remained unchanged after growing for two years on a certain medium does not prove that it will not vary in a longer period, or does not prove that it will not vary on some other medium. But the species of Penicillium stand in a different category from those of Gloeosporium. They have been growing for ages as saprophytes on various substances, and have become adapted to a wide range of media, and the forms have become fairly well fixed; but the forms of Gloeosporium are more plastic. Is it not just as possible to have variable forms among the low, fast-growing fungi as among the higher plants? There seems to be no other explanation for the condition that is present in the Glomerella type of anthracnose.

If the forms will vary in culture under constant conditions, should we not expect a greater variation in the open, where the extremes to which the plants are exposed must be many times as great as in the laboratory? In nature, they must at one time or another be subject to every sort of condition under which they will grow. If variation is possible, we should expect to find varieties or forms built up which will come true for at least several generations.

Furthermore, if we admit the fact of variation, we can explain the distribution of forms and also the constant discovery of forms of Gloeosporium on new hosts, or "new species" as they are generally called. Quite often a form is discovered which has never before been found, and immediately it becomes very prominent on account of the great damage it is doing. As a form varies, it may adapt itself to some new host and be able to grow with some vigor. A sudden mutation, like the one described above, might produce a form that would grow immediately on another host; or a form might take but poorly on a new host at first, but after many generations might become adapted to it. This sudden occurrence of new forms is a vexing question. It may be due to the naturalization of some exotic form, but this does not seem to be satisfactory. The best explanation, at least with variable forms, seems to be the production of new strains or forms from the old ones.

What needs to be done now, and what must be done before we can formulate any idea as to the limits of species among these forms, is to grow them on different hosts and different media for long periods, to see if they will adapt themselves to the new hosts and media and will become fixed. It seems probable from this study that such varieties could be built up, and perhaps sooner than we would expect.

NOMENCLATURE

Some doubt has been expressed lately as to the validity of the genus Glomerella. Some have thought that the forms might very well be put in some of the closely allied genera. There are three other genera which are quite similar, all of which doubtless have had described under them, at one time or another, forms that belong properly to Glomerella. These are Physalospora, Guignardia, and Phomatospora. None of these genera are understood very well by mycologists, and a thorough monograph is needed before we shall be sure of our ground. But there are some characters which seem to prevent the forms of Glomerella from being placed in the other genera. A stroma may be present in the forms of Glomerella, while it is entirely lacking in the others. Other distinguishing characters are the more or less well-developed neck of the perithecium and the lack of definite paraphyses.

The genus Glomerella is extremely variable, and there remains little doubt that some of the individuals would answer very well to the descriptions of the other genera; but it seems that the forms taken as a whole are distinct enough for a separate genus; at least this is the position I should take for the present. MAUBLANC and LASNIER (27) have recently described a perfect stage of a Gloeosporium on an orchid as a Physalospora. Although their material has not been seen, it seems probable that they had but a form of Glomerella. They described their specimen as possessing paraphyses, but their drawings show a few branched threads entirely outside of the asci; apparently these are the same as are seen in other forms of Glomerella.

The question of specific names is the most difficult one. We have a multitude of closely related forms with a greater number of names. What are we to do with all of these forms? If they were fixed and would vary but little or practically not at all, as seems to be the case with forms of Penicillium, it might be well to call them species, or at least varieties. But when it is difficult to find two collections with the same characters, or to find a form that will not vary on culture media, what is to be done with them? There seems to be little doubt that some of the forms are the same, and that many of the names should be reduced to synonyms; but it also seems that some of the forms are widely enough separated to be considered distinct species. It hardly seems possible that a form that produces large, hairy, steel-gray pustules when grown on apple, as SHELDON (**37**) has described for the guava anthracnose, and as is also the case with at least one form from the orange, could be put in the same species with the one originally found on apple, from which no such a pustule forms.

SHEAR and WOOD (**40**) believe that all the forms whose perfect stage has been found form a single species or at most varieties of one species. They say that all will be considered by them as *Glomerella rufomaculans* or varieties of it. Their use of names, however, is not always consistent. They use *rufomaculans* because it is the first name used for a Gloeosporium on a host of which the perfect stage has been found. Evidently they mean to use the first name applied to any stage. However, in another place in their paper they quote Miss STONEMAN'S species as if she were responsible for the specific name, as, for instance, *Glomerella cingulata* (Stoneman) Sp. & v. Schr. To have been consistent with their use of the name *rufomaculans*, they should have used *Glomerella cingulata* (Atk.) Sp. & v. Schr. ATKINSON (**4**) named the conidial stage and that was the first name. There is great difficulty in getting the right name for these forms. If we use the oldest name applied to the perfect stage, and if we believe, as do SHEAR and WOOD, that all of these forms are the same thing, we must use the name *Glomerella cingulata* (Stoneman) Sp. & v. Schr. But if we follow the other system of using the earliest name applied to any stage, we are in great difficulty. If we follow the latter, we may use *rufomaculans* until some one develops the perfect stage from some other host, the conidial stage of which was described before *rufomaculans;* and this older name would stand until another earlier one was found. Our names would be in an unstable condition until the perfect stage was developed from the first described Gloeosporium. Furthermore, if we consider the forms from different hosts distinct enough to be species, and also use the name first applied to the perfect stage, the name *Glomerella fructigena* (Clint.) Sacc. must stand for the form from apple.

While the various forms are morphologically very similar, some of them at least seem to be quite well separated from the rest. While the reactions to culture media are not very trustworthy, we can get some idea as to the relationship of the forms. There are some forms which do seem to be very closely related and very likely are the same; the forms from Dracaena, *Ficus elastica*, Anthurium, Coffea, and Sarracenia seem to be very close together and should be considered identical. Whether these are identical with the fungus causing the bitter rot of apples is somewhat questionable, though they are very close to it. There are some differences between them, as, for instance, the size of the germ tube in the germinating conidium, which is much larger in the apple form (*figs. 30, 36, 38, 39*). The forms on Sarracenia and Anthurium seem to be undescribed. The anthracnose, however, has not been found on the Sarracenia plants in the bogs, though it has been looked for. Species of both Gloeosporium and Colletotrichum have been described on the coffee tree. Of course it is impossible to identify the one studied here positively with any of them, but so far as the descriptions go it would fit either.

The form from cotton seems to be quite divergent from the other forms, and doubtless should be considered a distinct species. The cultural characters are quite distinct and it takes but poorly on apple. Also on Elfving's nutrient solution the mycelium breaks up into large cells (*fig. 34*) which are capable of growing into mycelium again, a character which has not been found in other forms. I was not able to bring the perithecia to maturity, that is, the asci did not develop in the perithecia, but SHEAR (40) has found a strain which would mature.

The forms from apple have been very perplexing to me. Until this time it has gone without question that these forms are all the same. SPAULDING and VON SCHRENK (**33**) state that they have seen specimens of the bitter rot on apples in Vermont as late as October. They might have seen apples affected with a Gloeosporium, but it is not certain that the apples were affected with the same form that grows in the south. As was stated above, the two forms on culture media are very distinct, and on the apple itself there is a tendency to divergence. On the apple from the south the conidial pustule as a rule is large, containing much pseudo-parenchymatous tissue which protrudes from the surface (*fig. 12*); while the northern form produces a pustule with little pseudo-parenchymatous tissue, similar to that illustrated from the tomato (*fig. 11*). This distinction does not always separate the two forms, as they often intergrade, but the characters are noticeable in studying the two. Furthermore, the southern form produces perithecia in abundance; while the northern form, so far as is known, never produces them. If we call these two forms the same, we might as well group all of the forms of Gloeosporium into one species and say that Glomerella is monotypic, growing on nearly everything. But if we call them distinct, what shall we name the forms? The names *rufomaculans* and *fructigenum* were given by BERKELEY (**6, 7**) to forms in England on grape and apple. These were found in a latitude comparable to our northern states, and consequently we should think that BERKELEY had a form comparable to our northern one. If this is true, the perfect stage of the true *Gloeosporium rufomaculans* has never been found. This northern form is known to occur on several other hosts, not entirely fruits, besides the apple. The anthracnose of tomatoes has generally been called *Gloeosporium phomoides*, and under this name it has been discussed by several authors. But the culture obtained by me was absolutely identical with the northern form on apple; although the the culture obtained by Miss STONEMAN differed somewhat from her culture from apple. GUEGUEN (**15**) in working on the true *G. phomoides* takes exception to Miss STONEMAN'S conception of the species. It seems that *G. phomoides* is an entirely different thing, and does not belong to the Glomerella type of anthracnoses; hence the name can no longer be used for the anthracnose commonly seen on tomatoes.

The forms from grape stems, raspberry stems, and Podophyllum fruit are probably only strains of the northern form on apple. At least they will be considered so at present.

While the form from bean has not been studied so much as the others as to its cultural characters, it seems to be distinct. Neither this, nor the form on melons, will grow on acid fruit like the apple. It would have been interesting if SHEAR had given the cultural characters of the form from bean from which he obtained the perfect stage. It should have been compared with the characters of the form from apples in the south. It is possible that the form from apple might develop occasionally on the bean. Even if he did have the true bean anthracnose and it developed a perithecium identical with the apple bitter rot, the habit of growth of the bean anthracnose is sufficiently different to continue calling it a distinct species.

From the evidence available at present, it does not seem advisable to call all these forms the same species. They may finally be placed in one, or at least in a limited number of species, but until the evidence is more certain, it seems best to consider some of them at least distinct. If we use the name first applied to the perfect stage, and we must do this to be consistent with the use of the name *Gnomonia veneta* used earlier in this paper, the names of the perfect stages found by the writer and brought to maturity would be as follows:

GLOMERELLA CINCTA (Stoneman) Sp. & v. Schr., including forms from orchid, *Sarracenia purpurea*, Dracaena, rubber plant, and Anthurium.

GLOMERELLA FRUCTIGENA (Clinton) Sacc. on apple and quince.

GLOMERELLA FUSARIOIDES, n. sp.[6] from *Asclepias syriaca*.

SHELDON (**38**) has recently described the form from Dracaena as a new species, calling it *Physalospora dracaenae*, but from my cultural work it cannot be considered distinct from the form on orchid, the perfect stage of which has already been described by Miss STONEMAN (**43**).

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[6] **Glomerella fusarioides,** n. sp. Perithecia nearly free, abundant on the surface of the substratum but more or less scattered, dark brown to black, sub-globose to pyriform, sometimes prolonged into a short beak at the apex, 150–200×140–175 μ. Asci numerous, clavate, 50–75×9–11 μ. Spores irregularly biseriate, straight or slightly curved, 12–18×3–4 μ. Many sterile threads in the perithecium and apparently outside of the asci.

Perfect stage of *Gloeosporium fusarioides* E. & K., which preceded it on the same stems. Stems of *Asclepias syriaca*, October 1907, Ithaca, N. Y.

SUMMARY

In closing the discussion of the Glomerella type, it may be well to summarize briefly the points that have been considered.

1. The Glomerella type is distinctly separated from the other types of anthracnoses by both perfect and imperfect stages.

2. The perfect stage seems to be distinct from nearly related genera of the Pyrenomycetes, is extremely variable, and without true paraphyses.

3. There seems to be a large number of closely related forms, and they are all extremely variable. Furthermore, many forms vary under artificial cultivation and doubtless under natural conditions. While many of the forms may be similar enough to be considered the same, some seem distinct enough to be considered distinct species; at least the evidence is not sufficient to consider them identical.

4. There seem to be two forms on the apple, the forms separated by thermal lines. The form in the southern part of the country differs in the presence of perithecia, a slightly different acervulus, and entirely different cultural characters.

5. From the investigation it seems that too much dependence should not be put on cultural characters for the determination of species. Some of the characters when well marked are useful, but many of the others are too variable.

In closing I wish to express my indebtedness to Professor ATKINSON for aid and criticisms during the progress of the study, and to Professor T. J. BURRILL, Dr. J. L. SHELDON, Dr. H. HASSELBRING, P. H. ROLFS, and Dr. ERNST BESSEY for material for study.

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EXPLANATION OF PLATE XI

Figs. 18–28. Gnomonia veneta

FIGS. 18, 19, 20.—Ripe asci, young ascus, and ascospores from sycamore leaf.

FIGS. 21, 22, 26.—Asci, ascospores, and germinating ascospores from *Quercus alba* leaves.

FIGS. 23, 24, 25.—Asci, ascospores and germinating ascospore from *Quercus velutina* leaves.

FIG. 27.—Conidia from sycamore leaf germinating.

FIG. 28.—Conidia from *Quercus alba* leaf germinating.

Figs. 29–47. Glomerella type of anthracnose

FIG. 29.—*Glomerella fructigena* on apple from Missouri, binucleate conidium.

FIG. 30.—Same, conidia germinating in bean agar.

FIG. 31.—Same, secondary spores formed in old cultures.

FIG. 32.—Same, secondary spores formed by germinating conidia.

FIG. 33.—Gloeosporium from apple from New York, secondary spores formed by germinating conidia.

FIG. 34.—*Colletotrichum gossypium* from cotton; large cells formed in Elfving's nutrient solution; one of these cells germinating, with a young spore at the end of the germ tube.

FIG. 35.—Conidia from quince, germinating in bean agar.

FIG. 36.—Conidia from tomato germinating in bean agar.

FIG. 37.—Conidia from apple from New York, germinating in bean agar.

FIG. 38.—Conidia from *Coffea arabica*, germinating in bean agar.

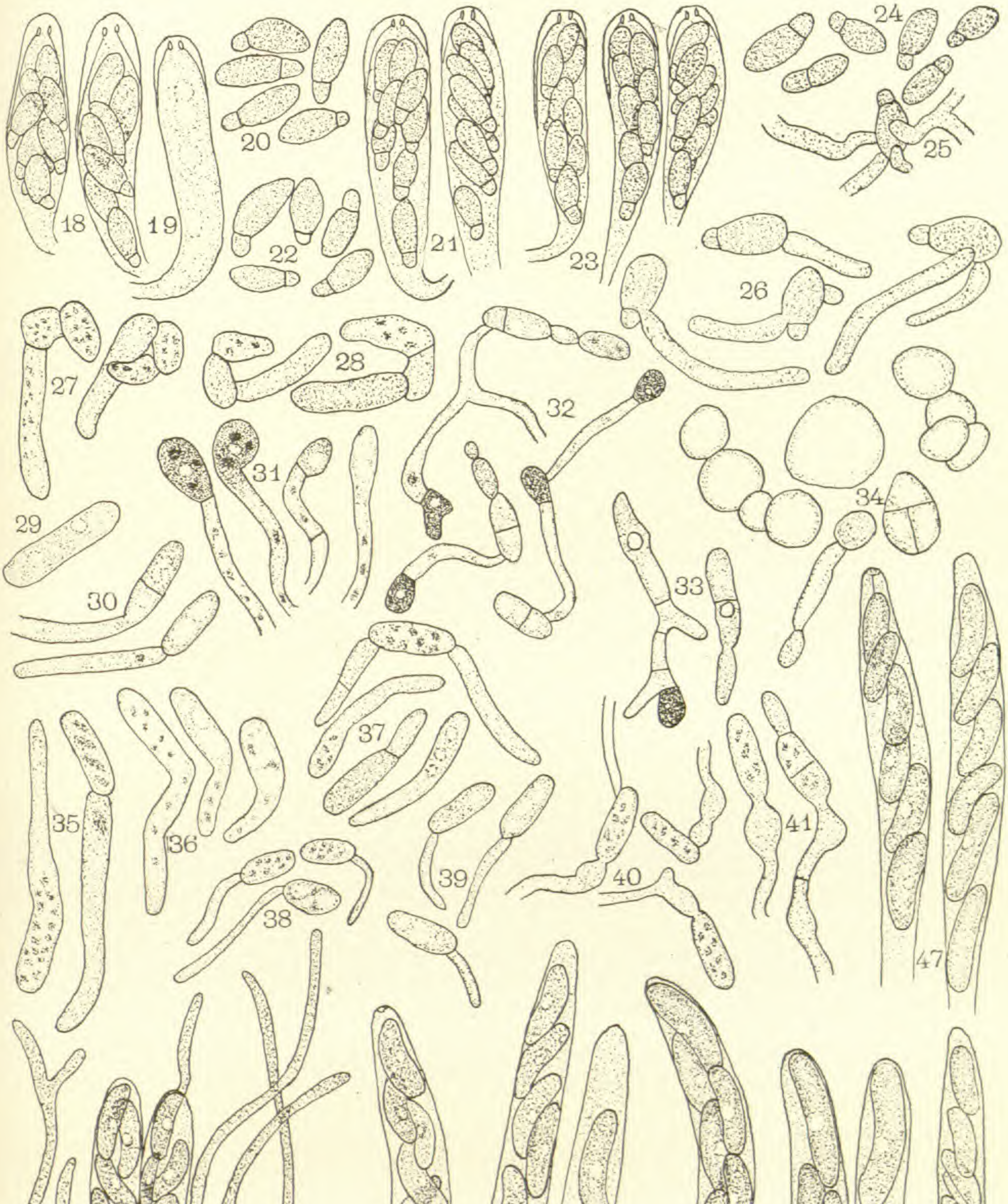
FIG. 39.—Conidia from *Sarracenia purpurea* germinating in bean agar.

FIG. 40.—Same, older stage.

FIG. 41.—Conidia from orchid germinating in bean agar.

FIG. 42, 43.—Asci and periphyses from Dracaena.

FIG. 44–47.—Asci, respectively from leaves of *Ficus elastica*, *Sarracenia purpurea*, quince, *Coffea arabica*.



EDGERTON on ANTHRACNOSES

BRIEFER ARTICLES

ON THE CONTENTS OF THE POLLEN CHAMBER OF A SPECIMEN OF *LAGENOSTOMA OVOIDES*

(WITH TWO FIGURES)

Lagenostoma ovoides is one of the commonest of the ovules occurring in the calcite nodules of the British Coal-measures. It is only specifically distinct from *L. Lomaxi*, which has been attributed by OLIVER and SCOTT[1] to *Lyginodendron Oldhamium*. It appears therefore probable that it belongs to one species of the wide group included under that name, and thus is the ovule of a pteridosperm.

In an oblique section through the micropyle and pollen chamber of a specimen of this ovule, sent me some years ago by Mr. LOMAX, I detected, besides the pollen grains which he recorded, several naked protoplasmic bodies of very characteristic form. One of these bodies is within one of the pollen grains, two are free and entire, and one is cut across. Protruding from three of the grains, which show a considerably ruptured epispore, are bladder-like structures which are clearly surrounded by the endospore. Thus I interpreted the grains as germinating, and (as I mentioned in a paper[2] I was writing at the time) "apparently in the very act of yielding antherozoids like those of Cycas and Ginkgo" (p. 168).

The slide was last year exhibited at the Linnaean Society's rooms, London, on the occasion of the celebration of the bicentenary of the birth of LINNAEUS, and my interpretation having met with general acceptance, I have decided that it should be figured. The many valuable contributions to our knowledge of spermatogenesis (including the recent work on Microcycas by CALDWELL[3]) that have appeared in the BOTANICAL GAZETTE, have induced me to send the account of this remarkable fossil to the same journal.

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[1] OLIVER, F. W., and SCOTT, D. H., On the structure of the palaeozoic seed *Lagenostoma Lomaxi*. Phil. Trans. Roy. Soc. London B **197**:193–247. *pls. 4–10.* 1904.

[2] BENSON, MARGARET, *Telangium Scotti*, a new species of Telangium (Calymmatotheca) showing structure. Annals of Botany **18**:161–177. *pl. 11.* 1904.

[3] CALDWELL, OTIS W., *Microcycas calocoma*. BOT. GAZETTE **44**:118–141. *figs. 14.* 1907.

The pollen grains in their enlarged state, owing to germination, measure $70\times 50\ \mu$.[4] The walls are reticulately thickened and the thinner areolae have in many cases given way and become perforations. The protruding endospore can be seen in two cases to contain cells (*fig. 2, t*), but their nature is difficult to determine. They are probably of the same nature as the cells that can be seen attached to one of the free antherozoids (*fig. 2,* t^1).

In the absence of any evidence as to their nature, I think it best to suggest that they are possibly of fungal origin, but, on the other hand, they bear a remarkable resemblance to the tissue observed by RENAULT in the pollen grains of Cordaites. If we had a case in which each of these cells gave rise to an antherozoid, it would then be obvious that they were gametophytic. The central body in each cell has the appearance of a nucleus, but is possibly merely the carbonized remains of the whole protoplast.

FIG. 1

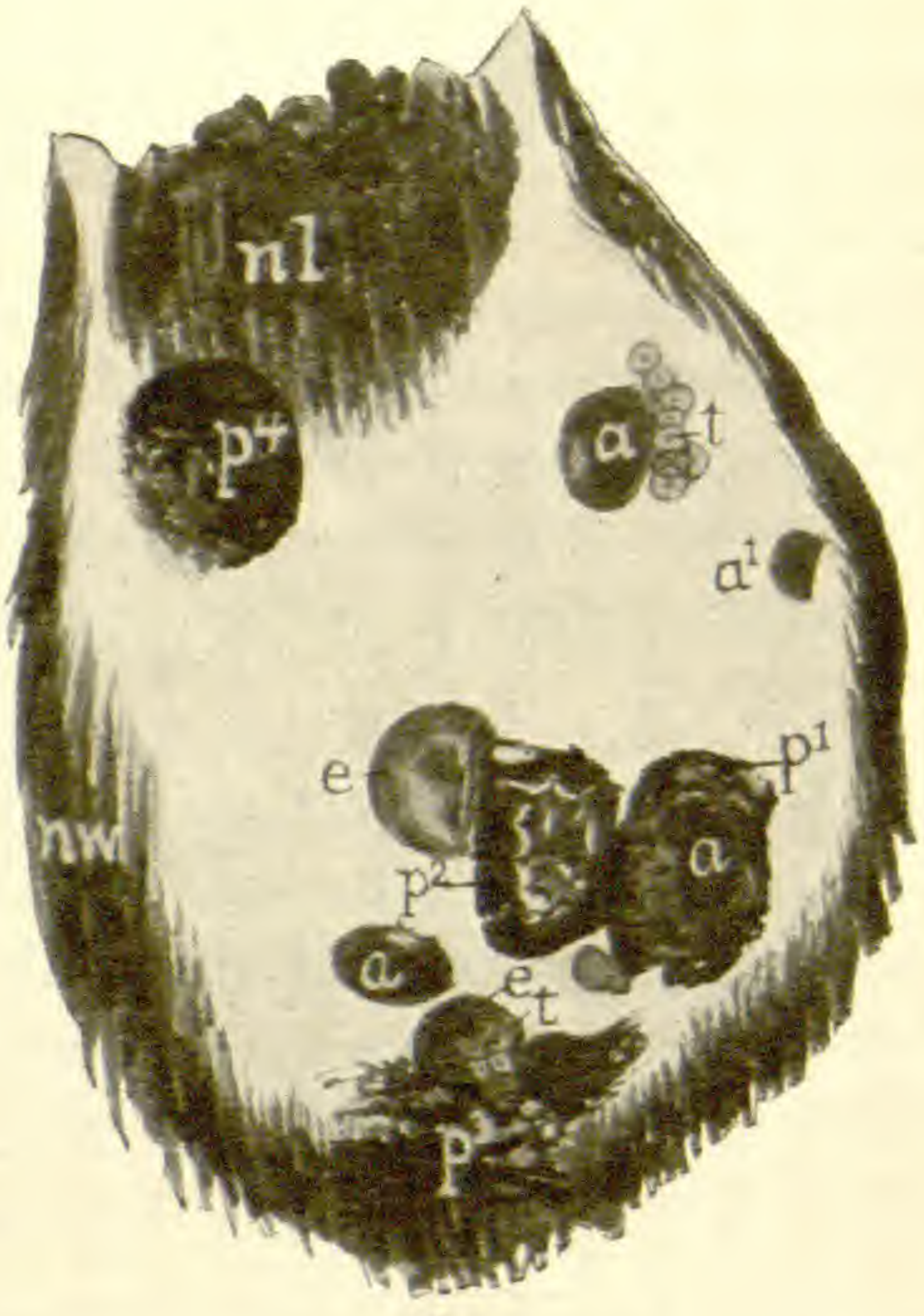

FIG. 2

FIG. 1.—An oblique section through the upper part of the ovule *Lagenostoma ovoides*, ×54; *pc*, pollen chamber; *c*, canopy or inner integument; *vb*, one of the vascular bundles of the integument.

FIG. 2.—The pollen chamber and its contents from the same ovule, ×232; *nl*, tongue of nucellar tissue which supports the pollen chamber; *nw*, nucellar wall of the pollen chamber; *a*, antherozoid; a^1, same cut across; *e*, endospore protrusion; p^1, p^2, p^3, p^4, four pollen grains; *t*, t^1, tissue which is possibly fungal.

Camera drawings from a slide in the R. H. College Collection, C. N. 63. Locality, Dulesgate.

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[4] By an oversight the dimensions were given ten times too small in BENSON, *loc. cit.*

The antherozoids measure 41μ across when free. They are thus about two-thirds the size of those of Microcycas, which appear from CALDWELL's drawings (*loc. cit.*) to measure 60μ across. Again, they are but one-sixth the size of those of Zamia as figured by WEBBER.[5]

That the antherozoids should be of smaller size than those of recent cycads is explicable when one considers the primitive nature of Lyginodendron and how many fern characteristics it retained. Moreover, there is considerable probability that the number of antherozoids produced by one pollen grain was not limited to two, although there is no evidence as to how many were produced. It is obvious, however, that this pteridosperm did not produce as many as CALDWELL has shown is the case with Microcycas.

Turning now to the figure (*fig. 2*), we see that the pollen chamber is bounded by a thin wall of elongated cells of nucellar origin (*nw*), and that the tongue of nucellar tissue characteristic of the Lagenostoma type of ovule is cut obliquely at *nl*. Within the space thus delimited lie the remains of four pollen grains (p^1, p^2, p^3, p^4). Of these p^1 still contains one antherozoid, and shows but little protrusion of endospore. The grain p^2 is empty; its epispore shows thin areolae, and at *e* the endospore has protruded and shows very faintly one hexagonal cell still preserved near its base. The grain p^3, at the bottom of the exposed part of the pollen chamber, shows clearly five hexagonal cells (*t*), each with a central black spot. The grain has been injured and its contents cannot be determined. Between the grains p^2 and p^3 lies one of the bodies I interpret as antherozoids. The hyaline area of this body appears to correspond with the apex of the coil, but cilia cannot be demonstrated.

A similar body appears on the right near the upper part of the pollen chamber, and this also shows a hyaline area. Accompanying this gamete are the problematical cells which apparently form a continuous tissue and therefore are not chytridiaceous. There are no fungal hyphae connected with them and each cell contains a minute central body. Not far off lies part of a fourth antherozoid (*fig. 2*, a^1), which has evidently been cut across in the process of making the slide.

CONCLUSION

That such bodies as pteridospermous antherozoids should be preserved in the calcite nodules of the Carboniferous rocks will not appear incredible to those who are familiar with the results of palaeobotanical research. In

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[5] WEBBER, H. J., Spermatogenesis and fecundation of Zamia. U. S. Dep. Agric., Bur. Pl. Ind., Bull. 2. pp. 100. *pls. 7.* 1901.

the silicified form we have had many records of archegonia from BRONGNIART[6] and RENAULT.[7] The latter devoted considerable time and attention to palaeozoic bacteria, and was the first to record tissue formation in a pollen grain. In calcified material archegonia are found preserved in the megaspores of the Lepidodendreae, and recently SCOTT[8] has recorded the early stages in the germination of fern spores. Still more recently I have had occasion to record a very early stage in the development of the embryo sac in the young megasporangium of Miadesmia.[9] But even with instances such as these, we should not have ventured to identify these antherozoids if it had not been for the antecedent discovery in Ginkgo and the cycads of this type of male gamete. As students of phylogeny, we may well congratulate ourselves that such forms as *Ginkgo biloba* and *Microcycas calocoma* persist to the present time to interpret to us the elaborate pollen chambers of the palaeozoic era. In their turn these paleozoic structures are now giving us some evidence of the great antiquity of the cycad type of male gamete.—MARGARET BENSON, *Royal Holloway College, London University.*

THE EMBRYO OF CERATOZAMIA: A PHYSIOLOGICAL STUDY

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 111

(WITH SEVEN FIGURES)

Ceratozamia is a genus of cycads of the American tropics. Its habit and habitat and the manner in which the ovules are shed shortly after fertilization are described by CHAMBERLAIN (1) in his preliminary note to the study of the reproductive structures.

The embryo has only one cotyledon. This fact was observed by VAN TIEGHEM (2) as early as 1873 in a form which he considered a hybrid between *C. longifolia* and *C. mexicana*, but which was probably pure *C. longifolia*. In 1878 WARMING (3) recorded the monocotyledonous condition of the embryo of *C. mexicana*, adding that the cotyledon arises at one side of the hypocotyl axis and little by little comes to surround it.

Being engaged in an anatomical study of the seedling, the first observation I made was naturally upon this character. In every case in over one

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[6] BRONGNIART, A., Recherches sur les graines fossiles silicifies. Paris. 1881.

[7] RENAULT, B., Flore fossile d'Autun. Paris. 1896.

[8] SCOTT, D. H., The occurrence of germinating spores in *Stauropteris Oldhamia*. New Phytol. 5:170–172. 1906.

[9] BENSON, MARGARET, *Miadesmia membranacea*, a new paleozoic lycopod with a seed-like structure. Phil. Trans. Roy. Soc. London B 199:409–425. *pls. 33–37*. 1908.

hundred ovules germinated in 1906, the embryos bore only one cotyledon (*figs. 1, 2, 3*).

When the anatomical study was completed, a preliminary note was received from MATTE (4), who had germinated some seeds provided by CHAMBERLAIN and had begun the study, not knowing that the work was being carried on in this laboratory. MATTE reports that the embryo is monocotyledonous. CHAMBERLAIN (14) in his recent report on Ceratozamia, read before the Chicago meeting of the Botanical Society of America, makes the same statement.

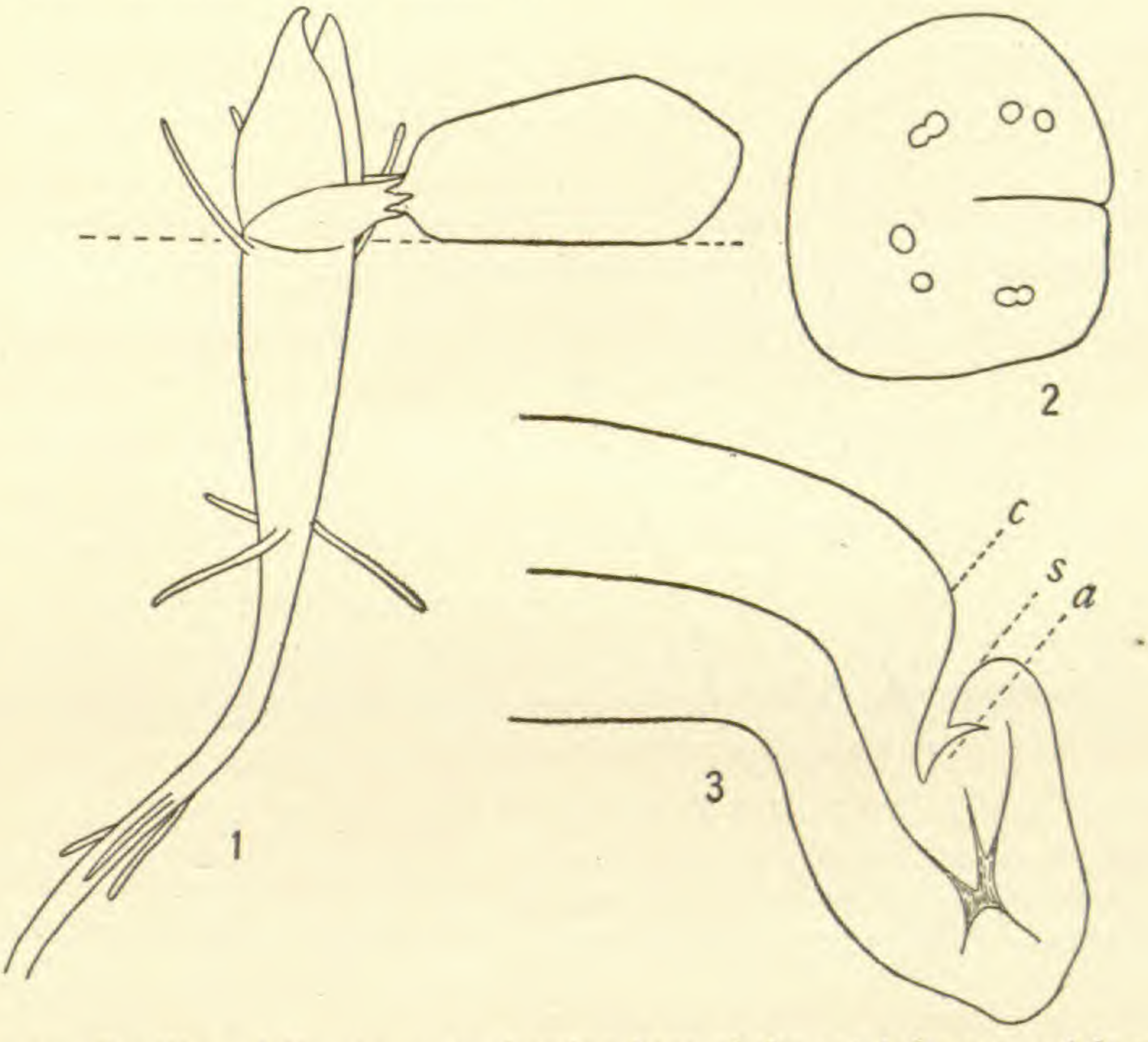
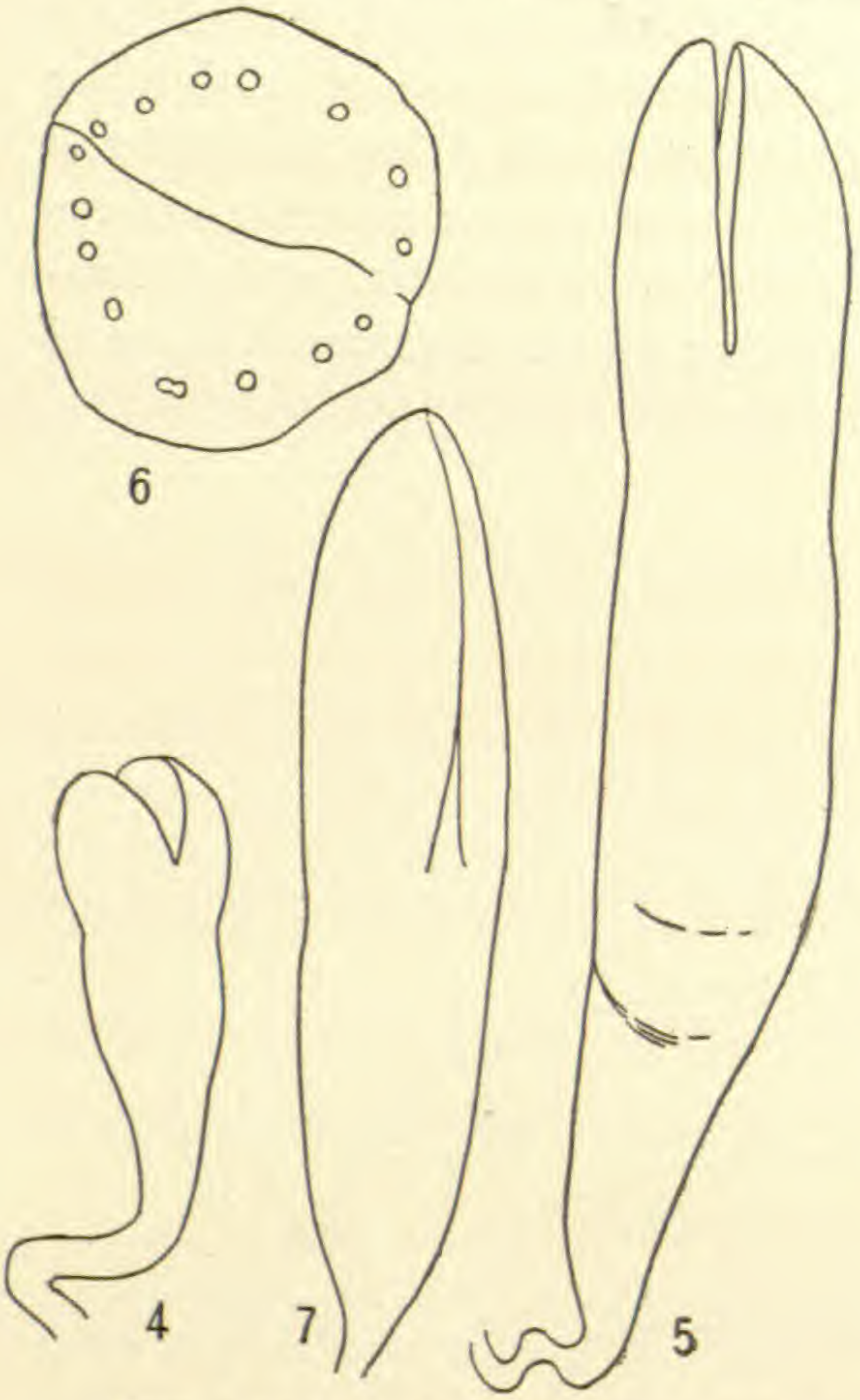


FIG. 1.—Seedling of Ceratozamia showing base of the cotyledonary petiole encircling the stem. Natural size.

FIG. 2.—Cross-section of middle region of cotyledon. ×8.

FIG. 3.—Longitudinal section of seedling in the emergent stage: *c*, cotyledon; *s*, scale; *a*, growing point of stem. ×8.

In other cycad embryos, the cotyledons are often reported as unequal in size. TREUB (5) observed and figured the inequality in *Cycas circinalis.* WORSDELL (6) described a seedling of *Cycas revoluta* in which one cotyledon was considerably shorter than the other. MATTE (7) reports a case in *Dioon edule* in which the vascular strands in the smaller cotyledon are reduced in number, only the rudiments of the missing ones occurring at the base of the cotyledon. He also mentions an inequality in the size of the cotyledons of *Encephalartos Barteri*. BOWER (**8**) recorded a difference in the size and anatomy of the two cotyledons of *Cycas Seemanni;* and LAND (Chicago) has noted (unpublished) a difference in the size of the cotyledons of *Zamia integrifolia*.



FIGS. 4 and 5.—Embryos developed on clinostat; removed February 3. ×8.

FIG. 6.—Cross-section of middle region of cotyledons of an embryo removed from clinostat February 26. ×8.

FIG. 7.—Embryo taken from soil in green house January 8. ×8.

Outside the cycads one finds the same tendency in seeds of similar habit. SOLMS-LAUBACH (**9**) figured it in an embryo of *Bennettites Gibsoniana*, a fossil relative of the cycads; and COULTER and CHAMBERLAIN (**10**) and LYON (**11**) have recorded it of Ginkgo.

In the course of my anatomical study, I noticed that the cotyledon was a lateral structure and that there was absolutely no external trace of another (*fig. 3*), but certain irregularities in the vascular system made me suspect that the second cotyledon had been suppressed. These irregularities consisted mainly in the suppression of one pole of the root, and the presence of certain rudimentary strands which joined the stem in normal fashion, but whose upper portions ended blindly in the cortex or fused with other bundles. The suspicion was strengthened by the observation that in every case the cotyledon developed on the under side as the seed lay. This latter circumstance led me to think that gravity was responsible for the monocotyledonous condition.

In the following autumn, therefore, fresh ovules were placed in broad pots, covered closely with sphagnum, and placed upon clinostats revolving at different rates of speed. In December the LIVINGSTON porous cup apparatus (**12**) was inserted in order to equalize the soil moisture. At the same time fresh seeds were planted in the greenhouse.

The apparently simple plan of planting the seeds end down was not adopted for two reasons. First, this would not give accurate results, because such a seed as that of Ceratozamia could not be placed and kept in an exactly vertical position, and there is no doubt that a variation from the vertical of only a few degrees is sufficient to bring an organ under the direct influence of gravity. Second, at the time of formation of the growing points, the embryo has been pushed half-way through the massive endosperm in a tortuous and winding path by the elongating suspensor, and it is probable that just at the time when it comes to rest, gravity will determine its position whether the seed be vertical or horizontal.

Rotation on the clinostat seems to have retarded development to a marked degree, but has not otherwise interfered with it. Early in February, the embryos developing on the clinostat were found to have two equal cotyledons (*figs. 4, 5, 6*), while those developing under normal conditions have only one (*fig. 7*). Further, the dicotyledonous embryos have a tetrarch stele in the root and the cotyledonary vascular system is of the usual cycad type.

It can scarcely be doubted that gravity is the main factor in bringing about the inequality reported in the cotyledons of other gymnosperms, and it may be that further experiment will throw some light upon the seeming suppression of root poles. It is possible also that it is to this cause we may attribute not only the monocotyledonous condition long known to exist in some dicotyledons, mentioned by COULTER and CHAMBERLAIN (**13**); but even the condition of certain true monocotyledons whose embryos from their very inception hold a practically unchanged one-sided relation to gravity.

The experimental work is under the direction of Professor CHARLES R. BARNES and Dr. WILLIAM CROCKER.—HELEN A. DORETY, *The University of Chicago.*

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2. VAN TIEGHEM, PH., Symétrie de structure des plantes. Ann. Sci. Nat. Bot. V. **13**:204. 1873.
3. WARMING, E., Ein Paar nachträgliche Notizen über die Entwicklung der Cycadeen. Bot. Zeit. **36**:737. 1878.
4. MATTE, HENRI, Note préliminaire sur des germinations des Cycadacées. Rennes. 1907.
5. TREUB, M., Embryogénie von *Cycas circinalis*. Ann. Jard. Bot. Buit. **4**:1. 1884.

6. WORSDELL, W. C., Comparative anatomy of the Cycadaceae. Jour. Linn. Soc. Lond. **33**:439. 1898.

7. MATTE, HENRI, Recherches sur l'appareil libero-ligneux des Cycadacées. Caen. 1904.

8. BOWER, F. O., Comparative morphology of the leaf of the vascular cryptogams and gymnosperms. Proc. Roy. Soc. Lond. B **175**:583. 1884.

9. SOLMS-LAUBACH, H., Fossil Botany, p. 96. Eng. ed. Oxford. 1891.

10. COULTER, JOHN M. and CHAMBERLAIN, C. J., Morphology of gymnosperms 48. 1901.

11. LYON, H., Embryogeny of Ginkgo. Minn. Bot. Studies. 1904.

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13. COULTER, J. M., and CHAMBERLAIN, C. J., Morphology of angiosperms 206. 1903.

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CURRENT LITERATURE

BOOK REVIEWS

North American gymnosperms

Professor PENHALLOW has written a very painstaking and a unique book[1] on gymnosperms. He has taken the North American species (exclusive of cycads), living and fossil, and has constructed a system of classification based upon the anatomy of the mature wood. The original plan had in view the identification of material used for structural purposes; but the investigation soon included the needs of paleobotany, and finally led to suggestions as to phylogeny. The final result includes detailed studies of 92 North American species, 21 Japanese, and 4 Australasian. The enormous mass of detail to be sifted and organized before any satisfactory differential characters based upon wood structure could be made evident can be imagined.

The book is divided into two parts, the first entitled "Anatomy," the second "Systematic." Under anatomy (192 pp.) a detailed account of the regions and elements of mature gymnosperm wood is given, including discussions of durability, decay, and general phylogeny. In the systematic part (157 pp.) a synopsis of the genera and species of Cordaitales, Ginkgoales, and Coniferales is presented, based entirely on wood characters, and including so far as possible the economic values.

Probably the most interesting portion of the book to the general botanist is the chapter devoted to phylogeny. The author has given an excellent illustration of the result of following one set of characters in reaching conclusions as to phylogeny. Morphologists and paleobotanists and anatomists of a different sort have done this before, and with results equally divergent. The morphologists have been checked more frequently than the others, perhaps, for they have been compelled to carry through many different structures that insist upon contradicting one another in reference to any suggested phylogenetic connection. In these latter days, however, morphology, anatomy, and paleobotany are beginning to combine their results in any conclusion as to phylogeny, and the contradictions are multiplying. Professor PENHALLOW has introduced another set of checks, which must either be incorporated in any general conclusions or explained away. He recognizes clearly that his scheme of phylogeny cannot stand, a statement which is true of any scheme based upon one set of characters; but he presents it to be combined with the others in the general result. When different schemes flatly

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[1] PENHALLOW, D. P., A manual of the North American gymnosperms, exclusive of the Cycadales but together with certain exotic species. 8vo. pp. viii+374. *pls. 55.* Boston: Ginn & Co. 1907.

contradict one another, it remains to discover the characters that should control, and this becomes a matter of opinion rather than of demonstration.

On the basis of wood anatomy, as interpreted by PENHALLOW, a phylogeny of the gymnosperms is constructed that certainly contradicts many of the conclusions that have been drawn from the other methods of attack. From the Cycadofilices two great divergent lines arise, one being the Cycadophytes, the other the general conifer-like plexus; this view seems reasonable enough. The conifer-like forms are thought to have been derived through such forms as Poroxylon, which in turn forked into Cordaitales and another branch which forked into Ginkgoales and Coniferales. It is interesting to note that the araucarians are made a branch of the Cordaitales stock. The Taxaceae (taxads and podocarps) are made the earliest side branch of the Coniferales stock; then come the Taxodineae, the Cupressineae, and finally the Abietineae, whose most modern and specialized expression is Pinus. Just how this phylogeny can be related to that of Jeffrey, for example, to say nothing of the phylogeny of modern morphology, is not clear. Either mature wood structure is not significant in phylogeny, or there will have to be an extensive revision of our former conclusions.

At all events, it is a good thing to have these facts before us; and the book will certainly be highly useful in its practical applications and also as a great assistance in the recognition of fossil material.—J. M. C.

NOTES FOR STUDENTS

Items of taxonomic interest.—W. N. CLUTE (Fern Bull. **16**: 1, 2. 1908) has described a new Asplenium from Arizona.—C. B. ROBINSON (Bull. Torr. Bot. Club **35**:63–75. 1908) has begun a series of contributions bearing the title "Alabastra philippinensis," the first one containing descriptions of 12 new species.—P. C. STANDLEY (*idem* 77–78) discusses certain New Mexican forms of Echinocereus and describes a new species.—H. D. HOUSE (*idem* 89–90) has described 2 new species of Evolvulus from the Bahama Islands.—E. GILG (Bot. Jahrb. **40**:444–518. 1908), in an account of the African Flacourtiaceae, describes numerous new species and the following new genera: *Trichostephanus*, *Marquesia*, and *Ophiobotrys*.—A. ENGLER (*idem* 519–572), among numerous new species of African plants, describes species under Gnetum, Loranthus (30), Viscum (7), and Garcinia (22), and a new genus (*Acanthotreculia*) of Moraceae.—B. L. ROBINSON (Rhodora **10**:29–35. 1908) has revised the nomenclature of a number of plants of the northeastern United States, chiefly of minor varieties and forms, and includes the description of a new Woodsia from Minnesota and Michigan.—G. HIERONYMUS (Hedwigia **47**:204–224. *pls. 1–4*. 1908), in an account of the pteridophytes of STÜBEL'S South American collections, describes new species under Diplazium (8) and Asplenium.—R. KELLER (Bull. Herb. Boiss. II. **8**:175–191. 1908), in a revision of Hypericum § Brathys, recognizes 21 species, 6 of which (from South America and Mexico) are described as new.—L. L. CAPITAINE (*idem* 251–253) has described a new genus (*Hyalocalyx*) of Turneraceae from Madagascar.—

M. L. Fernald (Rhodora **10**:46–55. 1908), in notes on some plants of northeastern America, publishes a number of new combinations, forms, and varieties, and also new species under Potamogeton, Fragaria, Callitriche, and Vaccinium.—A. Finet (Bull. Soc. Bot. France IV. **7**:531–538. 1908) has published a new genus (*Pseudoliparis*) of Orchidaceae from China, and also (*idem*, Mémoires, pp. 65. *pls. 12*) an account of African orchids of the tribe Sarcantheae, including many new species and the new genera *Monixus*, *Rhaphidorhynchus*, *Ancistrorhynchus*, and *Dicranotaenia*.—F. Gagnepain (*idem* 538–544) has described two new genera (*Oligolobos* and *Xystrolobos*) of Hydrocharitaceae from China.—T. Makino (Bot. Mag. Tokyo **22**:14–20. 1908) has described the new genera *Alectorurus* (Liliaceae) and *Tubocapsicum* (Solanaceae) from Japan.—S. Okamura (*idem* 29–31, 41–44. *pls. 2, 3*) has published two new genera (*Orthomniopsis* and *Okamuraea* Broth.) of mosses from Japan.—Marcus E. Jones (Contrib. Western Bot. No. 12. pp. 100. 1908) has described numerous (96) new species of western plants, including *Scopulophila* (Illecebraceae) and *Cusickia* (Umbelliferae) as new genera; and has critically reviewed the western Umbelliferae, adopting Cogswellia to replace Peucedanum.—W. R. Maxon (Contrib. U. S. Herb. **10**:473–503. *pls. 55, 56*. 1908), in the first number of a series of studies of tropical American ferns, has described as new genera *Holodictyum* (an "asplenioid fern") and *Ananthacorus* (allied to Vittaria), and several new species.—J. M. Greenman (Field Columb. Mus. Publ. Bot. **2**:247–287. 1908), in a paper on new or noteworthy spermatophytes from Mexico, Central America, and the West Indies, has described new species under numerous genera, 25 species of Senecio being new or fully characterized for the first time, and *Goldmania* (Coreopsideae) being a new genus.—D. Prain (Kew Bull. **1908**:114) has described a new genus (*Acrymia*) of Labiatae from Malaya.—J. M. C.

Diseases of apples.—Burrill[2] and Blair[3] have published two bulletins presenting what may be considered a summary of their extensive investigations of the bitter rot of apples, both as to its botanical relations and methods of control and eradication by removal of the cankers and proper spraying methods. Few orchard diseases have received such attention, and every orchardist and plant pathologist should read these two publications, which are too extensive to be summarized here.

Stevens and Hall[4] have recently published a description of several apple diseases. The volutella rot of the fruit is due to *Volutella fructi* Stevens and Hall. *Coniothyrium fuckelii* Sacc. is recorded as causing a fruit rot and also producing

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[2] Burrill, T. J., Bitter rot of apples. Botanical investigations. Bull. Ill. Exp. Stat. **118**:553–608. *pls. 10*. 1907.

[3] Blair, J. C., Bitter rot of apples. Horticultural investigations. Bull. Ill. Exp. Stat. **117**:481–552. *pls. 2*. 1907.

[4] Stevens, F. L., and Hall, J. G., Some apple diseases. Bull. N. Car. Exp. Stat. **196**:37–55. *figs. 5*. 1907.

cankers on the twigs. Twig cankers due to the black rot fungus (Sphaeropsis) is also reported as being frequent. Apple scurf, a twig disease, is described as due to Phyllosticta or *Phoma prunicola*.—E. MEAD WILCOX.

The roots of Lycopodium Selago.—Miss SAXELBY[5] has studied the origin of the roots of *Lycopodium Selago*, working with young plants grown from bulbils. She reports that the roots arise near the apex of the stem, but below the first leaves, and grow down through the cortex of the stem, emerging at the level of the ground. It is probable that "origin below the first leaves" is too sweeping a statement, for in preparations made by the reviewer from the same species the roots usually arise higher up than the first leaf. Miss SAXELBY finds that the dermatogen of the root arises from several cells of the innermost layer of the stem periblem; while the periblem and plerome arise from the plerome of the stem. It is interesting to note that the author finds three meristematic regions: plerome, periblem, and a dermatogen which forms both epidermis and root cap. The roots are usually diarch, with the metaxylem in the form of a horseshoe; but they may be tetrarch, with the metaxylem in two parallel bands; or there may be a transition between the two conditions.—ALMA G. STOKEY.

Germination of Fucus.—KNIEP finds ninety pages barely sufficient to relate and discuss the observations of three and a half months, at Bergen, on the physiology of fertilization and germination in *Fucus serratus*, *F. vesiculosus*, and *F. spiralis*.[6] After a serious attempt it appears impracticable for the reviewer to discover in this voluminous paper the author's results and conclusions, for he does not make clear the outcome of his work, nor anywhere give so much as a line by way of summary regarding a single topic. His observations were directed particularly to the influence of external factors on the gametes and sporelings. The main topics are the effect of concentration of the total salts in sea water upon the movement of sperms, fertilization, germination, and geographical distribution; the influence of temperature (brief); the directive and formative effects of light; certain phases of regeneration in sporelings; and finally the possible induction of polarity by chemical stimuli.—C. R. B.

Californian Hepaticae.—HUMPHREY publishes together a series of notes on the physiology and morphology of certain Californian Hepaticae.[7] He reports that *Fossombronia longiseta*, *Fimbriaria californica*, *Aneura multifida*, *Anthoceros Pearsoni*, and *Porella Bolanderi* are infested commonly with fungi, parasitic in the first case, symbiotic in the second, and epiphytic in the last three. In *Fegatella conica* fertilization occurs in early spring, the spores pass the dry

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[5] SAXELBY, E. MARY, The origin of the roots in *Lycopodium Selago*. Annals of Botany **22**:21–33. *pl. 3.* 1908.

[6] KNIEP, H., Beiträge zur Keimungs-Physiologie und -Biologie von Fucus. Jahrb. Wiss. Bot. **44**:635–724. *figs. 12.* 1907.

[7] HUMPHREY, H. B., Studies in the physiology and morphology of some California Hepaticae. Proc. Wash. Acad. Sci. **10**:1–50. *pls. 1, 2.* 1908.

season in the tetrad stage, and mature early in January. Various adaptations to a dry season are noted. Hygrophilous species are unable to recover from desiccation, but both thallus and spores of xerophilous ones grow promptly even after complete artificial desiccation. The remaining studies are concerned with the germination of spores of various species, and the brief life of the green or thin-walled spores, as contrasted with those of certain xerophilous species which are known to be viable after two years.—C. R. B.

Florida strangling figs.—BESSEY[8] has studied the germination and habits of the two native figs of Florida (*F. aurea* and *F. populnea*). In dense hammocks *F. aurea* begins only as an epiphyte, but eventually all trace of this habit is lost; while in open places it is often independent from the first. This seems to be explained by the fact that the seeds germinate only in the light, and experiments were performed to prove this peculiar habit. Following germination, the usual gradual "strangling" of the host plant occurs. Roots are sent down along the host plant and established in the ground, and finally by anastomosing and growth they completely incase the host. Both species have two or more crops of fruit each year, and are pollinated by different species of Blastophaga. Both kinds of flowers occur in the same receptacle, and the differentiation of the flowers is not so extreme as in the commercial fig.—J. M. C.

Morphology of Asimina triloba.—HERMS[9] has discovered the following facts in a study of *Asimina triloba:* the ovule passes the winter in a stage preceding the differentiation of the archesporial cell; the megaspore tetrad is occasionally tetrahedral instead of linear; the embryo sac becomes remarkably elongated; the polar nuclei remain in contact for three weeks or more; the antipodals are very ephemeral; the first segmentation of the egg does not occur for three or four weeks after fertilization; the first division of the endosperm nucleus is accompanied by transverse wall formation, dividing the linear sac into two equal chambers, the transverse divisions continuing until the sac contains a linear series of about twelve cells, when longitudinal division begins at the antipodal end; the embryo is very small and little organized even in the seed.—J. M. C.

Light and germination of fern spores.—LIFE[10] has been experimenting with the effect of light of different intensities upon the germination of fern spores. The spores of *Alsophila australis* germinated about a year after collection, but the spores of other ferns germinated as soon as they were dry. Under ordinary conditions the spores do not germinate in darkness; and at higher temperature

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[8] BESSEY, ERNST A., The Florida strangling figs. Rept. Mo. Bot. Garden **1908:** 25:33. *pls. 1–9.*

[9] HERMS, WILLIAM B., Contribution to the life history of *Asimina triloba.* Ohio Naturalist **8:**211–217. *pls. 15, 16.* 1907.

[10] LIFE, A. C., Effect of light upon the germination of spores and the gametophyte of ferns. Ann. Rep. Mo. Bot. Gardens **19:**109–122. 1907.

than ordinary room temperature the spores of Alsophila and Aneimia could not be induced to germinate in darkness. Germination was best in light of medium intensity (about 0.075 sunlight). Weaker light induced filamentous or ribbon-like prothallia, while strong light induced heart-shaped prothallia. Strong light (intensity 0.3) cause the production of only archegonia in Alsophila, while in the other ferns both sex organs were produced. Weak light inhibits the production of archegonia and favors the production of antheridia.—J. M. C.

Poisonous action of salts.—BENECKE[11] mingles with a strong polemic against LOEW an account of experiments which go to show that various salts collectively harmless are individually poisonous to Spirogyra, but have their toxic action neutralized by calcium salts. The chlorids, nitrates, sulfates, and phosphates of sodium, potassium, magnesium, and iron are more or less poisonous. The cations Fe and Mg are more poisonous than K, itself more poisonous than Na; of the anions named the phosphate, sulfate, and nitrate are more poisonous than chlorion. The toxicity of both anions and cations may be removed or diminished by the addition of Ca ions.

These results are essentially those obtained by OSTERHOUT and DUGGAR, the chief difference being that BENECKE ascribes to Ca a special antitoxic property toward other salts.—C. R. B.

Respiration and pollination.—When the gynaecium begins its second phase of development, after pollination, the respiratory activity might be expected to differ from that of an unpollinated one, but no comparative determinations seem to have been made. This has now been done by Miss JEAN WHITE,[12] using the Bonnier-Mangin apparatus for gas analysis. With due precautions she finds this apparatus, as modified by AUBERT, entirely satisfactory. "The net result of the whole work is to show that pollination not only produces a rapid rise of the respiratory activity, but also affects the respiratory quotient," which is usually less than unity and larger in the pollinated than in the unpollinated gynaecium. The numerical data show such extraordinary differences, under presumably like conditions, as to raise serious question regarding the value of the observations. —C. R. B.

Embryology of Oxalis.—HAMMOND[13] has investigated *O. corniculata* and records that the single hypodermal archesporial cell does not give rise to any parietal tissue, but functions directly as the megaspore mother cell, which he calls the megaspore; that the antipodals disappear soon after fertilization; and that the basal cells of the suspensor form a very active haustorium.—J. M. C.

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[11] BENECKE, W., Ueber die Giftwirkung verschiedener Salze auf Spirogyra, und ihre Entgiftung durch Calciumsalze. Ber. Deutsch. Bot. Gesells. **25**:322–337. 1907.

[12] WHITE, JEAN, The influence of pollination on the respiratory activity of the gynaecium. Annals of Botany **21**:487–499. 1907.

[13] HAMMOND, HOWARD S., The embryology of *Oxalis corniculata*. Ohio Nat. **8**:261–264. *pl. 18*. 1908.

NEWS

Dr. H. O. Juel, formerly assistant professor, becomes professor of botany in the University of Upsala.

Mr. L. L. Burlingame, The University of Chicago, has been appointed instructor in botany in Stanford University.

Miss Alma G. Stokey, The University of Chicago, has been appointed instructor in botany in Mount Holyoke College.

Dr. Carlton C. Curtis, Columbia University, has been advanced from an instructorship in botany to an adjunct professorship.

Miss E. N. Thomas, University College, London, has been appointed head of the department of botany in Bedford College for Women.

Dr. Laetitia M. Snow (Chicago), State Normal School, Farmville, Virginia, has been appointed instructor in botany in Wellesley College.

Dr. F. D. Heald, University of Nebraska, has been elected to a professorship in botany in the University of Texas, to succeed Dr. W. L. Bray, who has gone to Syracuse University.

Mr. H. Migliorato has undertaken to prepare a systematic dictionary of plant pathology, and asks botanists to send their papers to him at the Botanical Institute, via Panisperna 89B, Rome.

Carl Warnstorf (Berlin-Friedenau) announces for sale his large collection of Sphagnum, containing about 30,000 specimens from all parts of the world, and also a very large number of annotations and memoranda.

The twenty-fifth year of the professorship of Dr. Karl Goebel at the University of Munich was celebrated on March 7. More than fifty botanists were present, and Professor Goebel received a number of valuable presents from his former students, and greetings from many universities.

The committee in charge of securing a botanical foundation in honor of Leo Errera announces that the necessary amount has been subscribed. The income will be used for the encouragement of research, and information may be obtained from Professor J. Massart, rue Albert de Latour 44, Brussels.

Herbert F. Roberts, Kansas State Agricultural College, has received a commission from the Kansas Experiment Station to inspect the wheat regions of Southern and Central Europe during the coming summer. He will visit Italy, Greece, the Balkan states, and Hungary, and also the notable plant-breeding stations.

DR. OSKAR BREFELD, having been compelled by serious trouble with his eyes to give up the editorship of *Cohn's Beiträge zur Biologie der Pflanzen*, the duty has been assumed by Professor FELIX ROSEN of the University of Breslau. The second and concluding part of the ninth volume has recently appeared after an interval of several years.

THE LARGE and valuable herbarium of the late Mr. A. P. MORGAN has been presented to the State University of Iowa by his widow. It includes his botanical library, and is undoubtedly the most complete collection in existence of the mycologic flora of the lower valley of the Ohio River, and must always remain of importance on account of the numerous types it contains.

THEODOR OSWALD WEIGEL (Leipzig) has issued the first number of a paper entitled *Herbarium*, which will be published bimonthly, and is to be devoted to the distribution of *exsiccata*. It will announce continuations of current collections, collections that are wanted, and those that are offered. The publisher feels that this handling of dried plants in addition to botanical books will prove of advantage to many botanists and botanical establishments.

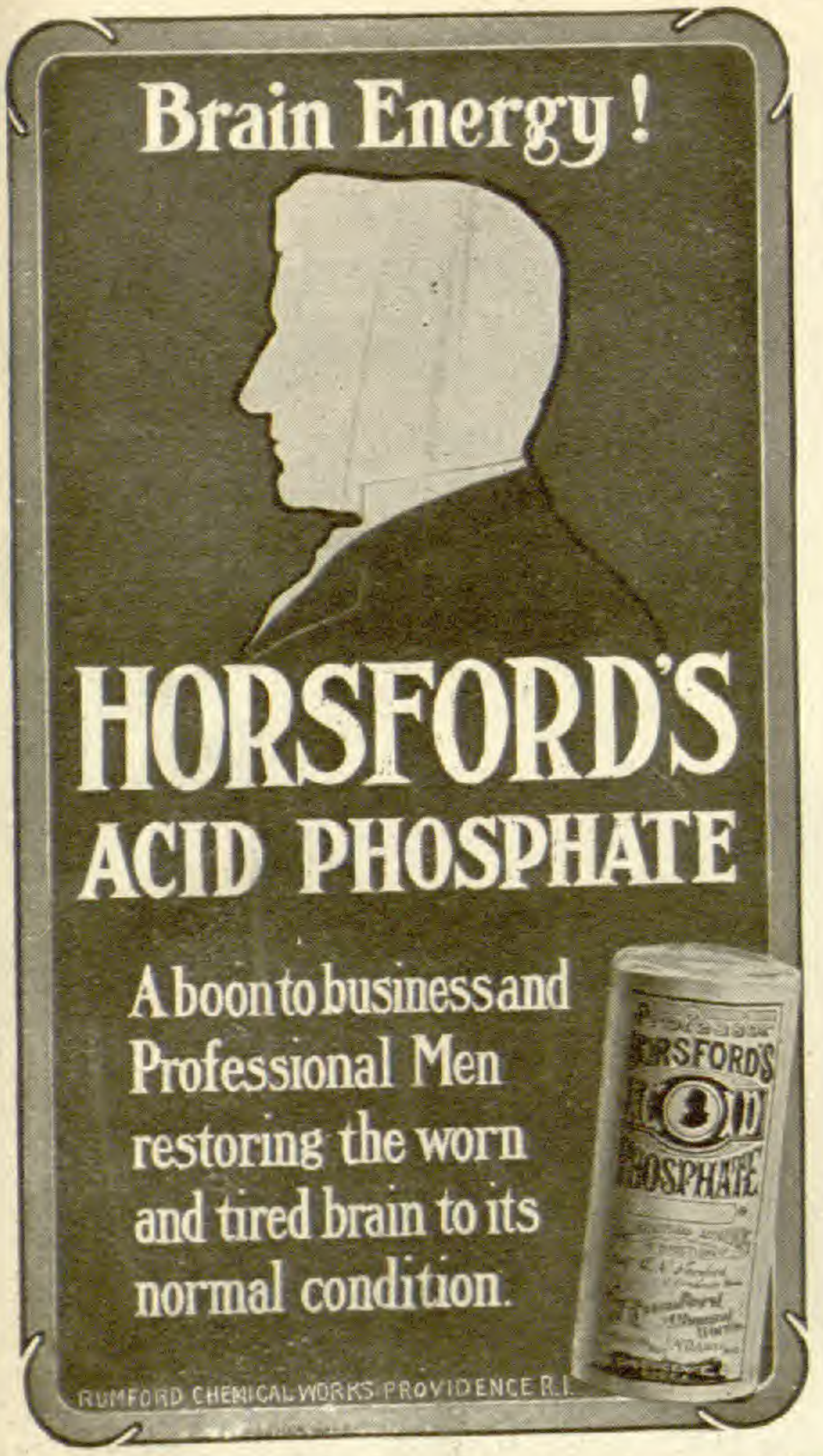
The following summer laboratories, in addition to those already noticed, announce botanical instruction and research: Marine Biological Laboratory, Woods Hole, Mass., July 1 to August 11 (GEORGE F. MOORE, JOHN M. COULTER, GEORGE R. LYMAN, C. J. CHAMBERLAIN, R. R. GATES, C. H. SHATTUCK, W. R. MAXON); Biological Laboratory, Cold Spring Harbor, L. I., July 1 to August 11 (D. S. JOHNSON, GEORGE D. FULLER, HARLAN H. YORK); Lake Laboratory, Cedar Point, Ohio, June 22 to July 31 (MALCOM E. STICKNEY); Harpswell Laboratory, South Harpswell, Maine, June 15 to September 15, purely research.

THE EDITORS OF THE BOTANICAL GAZETTE have decided to discontinue the **Items of taxonomic interest,** heretofore a feature of "Notes for Students." Their chief purpose was to serve taxonomists in calling attention to new genera and to new American species. There was not space enough to do this completely, and anything short of completeness in such a record is unsatisfactory. Besides, this useful service is now more fully rendered by the *Botanisches Centralblatt* and FEDDE'S *Repertorium novarum specierum*, to say nothing of the various current card indexes. This does not mean that taxonomic literature will be neglected, but that it will be reviewed selectively, as in the case of other literature.

WITH THE PRESENT NUMBER of the BOTANICAL GAZETTE, the department of "NEWS" will be discontinued. Botanical news is now more promptly announced for American readers in *Science* than it can be in a monthly journal; and foreign readers receive such information through journals nearer at hand.

GENERAL INDEX

The most important classified entries will be found under Contributors, Personals, and Reviews. New names and names of new genera, species, and varieties are printed in **bold-face** type; synonyms in *italic.*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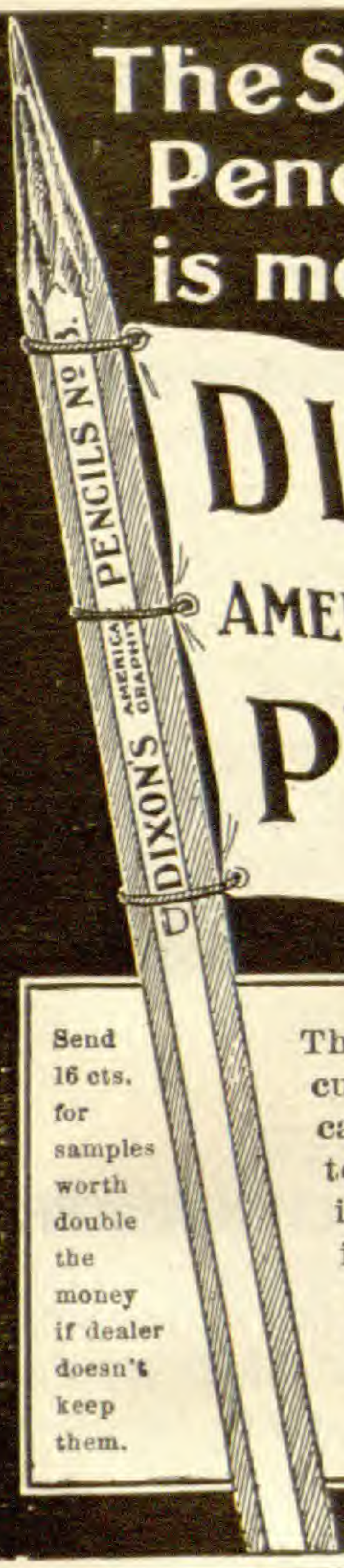
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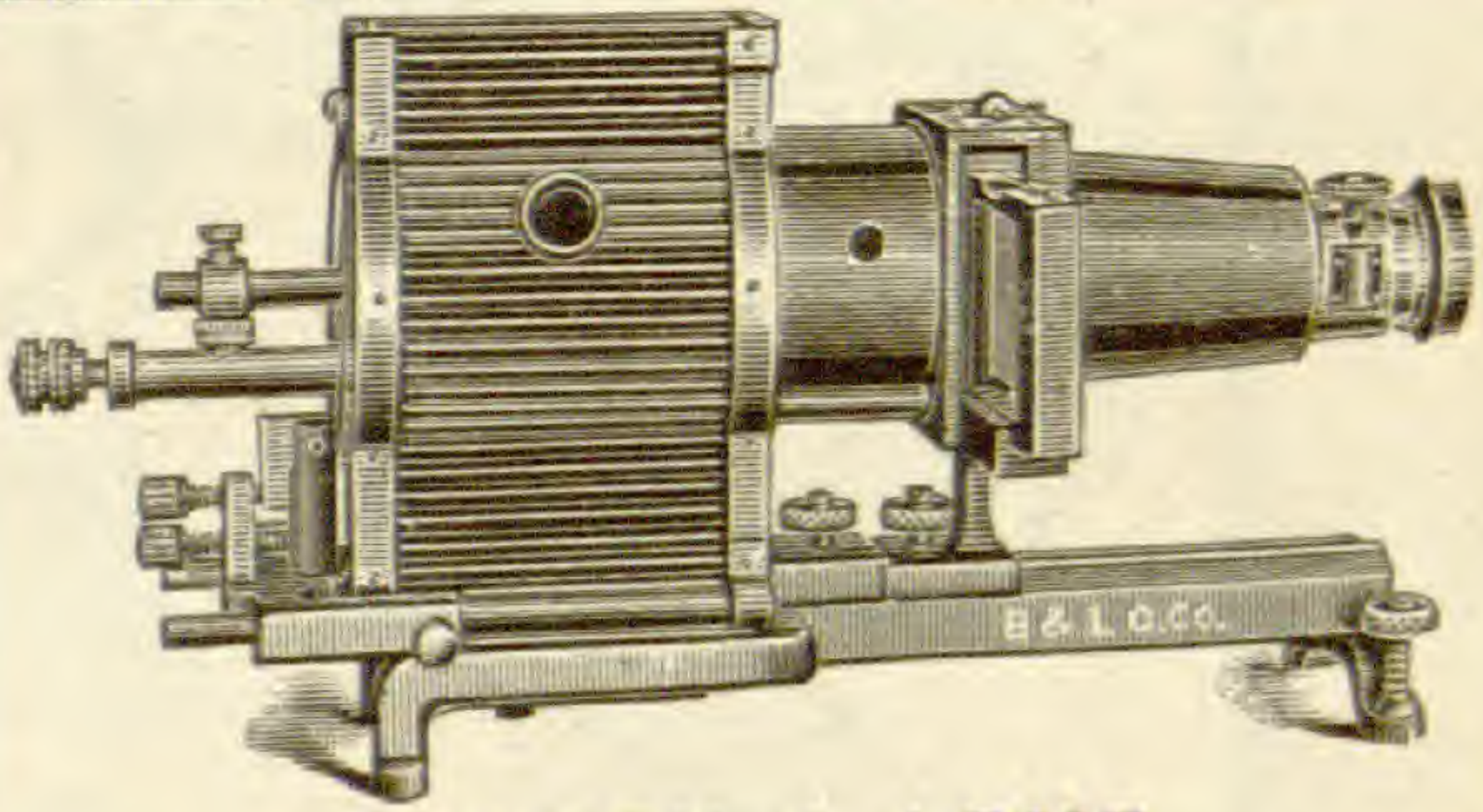
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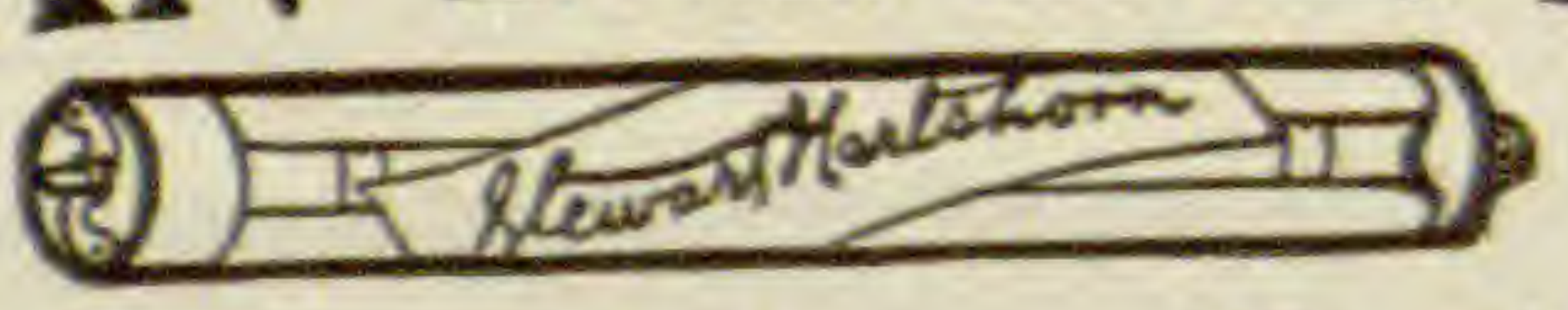

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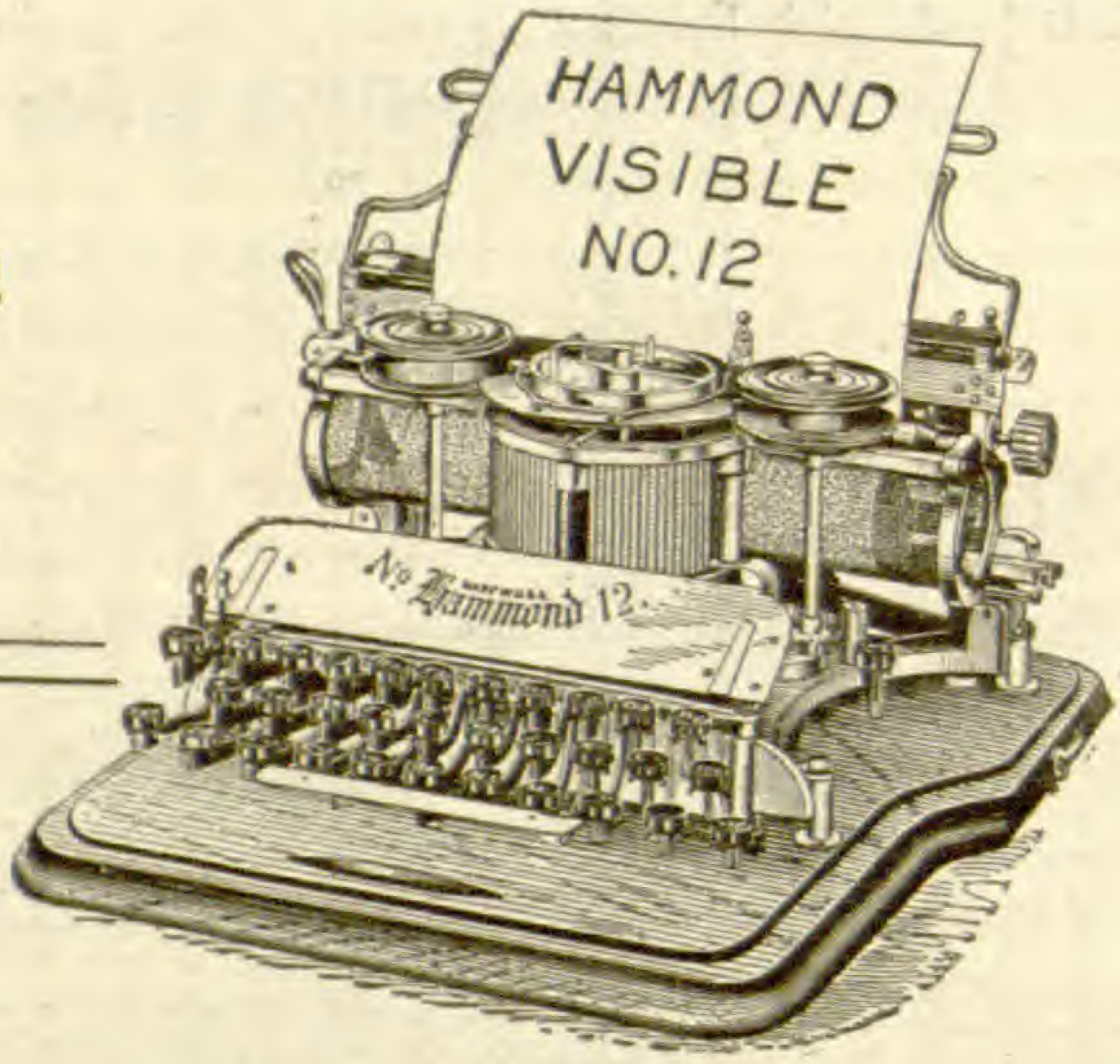
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